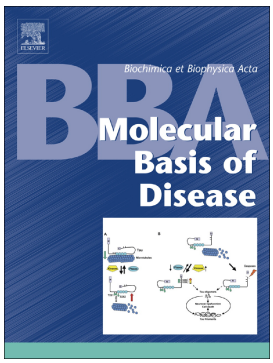

Epidemiology and pathogenesis of maternal-fetal transmission of Trypanosoma cruzi and a case for vaccine development against congenital Chagas disease

Lizette Rios, E. Emanuel Campos, Ramkumar Menon, M. Paola Zago, Nisha J. Garg

PII: S0925-4439(19)30314-X

DOI: https://doi.org/10.1016/j.bbadis.2019.165591

Reference: BBADIS 165591

To appear in: *BBA - Molecular Basis of Disease*

Received date: 12 July 2019

Revised date: 12 September 2019

Accepted date: 25 September 2019

Please cite this article as: L. Rios, E.E. Campos, R. Menon, et al., Epidemiology and pathogenesis of maternal-fetal transmission of Trypanosoma cruzi and a case for vaccine development against congenital Chagas disease, *BBA - Molecular Basis of Disease*(2019), https://doi.org/10.1016/j.bbadis.2019.165591

Epidemiology and pathogenesis of maternal-fetal transmission of *Trypanosoma cruzi* and a case for vaccine development against congenital Chagas disease

Lizette Rios [1,†], E. Emanuel Campos [2,†], Ramkumar Menon [3], M. Paola Zago [2,^], and Nisha J. Garg [1,4,^]

[1] Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston TX, USA

[2] Instituto de Patología Experimental, Universidad Nacional de Salta - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Salta, Argentina

[3] Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston TX, USA

[4] Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston TX, USA

[†] Equal first authors

[^] Corresponding authors
Dr. Maria Paola Zago, E: mpzago@conicet.gov.ar
Dr. Nisha Jain Garg, E: nigarg@utmb.edu

Abstract

Trypanosoma cruzi (*T. cruzi* or *Tc*) is the causative agent of Chagas disease (CD). It is common for patients to suffer from non-specific symptoms or be clinically asymptomatic with acute and chronic conditions acquired through various routes of transmission. The expecting women and their fetuses are vulnerable to congenital transmission of *Tc*. Pregnant women face formidable health challenges because the frontline antiparasitic drugs, benznidazole and nifurtimox, are contraindicated during pregnancy. However, it is worthwhile to highlight that newborns can be cured if they are diagnosed and given treatment in a timely manner. In this review, we discuss the pathogenesis of maternal-fetal transmission of *Tc* and provide a justification for the investment in the development of vaccines against congenital CD.

Keywords: Congenital; Chagas; Vaccine; *Trypanosoma cruzi*; Maternal-fetal transmission

Highlights

1. Every year, several *Trypanosoma cruzi*-infected babies are born to infected mothers in endemic and non-endemic countries.
2. Prompt diagnosis should be mandatory since the treatment of those babies would have a positive clinical and epidemiological impact.
3. Knowledge of the risk factors involved in maternal to fetal transmission would offer novel strategies for control of congenital Chagas disease.
4. Efforts to develop a vaccine that prevents vertical transmission of *T. cruzi* must be a public health priority.

Abbreviations: ASP – amastigote surface protein; BNZ – benznidazole; CD – Chagas disease; CCD – congenital Chagas disease; CTB – cytotrophoblast; DTU – discrete typing units; ECG – electrocardiogram; ELISA – enzyme-linked immunosorbent assay; GP63 – glycoprotein 63; GPx – glutathione peroxidase; HCMV – human cytomegalovirus; IFA – indirect immunofluorescence assay; IHA – indirect hemagglutination assay; LAMP – loop-mediated isothermal amplification; LVEF – left ventricular ejection fraction; MASPs – mucin-associated surface proteins; MnSOD – manganese superoxide dismutase; MVA – modified vaccinia ankara; MHC – major histocompatibility complex; NFX – nifurtimox; NLR – nod-like receptor; NO – nitric oxide; PRRs – pattern recognition receptors; PARP – poly(ADP-ribose) polymerase 1; PBN – phenyl-alpha-tert-butylnitrone; pPROM – preterm pre-labor rupture of the membranes; ROS – reactive oxygen species; RNS – reactive nitrogen species; STB – syncytiotrophoblast; SIRT1 – Sirtuin 1; SAPA – shed acute phase antigen; SOCS – suppressor of cytokine signaling; TLR – toll-like receptor; *T. cruzi* or *Tc* – *Trypanosoma cruzi*; TESA – trypomastigote excretory-secretory antigen; TS – *trans*-sialidase; TSA – trypomastigote surface antigen; uNK – uterine natural killer; WHO – World Health Organization.

1. Introduction

1.1. Epidemiology of T. cruzi infection and congenital Chagas disease

Trypanosoma cruzi (T. cruzi) is the causative agent of Chagas disease (CD). Transmission of *T. cruzi* occurs by triatomines in the natural environment, though this pathogen is also transmitted congenitally, or via blood transfusions, organ donation, lab accidents, and oral ingestion. The epidemiological update published by the World Health Organization (WHO) in 2015 [1] and other studies [2, 3] show that the prolonged burden of CD in twenty-one Latin American countries affects 6-7 million people and an additional 71 million people are at risk of infection every year. Due to the large-scale migration of Latin Americans over the last few decades, CD has also become an important health issue in the USA, Canada, Japan, and Europe [4]. In fact, autochthonous *T. cruzi* infection via vectorial transmission is well documented in the southern parts of the USA [5, 6]. In Europe, most migrants from CD endemic areas are concentrated in Spain, Italy, France, the United Kingdom, and Switzerland. Serology studies occasionally carried out in Europe, have recorded an overall 4.2% prevalence of *Tc* infection among immigrant Latin Americans, although the highest infection rate of 18.1% was noted among individuals from Bolivia [7]. However, in most European countries, the screening and treatment programs are almost absent [8].

In recent years, congenital Chagas disease (CCD) has emerged as a major health issue in endemic and non-endemic countries [7]. Though under-reported and under-estimated, globally >2-million women of fertile age are already infected with *T. cruzi*, and 1-10% of fetuses carried by infected mothers are born with CCD [9-11]. In 2010, WHO indicated 8668 new cases of CCD in Latin American countries that corresponded to 22% of all new, acute cases of *T. cruzi* infection that year [1, 12]. In the USA, a study conducted in 2009 estimated 40,000 women of child-bearing age with chronic CD are in the country, and 60-315 cases of CCD were expected to occur annually [13]. However, only two cases of CCD were reported [14, 15], likely due to a lack of awareness and an absence of policies requiring the training and reporting of CCD among USA pediatricians [16]. In Europe, physicians should also be aware of the risk of congenital transmission of *T. cruzi* to newborns by their infected mothers [17]. There is a consensus that the high rate of transmission occurs when women are acutely infected and/or exhibit reactivated infection [18]. Prenatal and/or perinatal transmission of *T. cruzi* to the fetus and newborns occurred in 53% of women who became infected during pregnancy [18-21]. Women, who were infected with *T. cruzi* before pregnancy and were exposed to HIV or received treatment with immunosuppressive drugs during pregnancy, transmitted *Tc* to their newborn with a much higher frequency [22, 23]. We surmise that the screening of pregnant women and newborns is important to restrict transmission and ensure timely treatment.

Historically, CCD has been associated with infected fetuses born with low birth weight, low Apgar score, hepatosplenomegaly, respiratory distress syndrome, myocarditis, or meningoencephalitis [24]. Recent data collected in Bolivia suggest that 29% of infants born with infection develop clinical symptoms of acute cardiac disease [25]. Other infected infants may not exhibit clinically symptomatic disease in their childhood; however, they remain at risk of developing digestive and neurological

disorders or chronic cardiomyopathy as they grow to be young adults [10, 26]. The severity of Chagas cardiac disease is categorized according to Kuschnir classification [27], depicted in **Figure 1**: class 0 displays reactive serology, normal electrocardiogram (ECG), and no cardiac enlargement; class 1 includes individuals with normal global ventricular function with minor ECG changes, e.g., arrhythmias or conduction disorders, and mild echocardiographic contractile abnormalities; class 2 includes patients exhibiting decreased left ventricular ejection fraction (LVEF) with no other signs of heart failure, as well as patients with compromised LVEF in combination with prior or current symptoms of heart failure; and class 3 include patients with apical aneurysm and symptoms of heart failure at rest that are refractory to maximized medical therapy and require specialized and intensive healthcare [28, 29].

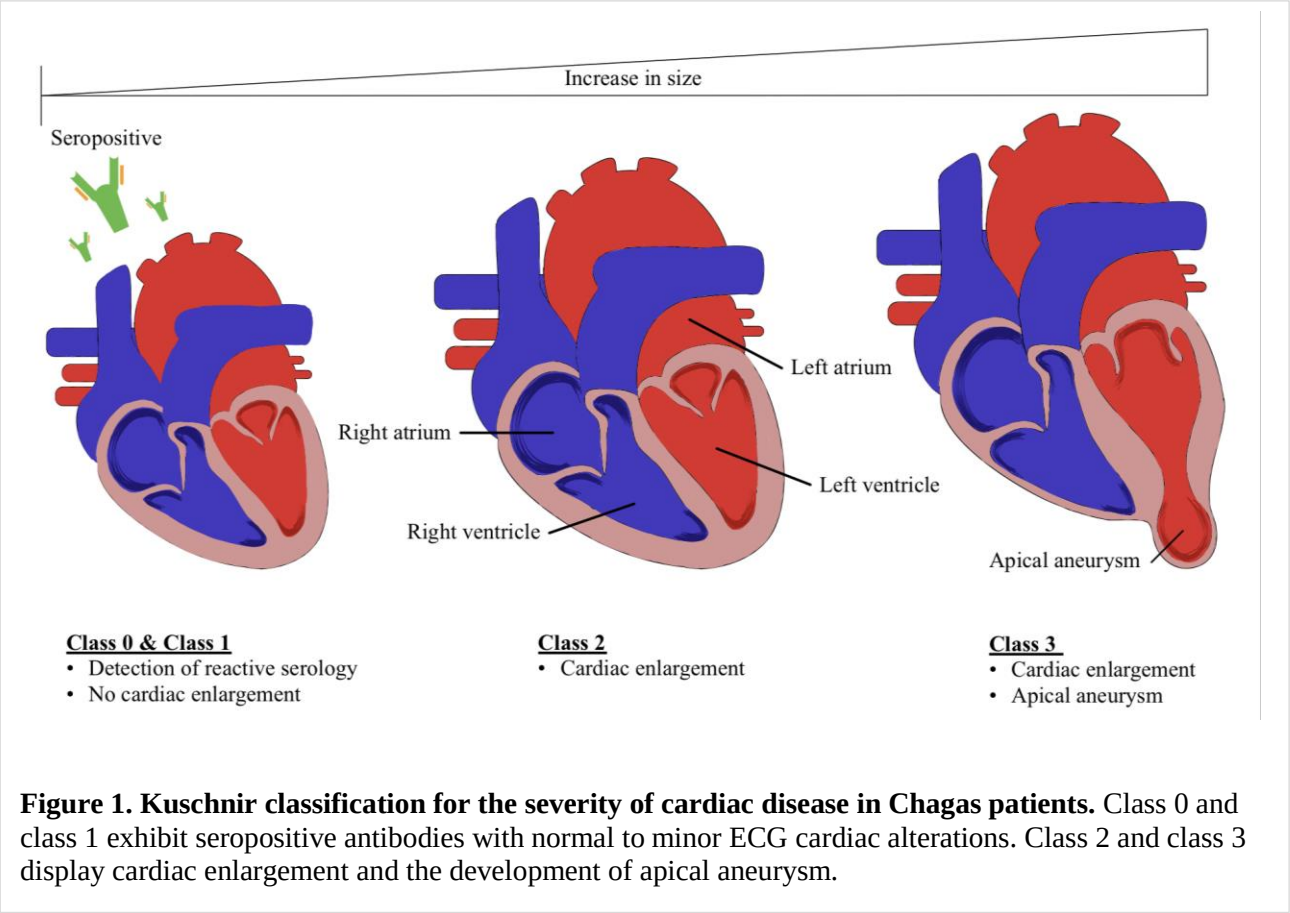

Figure 1. Kuschnir classification for the severity of cardiac disease in Chagas patients. Class 0 and class 1 exhibit seropositive antibodies with normal to minor ECG cardiac alterations. Class 2 and class 3 display cardiac enlargement and the development of apical aneurysm.

1.2. Current approaches to diagnosis

Upon *T. cruzi* infection, acute blood parasitemia can be detected for ~60 days. Direct microscopic visualization of circulating trypomastigotes in peripheral blood films or buffy coat smears remains the gold standard for diagnosing the acute infection; however, this protocol requires multiple blood draws and has relatively low sensitivity [30]. Molecular methods, e.g., traditional and quantitative PCR and loop-mediated isothermal amplification (LAMP), can offer a higher level of sensitivity and specificity in diagnosing acute *T. cruzi* infection [31]. Serology studies using trypomastigote excretory-secretory antigens (TESA) blot for the detection of IgM-specific shed acute-phase antigen (SAPA) bands were

able to detect *T. cruzi* infection in infants at 6 months of age [32, 33]. Other serological tests based on anti-*Tc* IgGs (e.g., indirect hemagglutination assay [34], indirect immunofluorescence assay [35, 36], or enzyme-linked immunosorbent assay [34, 37]) are used to identify exposure to *T. cruzi* in infants of 9 months or older. A summary of the diagnostic methods that are currently available or are in development is presented in **Table 1**.

Table 1. Summary of current methods in use or in development for the diagnosis of congenital transmission of *T. cruzi*

Method	Time after birth	Technique description	Drawbacks	Advantages	Reference
MicroStrout (micromethod)	0-3 months	Cord or peripheral blood of newborn collected in capillary tubes → microscopic observation of the parasites in buffy coat.	Suboptimal sensitivity, repeated sampling, many variables, e.g., operator´s skill, time of processing, sample quality can affect the outcome.	Direct observation of *Tc* is confirmatory, inexpensive, minimal equipment/ facilities requirements.	[38, 39]
PCR (conventional or qPCR)	0-3 months	*Tc*DNA amplification using cord or peripheral blood sample from newborn.	Expensive reagents and equipment, need trained personnel to avoid contamination.	Best test for early detection with high sensitivity and specificity in the absence of direct *Tc* observation.	[12, 38, 40]
Hemoculture	0-3 months	Cord or peripheral blood→ culture *Tc* in LIT medium or agar blood based medium.	Trained personnel, risk of contamination, time consuming, positive results take 2-3 months.	As sensitive as the micromethod (microStrout).	[37, 38]
LAMP	0-3 months	Isothermal *Tc*DNA amplification from blood samples. Results (+/-) can be visually recorded.	In experimental / development stage.	Potentially easy to apply in the field and resource limited settings.	[31, 41]
ChuNAP	0-3 months	Urine detection of *Tc* antigens with nanoparticles capture method.	In experimental / development stage, cost per test not known.	Reports of high sensitivity.	[37, 42]
IgM -TESA	1-6 months	Immune blotting for IgM for TESA antigens in serum of newborns.	Experimental stage, requires high operator´s skills.	Less expensive than qPCR, and will likely allow early diagnosis.	[25, 32]
SAPA-ELISA	1-12 months	α-SAPA IgG detection in serum by ELISA.	Available for research, not yet used for clinical diagnosis.	Likely allows early diagnosis and is less expensive than qPCR.	[25, 32]
IgG serology	After 8 months	Conventional detection of anti-*Tc* antibodies by ELISA, HAI, IFA.	No major concerns	Methods of choice for diagnosis in most clinical labs. Easy to perform and cost effective.	[37, 38, 40]

An ideal diagnostic algorithm for identifying congenital transmission of *T. cruzi* in infants is shown in **Figure 2**. The presented information was adapted from the algorithm developed by the

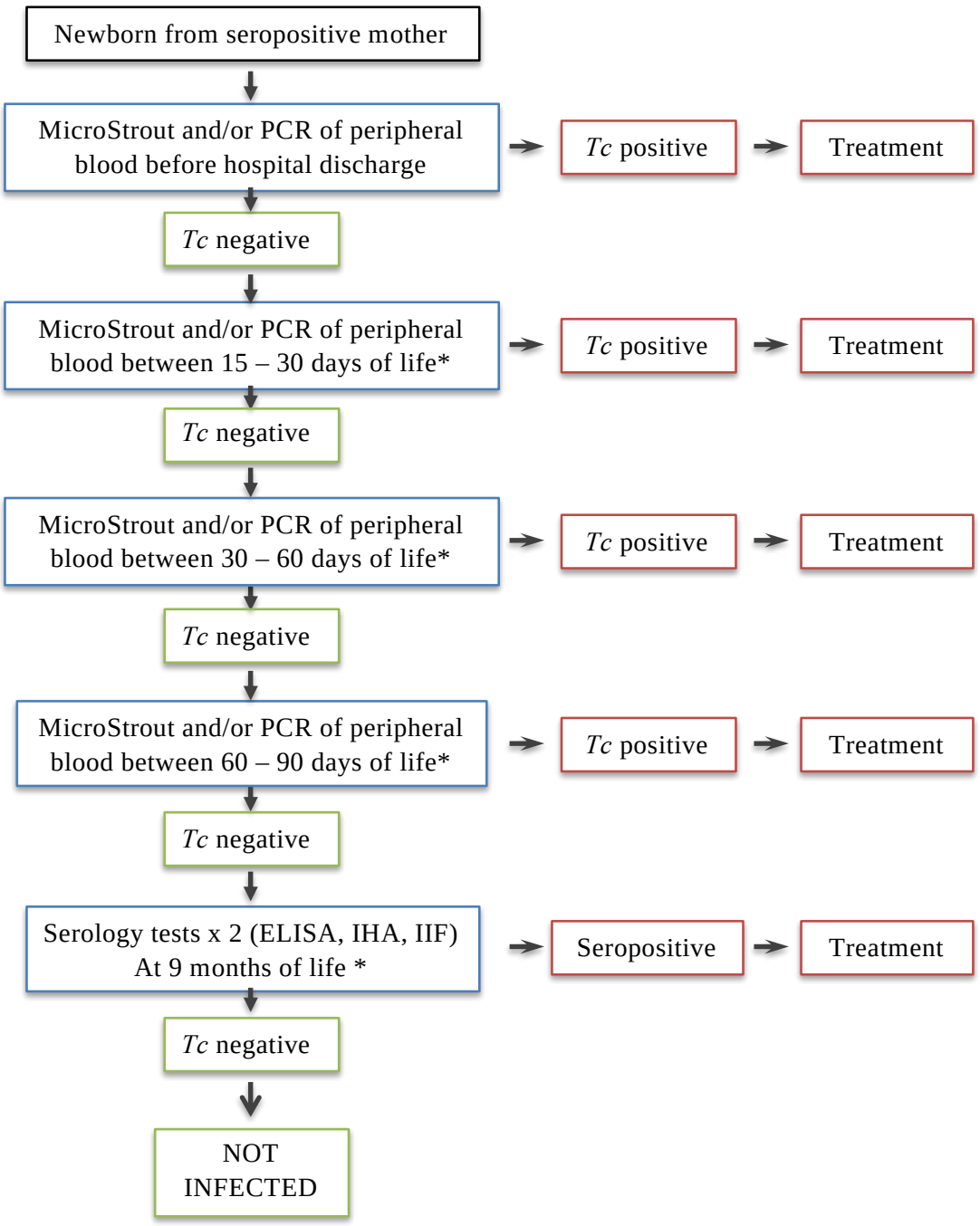

Figure 2. Diagnostic algorithm proposed for identification of *T. cruzi* infection in newborns in endemic countries. Abbreviations: PCR – polymerase chain reaction, *Tc* – *T. cruzi*, ELISA – enzyme linked immuno-sorbent assay, IHA – indirect hemagglutination assay, IIF – indirect immunofluorescence assay. ***** Tests for diagnosis of *T. cruzi* infection in infants can be performed at the time of mandatory vaccination, according to country-specific public health policies.

Hospital system in Salta, Argentina. Though not routinely employed in endemic countries due to a lack of resources, it is recommended that maternal seropositive status during pregnancy should be followed by testing of the infant for *T. cruzi* infection during the first year after birth. Until 3 months of age, newborn blood samples should be screened for circulating parasites', detection by microscopic micromethod is also known as microStrout, hemoculture, or qPCR [26]. In current practice, infants with an inconclusive diagnosis at birth may be screened serologically after 9 months when maternal transplacental antibodies have vanished [18]. This algorithm, when used for diagnosis, is very useful for treating children, however, only 20% of the infants in Bolivia follow up for nine months for unequivocal diagnosis [43]. In our experience, the loss of follow up and treatment of potentially infected infants was higher than 80% in an endemic province of Argentina (Campos et al, unpublished data).

1.3. Treatment

The currently available drugs for the treatment of *T. cruzi* infection are benznidazole (BNZ) and nifurtimox (NFX) **(Table 2)**. Infected children that are 14 years old or younger exhibit a cure rate of > 85% upon treatment with NFX [44, 45]. Unfortunately, NFX causes intense side effects, and therefore, children often do not complete the treatment resulting in increased incidences of NFX resistance [46]. Clinical studies have shown that BNZ is effective in curing up to 90% of acute or recent cases of infection [47-49] with fewer side effects. International guidelines recommend that acute cases (all ages) and children up to 14 years old should be treated with anti-parasitic drug therapies [44]. In the US, the Food and Drug Administration agency has recently approved BNZ for use in children 2–12 years of age [50].

The recommended dosage of BNZ and NFX for treatment of young children varies by country (**Table 2**). For example, the CDC recommends BNZ as an oral dose of 5-7.5 mg/kg per day in 2 divided doses for young children. CCD control programs in Bolivia and Argentina recommend treatment with BNZ at 5-10 mg/kg/day for 60 days, in two daily doses, except the first week when it is given at 7 mg/kg/day in two doses. Children are monitored to ensure compliance and doses are adjusted according to changes in weight and/or tolerance [49, 50]. However, a major issue regarding the treatment of infants and children include an absence of accurate diagnosis (discussed above) and a lack of drug accessibility. Both BNZ and NFX are contraindicated during pregnancy [51]. Many retrospective observational studies have indicated that anti-parasitic treatment of seropositive girls before they reach the childbearing age and of seropositive women before their first pregnancy would be useful in preventing the congenital transmission. These observational studies have led to strategic recommendation(s) that seropositive girls and women in reproductive age should be treated to reduce or eliminate the risk of congenital transmission [52-54].

Table 2: Current drug therapies approved for treatment of *T. cruzi* infection

Country	0-2 years	2-12 years	12 years or older	Remarks	References
Benznidazole recommended dosages					
Endemic countries (e.g., Argentina and Bolivia) *	5-7 mg/kg	5-7 mg/kg in Argentina and 10 mg/kg in Bolivia	200-300 mg Max: 400 mg	Some countries are testing slow release tablets of 12.5 mg tablets in children.	[43, 55] Argentina Ministry of Health⅄
US (FDA)	Not approved	5-7.5 mg/kg	Not approved		[50] CDC***
Non-endemic countries (e.g., Spain, Italy, Switzerland) *	7-10 mg/kg	10 mg/kg	150 mg		[7, 56]
Nifurtimox recommended dosages					
Argentina **	10-12 mg/kg	10-12 mg/kg in three doses per day	8-10 mg/kg in three doses, Max: 700 mg/day	Pre-term/low weight infants are treated with lower dose once a day, slowly increased to achieve therapeutic dose.	Argentina Ministry of Health⅄
Bolivia **	10-12 mg/kg	12.5-15 mg/kg	8-10 mg/kg in three doses		[57]
Spain **	15-20 mg/kg	12.5-15 mg/kg	8-10 mg/kg in three doses		[7, 58]

All dosages are listed per day.

* Unless stated, treatment is given in two doses per day; and recommended period of benznidazole treatment is 60 days. It is acceptable to halt treatment after 30 days in case drug intolerance is noted. If treatment is stopped before 30 days, it is recommended to control adverse effects and then start a new cycle of benznidazole treatment.

** Recommended period of treatment with nifurtimox is 60 days in endemic countries and 90 days in non-endemic countries. This drug is rarely used because of severe side effects.

***CDC: https://www.cdc.gov/parasites/chagas/health_professionals/tx.html

⅄Argentina Ministry of Health: http://www.msal.gob.ar/images/stories/bes/graficos/0000000622cnt-03-guia-para-la-atencion-al-paciente-con-chagas.pdf

2. Pathogenesis of congenital transmission

How *T. cruzi* evades the placental barrier and complex immune responses of the mother, placenta, and fetus to establish active congenital infection is not well understood. We discuss our current knowledge of the placental, parasitic, maternal, and fetal factors that likely play a role in the risk of congenital transmission.

2.1. Placenta

The placenta is the foremost organ to develop during embryogenesis. It is a maternal-fetal organization of tissue for the physiological exchange of gases, nutrients, and wastages. It provides hormones, growth factors and immunological protection required for pregnancy. It forms the primary barrier between the maternal and fetal compartments protecting the developing fetus from infectious agents [59, 60].

Decidua, trophoblastic and chorioamniotic cells establish the maternal-fetal interface of the placenta. Fetal trophoblasts differentiate into cytotrophoblasts (CTB), syncytiotrophoblasts (STB) and extravillous trophoblasts that form the placental villi and are in direct contact with maternal blood present in the intervillous space [59]. Parasites' invasion of the trophoblast layer is believed to be the most common route of transmission to fetal tissues [61] **(Figure 3A)**. Altemani et al [62] performed histological analysis comparing placentas from seronegative women without inflammation and seropositive placentas from stillbirths (n=4) and live births (n=4). In cases of stillbirths, authors noted numerous *T. cruzi* nests and diffused and severe villitis with presence of high number of macrophages and granulocytes, low CD4: CD8 ratio, and extensive trophoblastic necrosis. Furthermore, they were able to identify parasite infiltration of the trophoblastic barrier and parasitism of the trophoblast and stromal cells. In comparison, villitis in live births of seropositive mothers was focal with only a few parasites and no rupture of the trophoblastic barrier. These authors proposed that parasites invade not only the trophoblast but also the marginal zone of the hemochorial placenta. Indeed, Fernandez-Aguilar et al [63] collected placental tissues of infected newborns in Bolivia and showed that parasitic lesions were highest at the marginal sinus **(Figure 3B)**, and gradually decreased in the chorionic plate and distant membranes. If this is the case, infection can spread and weaken the membranes surrounding the fetus, causing pre-term premature rupture of the membranes (pPROM) and preterm birth, as reported by Torrico et al [64] **(Figure 3C)**. Host inflammatory reactions to infection in the membranes may also cause pPROM and preterm birth [65]. Other groups found parasites in placental areas devoid of trophoblast at chorionic plate and amastigotes were seen within membranes of amniotic epithelium [19], along with parasitism of the trophoblast and stromal cells at the trophoblastic barrier [66, 67]. The trophoblastic epithelium is not present at the marginal zone, and parasites can infect and replicate in fibroblasts and macrophages, and potentially transmit through blood vessels supplied to the fetus [24, 62].

Vertical transmission could also occur through ingestion of amniotic fluid containing *T. cruzi*, **(Figure 3D)** [18, 24] or via the hematogenous route from the mother during delivery when there is potential for a placental tear [18].

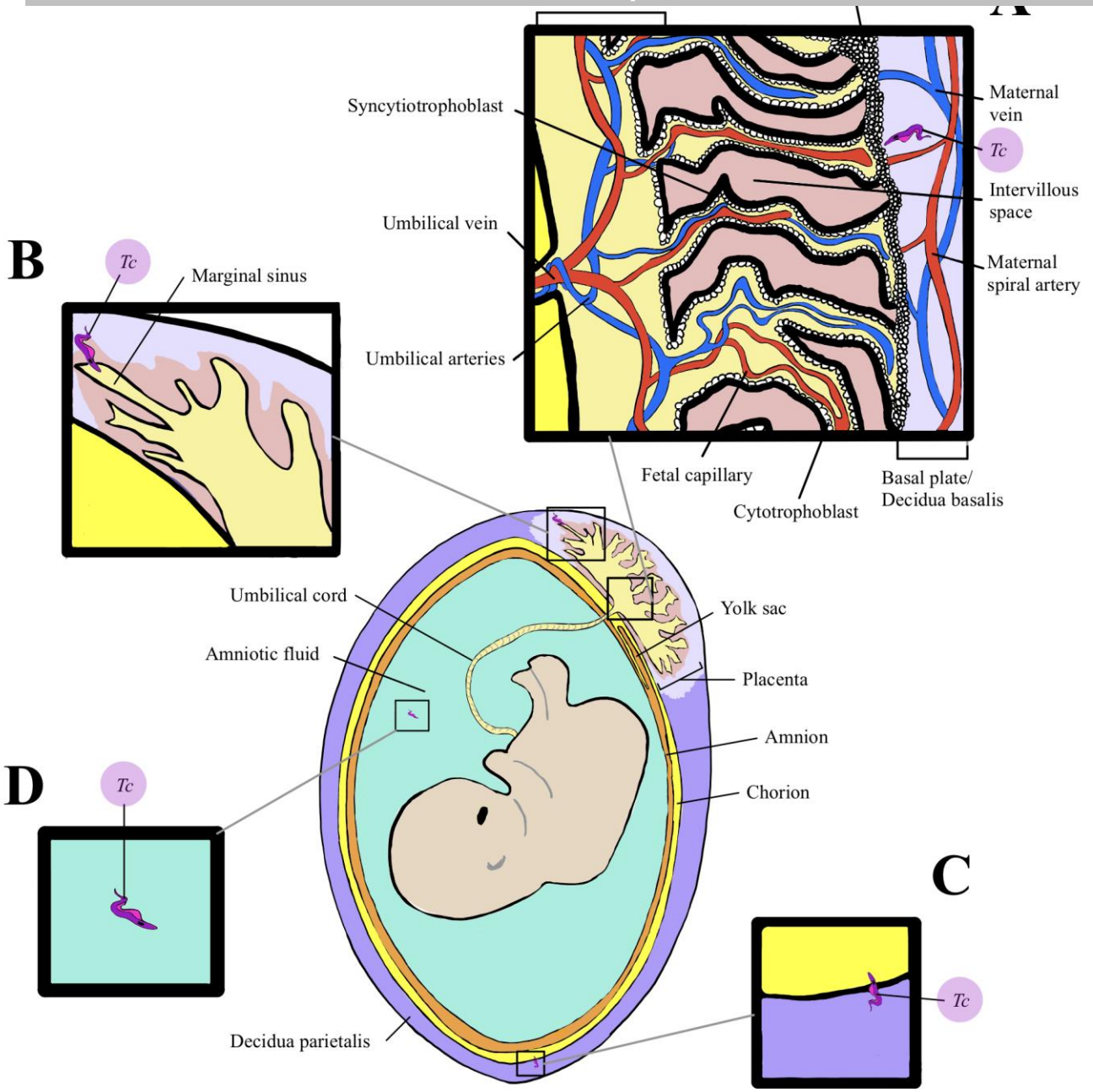

Figure 3. Potential routes of maternal-fetal transmission of *Trypanosoma cruzi* (*Tc*). (A) It is proposed that the parasite invades the intervillous space. **(B)**. Alternative transplacental route of vertical transmission through marginal sinus is shown. **(C)** It is also suggested that infection spreads through and weaken the fetal membranes that can cause premature rupture and early birth. **(D)** Congenital transmission may also occur via oral route through ingestion of amniotic fluid with circulating forms of *T. cruzi*.

The currently known anti-parasitic mechanisms of the placenta have been reviewed [68-70]. Briefly, studies in human placental explants suggest that chorionic villi denuded of STB were more susceptible to *T. cruzi* infection, thus, indicating that the detachment of the first structure of the placental barrier was detrimental for placental defense **(Figure 4)** [68-70].

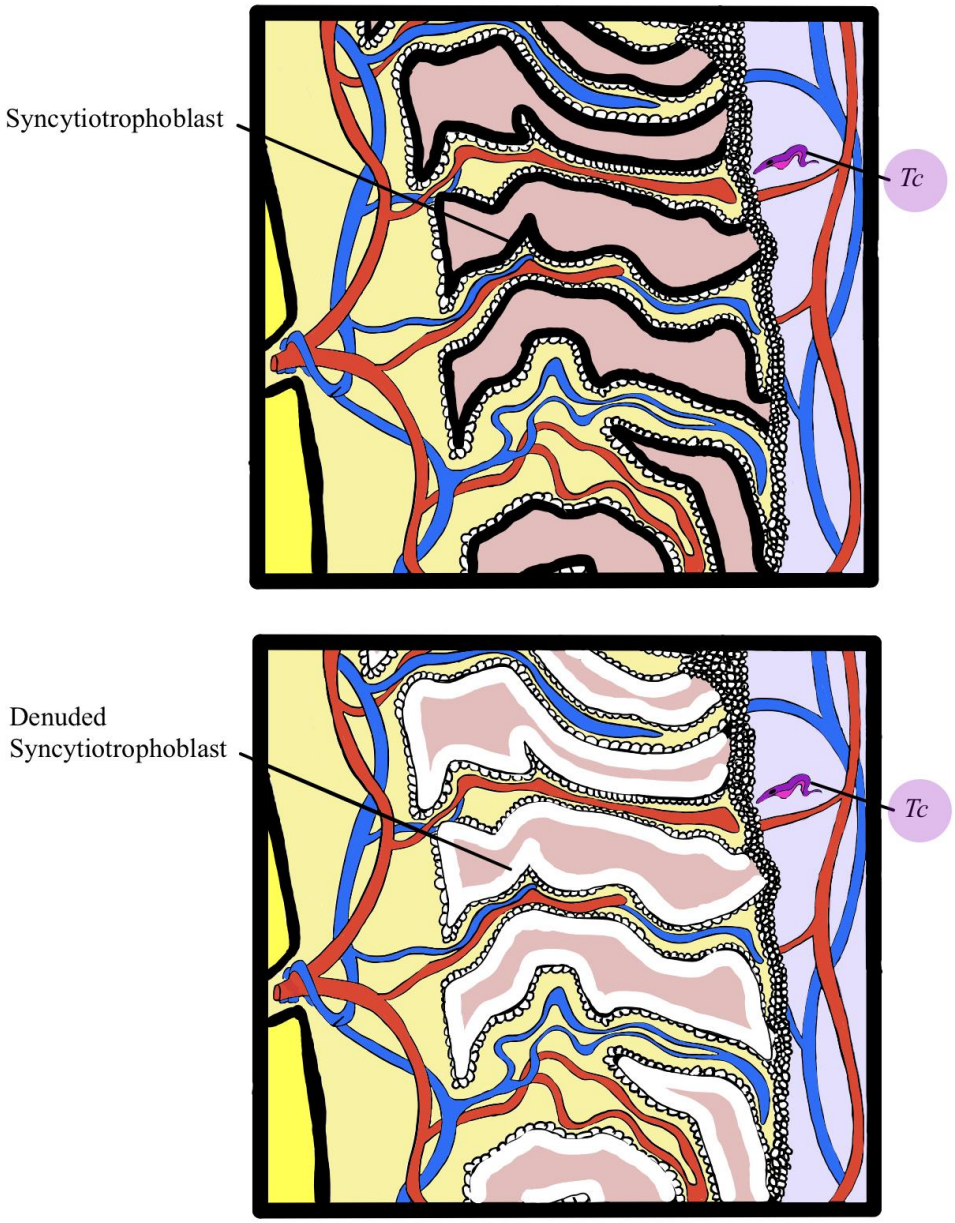

Figure 4. Diagrammatic presentation of increasing parasitic invasion due to the denudation of the chorionic villi syncytiotrophoblast. *Tc: Trypanosoma cruzi.*

Moreover, the placenta functions as an active immunological organ. Several innate immune receptors, including members of the toll-like receptor (TLR) and nod-like receptor (NLR) families, are expressed by extravillous trophoblasts **(Figure 5A)**. These pattern recognition receptors (PRRs) recognize the pathogen (or danger) associated molecular patterns (PAMPs and DAMPs, respectively) and respond by the activation of the innate immune response at the maternal-fetal interface [71, 72]. It was suggested that parasites in the intervillous space trigger the innate immune system, resulting in the production of pro-inflammatory cytokines, reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can damage the placental barrier [69, 73] and provide an entry for the parasite

(Figure 5B). Others showed using human placental chorionic villi explants that *T. cruzi* activated the TLR2 innate immune receptor resulting in placental secretion of IL-6 and IL-10 **(Figure 5C)** which can dysregulate trophoblastic turnover [74]. Overall, these studies show that the placenta represents an active surface for host-parasite interaction and provides the first barrier to *T. cruzi* infection. The detachment of the placental barrier would increase the chances of congenital transmission [69].

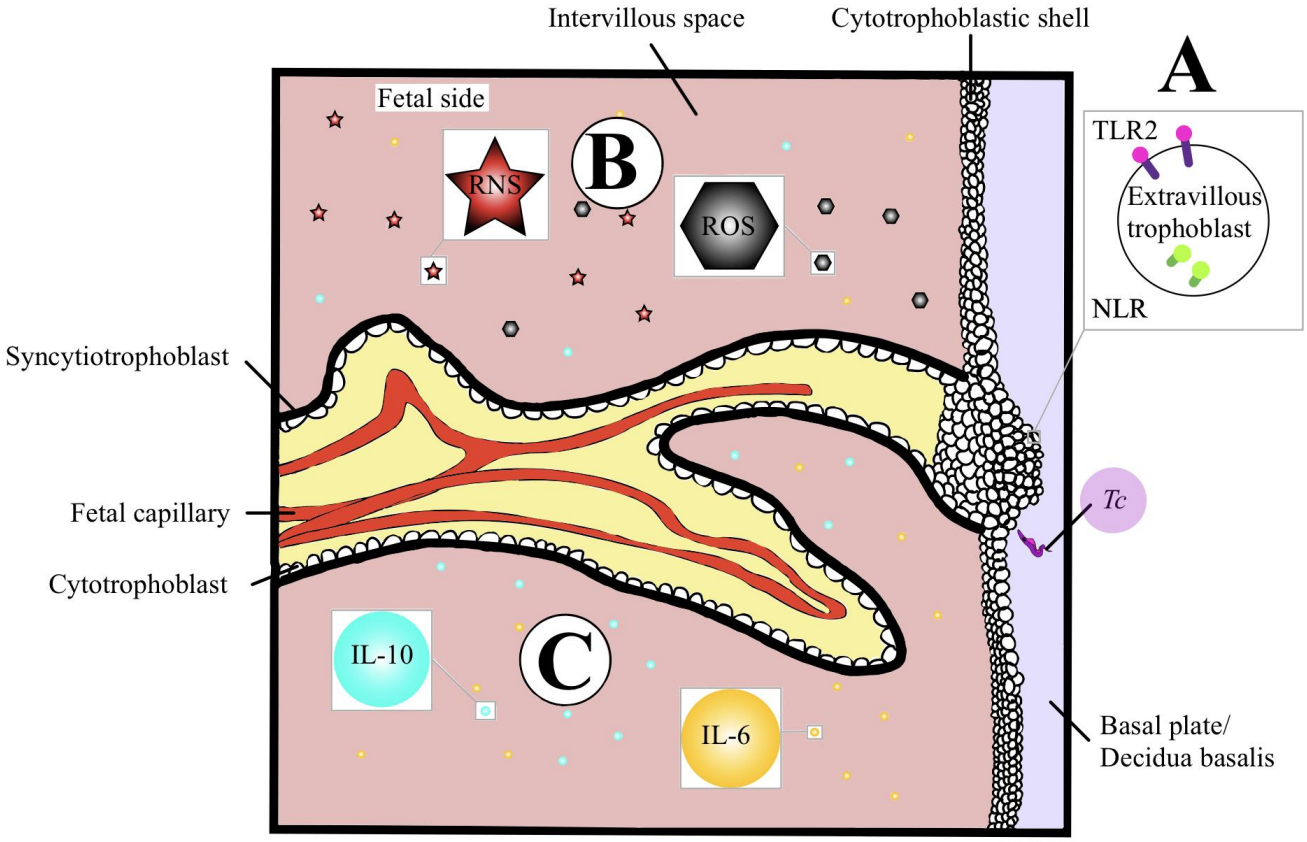

Figure 5. Current understanding of placental immune responses to *T. cruzi*. (A) Extravillous trophoblasts express innate immune receptors, e.g., toll-like receptor 2 (TLR2; purple) and nod-like receptor (NLR; green). **(B)** Reactive oxygen species (ROS; black hexagon) and reactive nitrogen species (RNS; red star) are released to kill the parasite, but these reactive species can also damage the placental barrier and provide the parasite access to the fetus. **(C)** Parasite exposure activated the TLR2 innate immune receptor and placental secretion of IL-6 and IL-10. *Tc: Trypanosoma cruzi.*

2.2. Parasitic factors

Currently, *T. cruzi* is classified into six phylogenic lineages known as DTUs (Discrete Typing Units). The DTUs identified in infants born to infected mothers were similar to those present in the local population indicating that all DTUs can be transmitted via congenital infection [75-77]. When mixed or multiclonal infections were identified in the mother, a predominance of different clones was noted in the mother and their newborns, suggesting that a process of natural selection of the

transmitted parasite might occur [75, 78, 79]. However, specific parasite factors associated with the risk of maternal-fetal transmission are not known.

2.3. Maternal factors

In humans, the congenital transmission of *Tc* may occur during any trimester of pregnancy. However, the transmission of the infectious agent(s) likely occurs after the placental intervillous space is opened in 12 weeks of gestation [80] and parasitic transmission probability further increases between 22 and 26 weeks of gestation [81]. Additionally, the parasite load of infected women during pregnancy is an indispensable factor. It is inferred from preclinical studies that women exhibiting parasitemia of 10-20 trypomastigotes/ml of blood will have a higher potential of endangering their fetus with *Tc* infection [11, 19, 24]. Some investigators believe that natural immune tolerance to prevent fetal rejection causes an increase in parasitemia in chronically infected women during the second and third trimester, and contributes to congenital transmission [82]. Other researchers have proposed that the immune system can be activated to control pathogen in pregnant women and thereby prevent congenital transmission [83]. This hypothesis is supported by the higher rate of congenital transmission in infected women living in non-endemic areas compared to women that are exposed to repeated, vector-borne *T. cruzi* infection, and most likely mount a robust immune response to the parasite [84, 85]. Likewise, transmission rates are higher when mothers acquire an acute infection during pregnancy or are immunocompromised due to HIV co-infection or other reasons and exhibit reactivated acute CD [22, 86]. Further, infected pregnant women who transmitted *Tc* not only had significantly higher parasitic load, but also produced less of IFN-γ and weak activation of monocytes and T cell response [87]. Other studies showed that non-transmitting mothers displayed higher levels of TNF-α compared to transmitting ones, suggesting that an inflammatory response is elicited to control the infection in order to prevent vertical transmission [88]. The role of age of the mother and number of pregnancies in increasing the risk of transmission needs further investigation [87, 89].

2.4. Fetal factors

Generally, neonatal parasitic loads are higher in comparison to maternal parasitemia and easily detected up to 3 months after birth. Parasitemia experienced by the infected newborns may also depend on the fetal/neonate innate and/or adaptive immune responses [10, 11]. It is described in the literature that infected neonates displayed weak innate responses, but stronger T cell responses based on cytotoxic T cells, IFN-γ producing type 1 immune response and increased plasma levels of IL-17 [90, 91]. These components of fetal immunity may control the parasitic load, but fail to completely protect from congenital infection [11]. Others have suggested that the fetal inflammatory response can contribute to adverse pregnancy outcomes such as pPROM and spontaneous preterm birth [64, 65].

In conclusion, the congenital transmission of *T. cruzi* is a complex process contingent on a combination of specific conditions for the parasite, placenta, and immune response of the fetus and the mother.

3. A case for vaccine development against CCD

3.1. Background

A systematic review of the literature shows that the pathogenesis of Chagas heart disease, irrespective of the route of acquisition of infection, is dependent on persistence of low-grade, systemic infection with documented adverse immune reactions [92-95]. We have shown that chronic *T. cruzi* infection also causes persistent mitochondrial dysfunction-related oxidative stress in the heart [96-109] and it is further exacerbated by inefficient antioxidant capacity [103, 106, 110-114]. Further, we have found that ROS directly signal cytokine and chemokine production in infected cardiomyocytes and murine hearts [98, 100, 115]. ROS-induced cellular debris and extracellular vesicles produced in CD have the capacity to stimulate a pro-inflammatory macrophage response [116]. Thus, the contributions of parasite persistence, mitochondrial dysfunction, oxidative stress, and inflammatory infiltrate are not mutually exclusive processes in the multi-faceted chronic Chagas disease.

A detailed review of the studies that have provided evidence for protective (vs. pathologic) immune responses are published elsewhere [117]. These studies conclude that 1) sub-par innate immune responses allow parasite dissemination and delayed kinetics of $CD8^+$ T cells associated with mixed type 1/type 2 cytokine responses fail to remove circulating and intracellular parasites and contribute to parasite persistence; and 2) an efficient protective response to *T. cruzi* requires elicitation of Th1 cytokines, lytic antibodies, and the concerted activities of phagocytes, T helper cells, and cytotoxic T lymphocytes. An important implication of these studies is that arresting infection or the acute parasite load below a threshold level would be effective in decreasing the tissue damage and clinical severity of CD. These studies provide an impetus for the development of immunotherapies against *T. cruzi.*

Potential vaccines for the prevention of congenital transmission would target trypomastigote- and amastigote-forms of the parasite. Vaccines against infective trypomastigotes that transfer from placenta to fetus will prevent the initiation or persistence of infection and limit the parasitemia level/incidence in fetal tissues. Vaccines against intracellular replicative amastigotes would arrest the propagation of the parasite in the mother as well as in fetal cells and prevent the parasite from re-entering the blood. Further, a vaccine should include antigens targeting all lineages of the parasite and be useful as a prophylactic as well as a therapeutic vaccine [118].

3.2. Efforts for preventive/therapeutic vaccine development against T. cruzi

The review of historical perspectives on vaccine development against *T. cruzi* is published elsewhere [118-120]. In recent studies, subunit vaccines have been considered as the best choice for vaccine development against *T. cruzi* [121-123]. This is because the *T. cruzi* genome estimated at > 100 Mb [124] is highly complex and consists of several large families that account for >18% of the total protein-encoding genome in *T. cruzi*. These families include *trans*-sialidase (TS) super family (737 genes), mucins (662 genes), mucin-associated surface proteins (MASPs, 944 genes), and

glycoprotein 63s (GP63s, 174 genes) [124]. Genes included in the large families express shared epitopes that can potentially tolerize the T cell response and result in sub-par parasite elimination [125]. Others propose that the potential synergistic immunologic advantage of a mixture of epitopes from a family with several genes would encourage an elevated frequency of immune effectors in heterogeneous host populations and will offer effective immunity against distinct parasite strains [119]. Accordingly, members of the TS superfamily, including, trypomastigote surface antigen (TSA-1), TS, and amastigote surface proteins (ASP-1, ASP-2, ASP-9) were considered as potential vaccine candidates because they have been shown to be recognized by antibody response and $CD8^+$ T lymphocytes in infected mice and humans [126-135]. Other antigens, including complement regulatory protein [136, 137], lysosomal cysteine proteinase (cruzipain) [138, 139], flagellar calcium-binding protein [140, 141], GP90 and GP82 [142-145], kinetoplastid membrane protein 11 [146-148], LYT1 [149], paraflagellar rod proteins [150-153], Tc52 (glutathione *S*-transferases) [154] etc. were also tested as vaccine candidates in different models of experimental *T. cruzi* infection. Recently, α-Gal glycotype, Galα1,3Galβ1,4GlcNAc (Galα3LN) was used as a candidate vaccine [155]. In conjunction with vaccine studies, multiple adjuvants (e.g., IL-12, CD40, HSP70, MALP-2, and CpG oligodeoxynucleotides), routes of delivery, concentration and number of doses, and prime-boost strategies were tested to skew the vaccine-induced immune responses toward Th1 type and enhance the protective long-term efficacy [149, 156, 157]. These candidate prophylactic vaccines provided a variable degree of protection, measured based on increased survival and control of blood parasitemia, tissue parasite load, and cardiac inflammation (discussed in [158]).

Others have used immunoinformatics to identify potential antigens and epitopes of the major histocompatibility complex (MHC) class I. Of the 172 epitopes identified by this approach, 26 were tested in mice. Ten of the 26 tested epitopes induced IFN-γ and provided protection from parasitemia, cardiac inflammation, and tissue parasite burden [159].

We utilized a computational algorithm to investigate the *Tc* sequence database for suitable B and T epitopes [160, 161]. Next, we implemented a biological screen to locate those that were identified by IgGs and/or provoked a type 1 $CD8^+$T cell response in infected mice, dogs and humans [160-164]. Among the 11 antigens identified, TcG1, TcG2, and TcG4 qualified for vaccine design. These antigens **a)** contained mRNA/protein expression during the mammalian stages of *Tc* [160, 161], **b)** are discharged in the cell cytoplasm of the host during parasite differentiation [160, 161] and **c)** consisted of epitopes offered by MHC alleles of mice [161], dogs [163, 164], and humans [162]. Remarkably, TcG2 and TcG4 were preserved in five of the six *Tc* lineages (80-96% homology), affording us confidence that TcG2/TcG4-vaccine can offer protection against various *Tc* isolates in the USA and Latin America [160].

We followed the protective ability of three antigens TcG1, TcG2, TcG4 in mice and canine models. In mice, experiments were conducted with the delivery of the three antigens individually or in combination by various prime/boost methods (e.g., DNA/DNA [161], DNA/protein [165], or DNA/ Modified Vaccinia Ankara (MVA) [166]). In all cases, prophylactic vaccine provided 80-95% protection from challenge infection and chronic myocarditis in mice **(Table 3)** [160, 161, 165, 166].

Protection was associated with the stimulation of Th1 cytokine response, trypanolytic antibodies, and cytolytic activity of $CD8^+$ T cells. Notably, these studies indicated an elevated inflammatory response in the vaccinated mice closely after challenge infection. Contrarily, chronic inflammatory infiltrates were decreased in the vaccinated mice. DNA-DNA prophylactic vaccine was the simplest in design [161], while DNA-protein prophylactic vaccine was multifaceted, requiring two doses of six DNA-encoding plasmids and two doses of three recombinant proteins [165]. MVA has an excellent safety record, and MVA itself can act as an adjuvant to signal the innate immune system and boost T cells

Table 3: Summary of authors' subunit vaccine efficacy studies in mice.

Response	Empty vector	DNA-DNA	DNA-protein	DNA-MVA	DNA-MVA w/o cytokines
Vaccine-induced immune responses (14 days after delivery of last vaccine dose)					
Antibody levels	–	+	+	+	+
Lytic Antibodies	–	+	++	+++	++++
Cytokines	–	ND	T_H1 low	$T_H1>T_H2$	$T_H1>T_H2$
$CD8^+$ T cells	–	+	+	+	+
Immune responses post-challenge infection (10,000 *Tc*/mouse, 30 dpi)					
Antibody levels	+	++	+++	++++	++++
Lytic Antibodies	–	+	++	+++	ND
Cytokines	mixed	T_H1/T_H2	T_H1	$T_H1>T_H2$	$T_H1>T_H2$
T cell proliferation	+	ND	++	++++	++++
$CD8^+$T cells	28%	ND	62%	40%	45%
$CD8^+IFN\gamma^+$	0.5%	ND	26%	20%	23%
$CD8^+$ CTLs	1.5%	ND	12%	10%	9.5%
Tc (30 dpi)	ND	90%↓	91%↓	89%↓	90%↓
Tc (120 dpi)	ND	92%↓	98%↓	92%↓	94%↓
Myocarditis	1-3	1-2	0-1	0-2	0-1
Fibrosis	2-4	0-2	0-1	0-1	0-1

Prophylactic delivery was used in all studies.

DNA-DNA: pCDNA3-TcG1, -TcG2, -TcG4 (+ IL-12 and GM-CSF encoding plasmids), 2-4 doses.

DNA-protein: DNA as above/recombinant TcG1, TcG2, TcG4 proteins, 2-4 doses.

DNA-MVA: DNA as above/rMVA encoding TcG2 and TcG4, 2 doses.

DNA: 25-µg each/mouse, intramuscular; Proteins: 25-µg each/mouse, intradermal, MVA: 10^7pfu/mouse, intradermal. Vaccine doses were delivered at 3-week interval.

+, ++, +++, and ++++ define the extent of antibody responses, + being lowest level, and ++++ being highest level of anti-*T. cruzi* antibodies.

Percent increase in splenic $CD8^+$T cell frequency (compared to normal controls) was determined by flow cytometry.

ND – not determined;

Myocarditis/inflammation scoring: (0) - absent/none, (1) - focal or mild with ≤1 foci, (2) - moderate with ≥ 2 inflammatory foci, (3) - extensive with generalized coalescing of inflammatory foci or disseminated inflammation, and (4) - severe with diffused inflammation, interstitial edema, and loss of tissue integrity.

% fibrotic area: (0) <1%, (1) 1 – 5%, (2) 5 – 10%, (3) 10 – 15%, and (4) >15%.

[167, 168].

Likewise, dogs immunized with TcG1, TcG2, and TcG4- based prophylactic vaccine exhibited significant protection from challenge infection and acute myocarditis [163, 164]. Others have shown the TcSP- and TcSSP4-encoding DNA vaccine stimulated anti-parasite IgG2 and IFN-γ-producing lymphocytic cell proliferation in dogs [169]. Upon challenge infection, dogs immunized with TcSP- and TcSSP4-encoding plasmids exhibited a modest control of tissue parasites and electrocardiographic irregularities.

Therapeutic vaccines are envisioned to modulate or enhance the multiple effector mechanisms against *T. cruzi* so as to clear the parasite's persistence in the infected host. Some studies have tested TSA-1, TS, and ASP-2 pertaining to the *trans*-sialidase family as well as Tc52 (glutathione *S*-transferases) and Tc24 (a Ca^{2+} binding protein) for therapeutic efficacy in acutely or chronically infected mice. When injected immediately after infection or within two weeks post-infection, *Tc52-*, *TSA-1-*, and *Tc24*-based DNA therapeutic vaccines decreased the parasitemia and mortality from infection in mice [170, 171]. This protection was associated with vaccine-induced increase of $CD4^+$ and $CD8^+$ T cells [172]. However, these same candidates did not control cardiomyopathy in chronically infected mice or dogs (reviewed in [173]). ASP2 and TS (individually or in combination as a DNA therapeutic vaccine) did not limit parasitemia or improve the survival rate in infected mice [171, 174] despite their known efficacy as a prophylactic vaccine [156]. Some studies also observed an increase in myocarditis in infected mice inoculated with TSA1 DNA therapeutic vaccine [172].

In our experience, a therapeutic approach focused on controlling only parasites is not sufficient to arrest the progression of chronic disease [122]. Treatment with anti-parasitic drug BNZ after immune control of acute parasitemia controlled parasite persistence; however, it was not sufficient to avert cardiac remodeling and deterioration of ventricular contractility in infected mice and rats [175]. Instead, maximal benefits of preserving the structure and function of Chagas heart in rodents were obtained when along with parasite control by BNZ; oxidative insult was also controlled by co-delivery of PBN (phenyl-alpha-tert-butylnitrone) or vitamin A antioxidants, or by genetic overexpression of MnSOD (manganese superoxide dismutase) or GPx (glutathione peroxidase) antioxidants [106, 122, 176, 177]. Likewise, treatment with sildenafil, an inhibitor of phosphodiesterase 5 provided cardioprotection through the preservation of cGMP-PKG activity and antioxidant-oxidant balance in chronically infected mice [178]. Sirtuin 1 (SIRT1) maintains mitochondrial metabolic homeostasis and prevents the over-activation of inflammation [113]. SIRT1 activity was decreased in CD, and treatment with SIRT1 agonist (SRT1720) or inhibition of PARP1 (poly(ADP-ribose) polymerase 1) that competes with SIRT1 for substrates reduced oxidative and inflammatory stress in infected cells and mice [113, 179]. Moreover, we noted a better efficacy of a therapeutic vaccine in infected GPx transgenic mice than in infected/WT mice under similar conditions [122].

Oxidative stress during CCD [73] could potentially be ameliorated with antioxidants delivered in conjunction with a therapeutic vaccine. Indeed, several studies have shown the importance of free-radical scavengers during pregnancy. According to a meta-analysis study, decreased maternal levels of

antioxidant vitamin A were associated with the risk of preeclampsia [180]. Schoots et al have discussed the adverse consequences of MnSOD deficiency on the integrity of syncytiotrophoblast of the placenta [181]. Recently, PBN antioxidant was shown to control the teratogenicity effects of ROS in pregnant rats [182] and vitamin C and vitamin E were found to be safe supplements during pregnancy [183, 184]. Resveratrol treatment improved the glucose and lipid profile of pregnant mice [185]. We, therefore, propose that therapeutic vaccines designed to achieve a rapid, short-term stimulation of type 1 cellular immunity to attack the persistent parasites, along with adjunct therapies (e.g., PBN or resveratrol) to control the oxidative insult and mitochondrial deficiencies, would prove to be maximally beneficial in preserving the tissue homeostasis in congenital and other forms of Chagas disease.

3.3. Vaccine and vaccine-induced immunity during pregnancy

As congenital transmission is becoming a global public health issue, strategies towards reducing this burden are a priority [7]. Considering anti-parasite drugs are not administered during pregnancy because of potential teratogenic effects, we envision three time-points when a vaccine could be useful as a public health strategy towards CD elimination. One, a prophylactic vaccine can be used in women of childbearing age. Two, a therapeutic vaccine could be given to women who have been chronically infected with *T. cruzi*. Ideally, these women should be vaccinated before conception; however, a vaccine designed to enhance maternal immune response, decrease parasitemia, and arrest pathologic inflammation and oxidative stress can be given any time during pregnancy. In these women-centric approaches, the purpose would be to prevent the exposure to pathogen before or during pregnancy or control the parasite burden below a threshold level so that women are protected themselves and they do not transmit the parasite to fetus. Three, a therapeutic vaccine can be given to infants born with acute *T. cruzi* infection. Though BNZ is highly effective in treating infection in infants, many times infants and children are not followed up because diagnosis of infection and BNZ treatment requires multiple visits to the healthcare providers [186]. We believe that anti-parasite immune therapy for children will be safe, and it will help to build the protective immunity to control parasitemia and thereby prevent the onset of the clinically symptomatic CD in their adult years.

There are potential limitations that need to be overcome in designing a vaccine against CCD. The historical concept that pregnancy is associated with immune suppression and increases the susceptibility to infection has not been supported by more recent studies [83, 187]. Current studies suggest that maternal and placental immune systems cooperate to create a pregnancy-supportive immune environment [188]. This relationship prevents a reaction of the mother's immune response against paternal antigens in order to avoid fetal rejection, but simultaneously supports the mother and the fetal capability to defend against invading pathogens [187, 188]. However, our understanding of the effects of acute or chronic *T. cruzi* infection on maternal immunity is very limited and remains to be further investigated in appropriate experimental models and in pregnant women.

Current literature indicates that TLRs and type I interferon play a key role in maintaining placental function. The uterine natural killer (uNK) cells (70% of the total leukocytes) are suggested to

differentiate to pro-pregnancy in an IL-10-dependent manner, and produce pregnancy-promoting cytokines that regulate spiral artery remodeling and control invasion of trophoblast into the endometrium, and thus support placental development **(Figure 6A)** [189]. The same uNK cells can exhibit cytotoxic activity when activated in response to pathogen exposure [190]. Studies evaluating the microenvironment of NK cells in the uterus and other tissues (e.g. spleen and muscle tissues for *T. cruzi*) will shed light on how a successful pregnancy is promoted by IL-10-uNK while keeping a balance of anti-parasite immunity with vaccination.

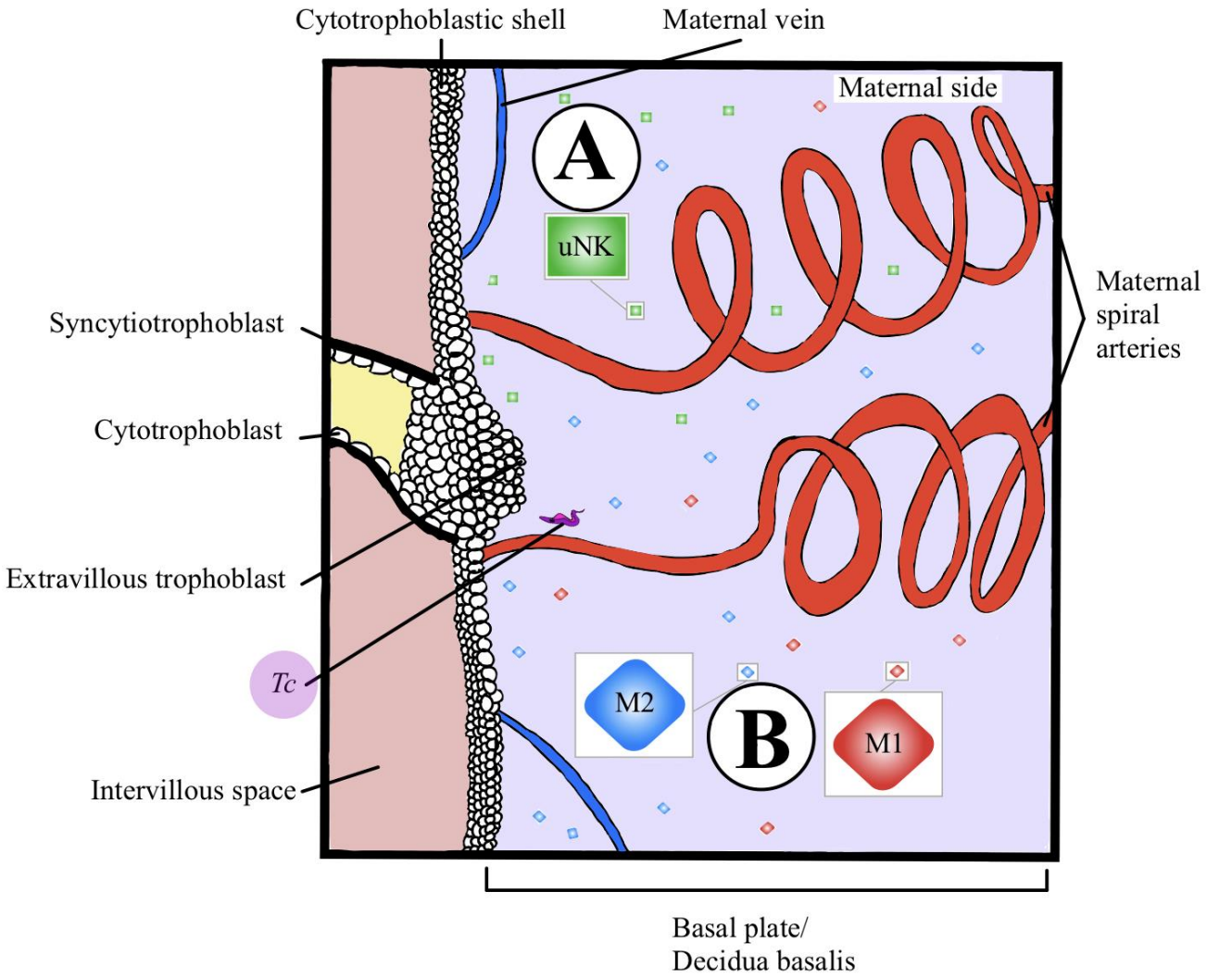

Figure 6. Potential maternal immune responses to *T. cruzi.* (6A) The uterine natural killer (uNK) cells differentiate to pro-pregnancy and produce pregnancy-promoting cytokines that regulate and support placental development. **(6B)** In decidua, macrophages of M1 and M2 phenotypes are involved in inflammatory responses capable of killing the parasite and tissue renewal and repair critical for normal pregnancy, respectively. *Tc: Trypanosoma cruzi.*

The second most abundant immune cells in human decidua are macrophages (20-25% of total leukocytes). In general, M2-like (also referred as alternatively-activated) macrophages involved in tissue renewal and repair are critical for normal pregnancy [191]. VEGF (vascular endothelial growth factor) plays a significant role in macrophage recruitment and M2 polarization, and inhibition of VEGF signaling is suggested to contribute to the shift in macrophage polarity towards M1 (classically-activated, proinflammatory) phenotype observed in different pregnancy disorders, including preeclampsia [192, 193]. Others have indicated that trophoblast secreted factors participate in the differentiation of macrophages to a pro-pregnancy phenotype [194]. Zhao and colleagues found that IL-10 treatment inhibited apoptosis of human primary trophoblast cells and abnormal pregnancy in mice infected with *Toxoplasma gondii* [195, 196]. In context to *T. cruzi* infection, pro-inflammatory macrophages are necessary for parasite clearance through the induction of ROS and NO (nitric oxide) **(Figure 6B)**. Furthermore, macrophages also serve as antigen-presenting cells to stimulate T cell immunity, which is required for controlling intracellular parasite replication (reviewed in [102]). Thus, our challenge would be to adjuvant the vaccine such that decidual macrophages can be educated to differentiate to M1 phenotype for clearance of the pathogen while simultaneously preserving the M2 phenotype in the placental environment to support fetal development. This phenomenon remains to be researched in future studies.

Type I interferons, recognized as potent anti-parasite and anti-viral molecules, also have an immunomodulatory function as they activate the suppressor of cytokine signaling (SOCS) to regulate the overwhelming activation of inflammatory pathways [197]. Placental extracts consist of high amounts of type I IFNs, and these molecules are suggested to play an important role as a modulator of the maternal immune system [83]. Future studies are needed to understand how type I IFNs affect placental function in the presence of infection and/or anti-parasite vaccine.

An accumulating body of evidence shows that congenital human cytomegalovirus (HCMV) vaccination can influence the incidence of infection and congenital disease (reviewed in [198]). Researchers working to develop an HCMV vaccine faced similar concerns as we face today against a vaccine for CCD. Yet, both the public and private sectors are heavily engaged in an HCMV vaccine, and several vaccine candidates have entered the clinical trials. Thus, we hope that researchers will engage and continue to develop unique vaccine designs that will bring an end to the devastating CCD in the near future. Our goal would be to design an effective vaccine that can be administered to women before or during pregnancy to prevent vertical *T. cruzi* transmission. The influenza vaccine is shown to be harmless and is recommended to be given to pregnant women; comparably we aspire to develop an anti-*T. cruzi* vaccine that would be safe to administer during pregnancy [199].

Acknowledgements: MPZ (a researcher from Carrera del Investigador Científico and EEC (graduate student) are supported by CONICET, Argentina. NJG is supported by grants from the National Institute of Allergy and Infectious Diseases (R01AI054578; R01AI136031) of the National Institutes of Health.

4. References

[1] World Health Organization, Chagas disease in Latin America: an epidemiological update based on 2010 estimates, 2015. https://www.who.int/wer/2015/wer9006.pdf?ua=1. (Accessed 9/1/2019).

[2] J.A. Perez-Molina, I. Molina, Chagas disease, Lancet 391(10115) (2018) 82-94.

[3] J.D. Stanaway, G. Roth, The burden of Chagas disease: estimates and challenges, Glob Heart 10(3) (2015) 139-44.

[4] H.B. Tanowitz, L.M. Weiss, S.P. Montgomery, Chagas disease has now gone global, PLoS Negl Trop Dis 5(4) (2011) e1136.

[5] C. Bern, S. Kjos, M.J. Yabsley, S.P. Montgomery, *Trypanosoma cruzi* and Chagas' disease in the United States, Clin Microbiol Rev 24(4) (2011) 655-81.

[6] P.T. Cantey, S.L. Stramer, R.L. Townsend, H. Kamel, K. Ofafa, C.W. Todd, M. Currier, S. Hand, W. Varnado, E. Dotson, C. Hall, P.L. Jett, S.P. Montgomery, The United States *Trypanosoma cruzi* infection study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors, Transfusion 52(9) (2012) 1922-30.

[7] S. Antinori, L. Galimberti, R. Bianco, R. Grande, M. Galli, M. Corbellino, Chagas disease in Europe: a review for the internist in the globalized world, Eur J Intern Med 43 (2017) 6-15.

[8] Q. Liu, X.N. Zhou, Preventing the transmission of American trypanosomiasis and its spread into non-endemic countries, Infect Dis Poverty 4(1) (2015) 60.

[9] I. Oliveira, F. Torrico, J. Munoz, J. Gascon, Congenital transmission of Chagas disease: a clinical approach, Expert Rev Anti Infect Ther 8(8) (2010) 945-56.

[10] Y. Carlier, S. Sosa-Estani, A.O. Luquetti, P. Buekens, Congenital Chagas disease: an update, Mem Inst Oswaldo Cruz 110(3) (2015) 363-8.

[11] Y. Carlier, C. Truyens, Congenital Chagas disease as an ecological model of interactions between *Trypanosoma cruzi* parasites, pregnant women, placenta and fetuses, Acta Tropica 151 (2015) 103-115.

[12] A. Picado, I. Cruz, M. Redard-Jacot, A.G. Schijman, F. Torrico, S. Sosa-Estani, Z. Katz, J.M. Ndung'u, The burden of congenital Chagas disease and implementation of molecular diagnostic tools in Latin America, BMJ Glob Health 3(5) (2018) e001069.

[13] C. Bern, S.P. Montgomery, An estimate of the burden of Chagas disease in the United States, Clin Infect Dis 49(5) (2009) e52-4.

[14] A. Alarcon, M. Morgan, S.P. Montgomery, L. Scavo, E.C. Wong, A. Hahn, B. Jantausch, Diagnosis and treatment of congenital Chagas disease in a premature infant, J Pediatric Infect Dis Soc 5(4) (2016) e28-e31.

[15] R. Voelker, Congenital Chagas disease reported in United States, JAMA 308(5) (2012) 443.

[16] M.S. Edwards, F.A. Abanyie, S.P. Montgomery, Survey of pediatric infectious diseases society members about congenital Chagas disease, Pediatr Infect Dis J 37(1) (2018) e24-e27.

[17] J.M. Ramos, Chagas diseases: opportunities for internists, Eur J Intern Med 48 (2018) e28.

[18] A.M. Cevallos, R. Hernández, Chagas' disease: pregnancy and congenital transmission, Biomed Res Int 2014 (2014) 10.

[19] P.L. Bustos, N. Milduberger, B.J. Volta, A.E. Perrone, S.A. Laucella, J. Bua, *Trypanosoma cruzi* infection at the maternal-fetal interface: implications of parasite load in the congenital transmission and challenges in the diagnosis of infected newborns, Frontiers in Microbiology 10 (2019) 1250.

[20] P. Buekens, M.L. Cafferata, J. Alger, F. Althabe, J.M. Belizan, N. Bustamante, Y. Carlier, A. Ciganda, J.H. Del Cid, E. Dumonteil, R. Gamboa-Leon, J.A. Garcia, L. Gibbons, O. Graiff, J.G. Maldonado, C. Herrera, E. Howard, L.S. Lara, B. Lopez, M.L. Matute, M.J. Ramirez-Sierra, M.C. Robles, S. Sosa-Estani, C. Truyens, C. Valladares, D.M. Wesson, C. Zuniga, Congenital transmission of *Trypanosoma cruzi* in Argentina, Honduras, and Mexico: an observational prospective study, Am J Trop Med Hyg 98(2) (2018) 478-485.

[21] P. Moya, B. Basso, E. Moretti, Congenital Chagas disease in Cordoba, Argentina: epidemiological, clinical, diagnostic, and therapeutic aspects. Experience of 30 years of follow up, Rev Soc Bras Med Trop 38 Suppl 2 (2005) 33-40.

[22] P.G. Scapellato, E.G. Bottaro, M.T. Rodriguez-Brieschke, Mother-child transmission of Chagas disease: could coinfection with human immunodeficiency virus increase the risk?, Rev Soc Bras Med Trop 42(2) (2009) 107-9.

[23] M.R. Agosti, P. Ercoli, G. Dolcini, G. Andreani, L.M. Peralta, S.G. Ayala, Two cases of mother-to-child transmission of HIV and *Trypanosoma cruzi* in Argentina- ClinicalKey, Brazilian Journal of Infectious Diseases 16(4) (2012) 398-399.

[24] C. Diaz-Luján, M. Fernanda Triquell, L. Mezzano, R.E. Fretes, Placental infection by *Trypanosoma cruzi*, the causal agent of congenital Chagas disease,, in: J. Zheng (Ed.), Recent Advances in Research on the Human Placenta, InTech, 2012, pp. 127-148.

[25] L.A. Messenger, R.H. Gilman, M. Verastegui, G. Galdos-Cardenas, G. Sanchez, E. Valencia, L. Sanchez, E. Malaga, V.R. Rendell, M. Jois, V. Shah, N. Santos, M.D.C. Abastoflor, C. LaFuente, R. Colanzi, R. Bozo, C. Bern, Toward improving early diagnosis of congenital Chagas disease in an endemic setting, Clin Infect Dis 65(2) (2017) 268-275.

[26] L.A. Messenger, C. Bern, Congenital Chagas disease: current diagnostics, limitations and future perspectives, Curr Opin Infect Dis 31(5) (2018) 415-421.

[27] E. Kuschnir, H. Sgammini, R. Castro, C. Evequoz, R. Ledesma, J. Brunetto, Evaluation of cardiac function by radioisotopic angiography, in patients with chronic Chagas cardiopathy, Arq Bras Cardiol 45(4) (1985) 249-56.

[28] J.P. Andrade, J.A. Marin Neto, A.A. Paola, F. Vilas-Boas, G.M. Oliveira, F. Bacal, E.A. Bocchi, D.R. Almeida, A.A. Fragata Filho, C. Moreira Mda, S.S. Xavier, W.A. Oliveira Junior, J.C. Dias, I

Latin American guidelines for the diagnosis and treatment of Chagas' heart disease: executive summary, Arq Bras Cardiol 96(6) (2011) 434-42.

[29] M.C. Nunes, W. Dones, C.A. Morillo, J.J. Encina, A.L. Ribeiro, Chagas disease: an overview of clinical and epidemiological aspects, J Am Coll Cardiol 62(9) (2013) 767-76.

[30] Y.M. Gomes, V.M. Lorena, A.O. Luquetti, Diagnosis of Chagas disease: what has been achieved? What remains to be done with regard to diagnosis and follow up studies?, Mem Inst Oswaldo Cruz 104 Suppl 1 (2009) 115-21.

[31] S.A. Besuschio, M. Llano Murcia, A.F. Benatar, S. Monnerat, I. Cruz, A. Picado, M.L.A. Curto, Y. Kubota, D.P. Wehrendt, P. Pavia, Y. Mori, C. Puerta, J.M. Ndung'u, A.G. Schijman, Analytical sensitivity and specificity of a loop-mediated isothermal amplification (LAMP) kit prototype for detection of *Trypanosoma cruzi* DNA in human blood samples, PLoS Negl Trop Dis 11(7) (2017) e0005779.

[32] S. Noazin, J.A. Lee, E.S. Malaga, E. Valencia Ayala, B.J. Condori, C. Roca, A.G. Lescano, C. Bern, W. Castillo, H. Mayta, M.C. Menduina, M.R. Verastegui, F. Tinajeros, R.H. Gilman, Trypomastigote excretory secretory antigen blot is associated with *Trypanosoma cruzi* load and detects congenital *T. cruzi* infection in neonates, using anti-shed acute phase antigen immunoglobulin M, J Infect Dis 219(4) (2019) 609-618.

[33] B.J. Volta, G. Russomando, P.L. Bustos, K. Scollo, A.M. De Rissio, Z. Sanchez, R.L. Cardoni, J. Bua, Diagnosis of congenital *Trypanosoma cruzi* infection: a serologic test using shed acute phase antigen (SAPA) in mother-child binomial samples, Acta Trop 147 (2015) 31-7.

[34] K.E. Eguez, J. Alonso-Padilla, C. Teran, Z. Chipana, W. Garcia, F. Torrico, J. Gascon, D.F. Lozano-Beltran, M.J. Pinazo, Rapid diagnostic tests duo as alternative to conventional serological assays for conclusive Chagas disease diagnosis, PLoS Negl Trop Dis 11(4) (2017) e0005501.

[35] A. Taibi, B. Plumas-Marty, A. Guevara-Espinoza, R. Schöneck, H. Pessoa, M. Loyens, R. Piras, T. Aguirre, H. Gras-Masse, M. Bossus, *Trypanosoma cruzi*: immunity-induced in mice and rats by trypomastigote excretory-secretory antigens and identification of a peptide sequence containing a T cell epitope with protective activity, The Journal of Immunology 151(5) (1993) 2676-2689.

[36] A.C. Pereiro, Guidelines for the diagnosis and treatment of Chagas disease, Lancet 393(10180) (2019) 1486-1487.

[37] V. Balouz, F. Aguero, C.A. Buscaglia, Chagas disease diagnostic applications: present knowledge and future steps, Adv Parasitol 97 (2017) 1-45.

[38] M.C. Mora, O. Sanchez Negrette, D. Marco, A. Barrio, M. Ciaccio, M.A. Segura, M.A. Basombrio, Early diagnosis of congenital *Trypanosoma cruzi* infection using PCR, hemoculture, and capillary concentration, as compared with delayed serology, J Parasitol 91(6) (2005) 1468-73.

[39] R.G. Strout, A method for concentrating hemoflagellates, J Parasitol 48 (1962) 100.

[40] J. Bua, B.J. Volta, A.E. Perrone, K. Scollo, E.B. Velazquez, A.M. Ruiz, A.M. De Rissio, R.L. Cardoni, How to improve the early diagnosis of *Trypanosoma cruzi* infection: relationship between

validated conventional diagnosis and quantitative DNA amplification in congenitally infected children, PLoS Negl Trop Dis 7(10) (2013) e2476.

[41] R. Rivero, M. Bisio, E.B. Velazquez, M.I. Esteva, K. Scollo, N.L. Gonzalez, J. Altcheh, A.M. Ruiz, Rapid detection of *Trypanosoma cruzi* by colorimetric loop-mediated isothermal amplification (LAMP): a potential novel tool for the detection of congenital Chagas infection, Diagn Microbiol Infect Dis 89(1) (2017) 26-28.

[42] Y.E. Castro-Sesquen, R.H. Gilman, G. Galdos-Cardenas, L. Ferrufino, G. Sanchez, E. Valencia Ayala, L. Liotta, C. Bern, A. Luchini, Use of a novel Chagas urine nanoparticle test (chunap) for diagnosis of congenital Chagas disease, PLoS Negl Trop Dis 8(10) (2014) e3211.

[43] C. Alonso-Vega, C. Billot, F. Torrico, Achievements and challenges upon the implementation of a program for national control of congenital Chagas in Bolivia: results 2004-2009, PLoS Negl Trop Dis 7(7) (2013) e2304.

[44] P.A. Sales Junior, I. Molina, S.M. Fonseca Murta, A. Sanchez-Montalva, F. Salvador, R. Correa-Oliveira, C.M. Carneiro, Experimental and clinical treatment of Chagas disease: a review, Am J Trop Med Hyg 97(5) (2017) 1289-1303.

[45] F. Bianchi, Z. Cucunuba, F. Guhl, N.L. Gonzalez, H. Freilij, R.S. Nicholls, J.D. Ramirez, M. Montilla, A.C. Florez, F. Rosas, V. Saavedra, N. Silva, Follow-up of an asymptomatic Chagas disease population of children after treatment with nifurtimox (Lampit) in a sylvatic endemic transmission area of Colombia, PLoS Negl Trop Dis 9(2) (2015) e0003465.

[46] C. Muñoz, I. Zulantay, W. Apt, S. Ortiz, A.G. Schijman, M. Bisio, V. Ferrada, C. Herrera, G. Martínez, A. Solari, Evaluation of nifurtimox treatment of chronic Chagas Disease by means of several parasitological methods, Antimicrob Agents Chemother 57(9) (2013) 4518-23.

[47] A.L. de Andrade, F. Zicker, R.M. de Oliveira, S. Almeida Silva, A. Luquetti, L.R. Travassos, I.C. Almeida, S.S. de Andrade, J.G. de Andrade, C.M. Martelli, Randomised trial of efficacy of Benznidazole in treatment of early *Trypanosoma cruzi* infection, Lancet 348(9039) (1996) 1407-13.

[48] J.R. Coura, Current prospects of specific treatment of Chagas' disease, Bol Chil Parasitol 51(3-4) (1996) 69-75.

[49] J. Bermudez, C. Davies, A. Simonazzi, J.P. Real, S. Palma, Current drug therapy and pharmaceutical challenges for Chagas disease, Acta Trop 156 (2016) 1-16.

[50] Centers for Disease Control and Prevention, Antiparasitic Treatment, 2019. https://www.cdc.gov/parasites/chagas/health_professionals/tx.html.

[51] S. Meymandi, S. Hernandez, S. Park, D.R. Sanchez, C. Forsyth, Treatment of Chagas disease in the United States, Curr Treat Options Infect Dis 10(3) (2018) 373-88.

[52] M.G. Alvarez, C. Vigliano, B. Lococo, G. Bertocchi, R. Viotti, Prevention of congenital Chagas disease by Benznidazole treatment in reproductive-age women. An observational study, Acta Trop 174 (2017) 149-152.

[53] D.L. Fabbro, E. Danesi, V. Olivera, M.O. Codebo, S. Denner, C. Heredia, M. Streiger, S. Sosa-Estani, Trypanocide treatment of women infected with *Trypanosoma cruzi* and its effect on preventing congenital Chagas, PLoS Negl Trop Dis 8(11) (2014) e3312.

[54] L. Murcia, M. Simon, B. Carrilero, M. Roig, M. Segovia, Treatment of infected women of childbearing age prevents congenital *Trypanosoma cruzi i*nfection by eliminating the parasitemia detected by PCR, J Infect Dis 215(9) (2017) 1452-1458.

[55] M.G. Davanço, M.L. Campos, T.A. Rosa, E.C. Padilha, A.H. Alzate, L.A. Rolim, P.J. Rolim-Neto, R.G. Peccinini, Benznidazole extended-release tablets for improved treatment of Chagas disease: preclinical pharmacokinetic study, Antimicrobial Agents and Chemotherapy 60(4) (2016) 2492.

[56] P. Rodari, A. Angheben, G. Gennati, L. Trezzi, G. Bargiggia, M. Maino, M. Ruggeri, S. Rampello, L. Soavi, M. Rizzi, Congenital Chagas disease in a non-endemic area: results from a control programme in Bergamo province, Northern Italy, Travel Med Infect Dis 25 (2018) 31-34.

[57] C. Bern, D.L. Martin, R.H. Gilman, Acute and congenital Chagas disease, Adv Parasitol 75 (2011) 19-47.

[58] L. Murcia, B. Carrilero, P. Albajar Vinas, M. Segovia, Nifurtimox chemotherapy: collateral effects in treated *Trypanosoma cruzi* infected patients, Rev Esp Quimioter 25(1) (2012) 74-5.

[59] S. Zhang, T.R. Regnault, P.L. Barker, K.J. Botting, I.C. McMillen, C.M. McMillan, C.T. Roberts, J.L. Morrison, Placental adaptations in growth restriction, Nutrients 7(1) (2015) 360-89.

[60] N. Arora, Y. Sadovsky, T.S. Dermody, C.B. Coyne, Microbial vertical transmission during human pregnancy, Cell Host Microbe 21(5) (2017) 561-7.

[61] J. Blaszkowska, K. Goralska, Parasites and fungi as a threat for prenatal and postnatal human development, Ann Parasitol 60(4) (2014) 225-34.

[62] A.M. Altemani, A.L. Bittencourt, A.M. Lana, Immunohistochemical characterization of the inflammatory infiltrate in placental Chagas' disease: a qualitative and quantitative analysis, Am J Trop Med Hyg 62(2) (2000) 319-24.

[63] S. Fernandez-Aguilar, M.A. Lambot, F. Torrico, C. Alonso-Vega, M. Cordoba, E. Suarez, J.C. Noel, Y. Carlier, Placental lesions in human *Trypanosoma cruzi* infection, Rev Soc Bras Med Trop 38 Suppl 2 (2005) 84-6.

[64] F. Torrico, C. Alonso-Vega, E. Suarez, P. Rodriguez, M.C. Torrico, M. Dramaix, C. Truyens, Y. Carlier, Maternal *Trypanosoma cruzi* infection, pregnancy outcome, morbidity, and mortality of congenitally infected and non-infected newborns in Bolivia, Am J Trop Med Hyg 70(2) (2004) 201-9.

[65] R. Menon, L.S. Richardson, Preterm Prelabor Rupture of the Membranes: A Disease of the Fetal Membranes, Semin Perinatol 41(7) (2017) 409-19.

[66] A.L. Bittencourt, L.A. Rodrigues de Freitas, M.O. Galvao de Araujo, K. Jacomo, Pneumonitis in congenital Chagas' disease. A study of ten cases., Am J Trop Med Hyg 30(1) (1981) 38-42.

[67] A. Heerema-McKenney, Defense and infection of the human placenta, APMIS 126(7) (2018) 570-588.

[68] R.E. Fretes, U. Kemmerling, Mechanism of *Trypanosoma cruzi* placenta invasion and infection: the use of human chorionic villi explants, J Trop Med 2012 (2012) 614820.

[69] C. Diaz-Lujan, M.F. Triquell, C. Castillo, D. Hardisson, U. Kemmerling, R.E. Fretes, Role of placental barrier integrity in infection by *Trypanosoma cruzi*, Acta Trop 164 (2016) 360-368.

[70] A. Liempi, C. Castillo, I. Carrillo, L. Munoz, D. Droguett, N. Galanti, J.D. Maya, U. Kemmerling, A local innate immune response against *Trypanosoma cruzi* in the human placenta: the epithelial turnover of the trophoblast, Microb Pathog 99 (2016) 123-129.

[71] V.M. Abrahams, The role of the Nod-like receptor family in trophoblast innate immune responses, J Reprod Immunol 88(2) (2011) 112-7.

[72] J.V. Ilekis, E. Tsilou, S. Fisher, V.M. Abrahams, M.J. Soares, J.C. Cross, S. Zamudio, N.P. Illsley, L. Myatt, C. Colvis, M.M. Costantine, D.M. Haas, Y. Sadovsky, C. Weiner, E. Rytting, G. Bidwell, Placental origins of adverse pregnancy outcomes: potential molecular targets: an executive workshop summary of the Eunice Kennedy Shriver national institute of child health and human development, Am J Obstet Gynecol 215(1 Suppl) (2016) S1-s46.

[73] M.F. Triquell, C. Díaz-Luján, M.C. Romanini, J.C. Ramirez, P. Paglini-Oliva, A.G. Schijman, R.E. Fretes, Nitric oxide synthase and oxidative-nitrosative stress play a key role in placental infection by *Trypanosoma cruzi*, American Journal of Reproductive Immunology 80(1) (2018) e12852.

[74] C. Castillo, L. Munoz, I. Carrillo, A. Liempi, L. Medina, N. Galanti, J.D. Maya, U. Kemmerling, Toll-like receptor-2 mediates local innate immune response against *Trypanosoma cruzi* in ex vivo infected human placental chorionic villi explants, Placenta 60 (2017) 40-46.

[75] S. Ortiz, I. Zulantay, A. Solari, M. Bisio, A. Schijman, Y. Carlier, W. Apt, Presence of *Trypanosoma cruzi* in pregnant women and typing of lineages in congenital cases, Acta Trop 124(3) (2012) 243-6.

[76] C. Herrera, C. Truyens, E. Dumonteil, J. Alger, S. Sosa-Estani, M.L. Cafferata, L. Gibbons, A. Ciganda, M.L. Matute, C. Zuniga, Y. Carlier, P. Buekens, Phylogenetic analysis of *Trypanosoma cruzi* from pregnant women and newborns from Argentina, Honduras, and Mexico suggests an association of parasite haplotypes with congenital transmission of the parasite, J Mol Diagn S1525-1578(19) (2019) 30367-8.

[77] R.M. Corrales, M.C. Mora, O.S. Negrette, P. Diosque, D. Lacunza, M. Virreira, S.F. Breniere, M.A. Basombrio, Congenital Chagas disease involves *Trypanosoma cruzi* sub-lineage IId in the northwestern province of Salta, Argentina, Infect Genet Evol 9(2) (2009) 278-82.

[78] M.S. Llewellyn, L.A. Messenger, A.O. Luquetti, L. Garcia, F. Torrico, S.B. Tavares, B. Cheaib, N. Derome, M. Delepine, C. Baulard, J.F. Deleuze, S. Sauer, M.A. Miles, Deep sequencing of the *Trypanosoma cruzi* GP63 surface proteases reveals diversity and diversifying selection among chronic and congenital Chagas disease patients, PLoS Negl Trop Dis 9(4) (2015) e0003458.

[79] J.M. Burgos, J. Altcheh, N. Petrucelli, M. Bisio, M.J. Levin, H. Freilij, A.G. Schijman, Molecular diagnosis and treatment monitoring of congenital transmission of *Trypanosoma cruzi* to twins of a triplet delivery, Diagn Microbiol Infect Dis 65(1) (2009) 58-61.

[80] Y. Carlier, C. Truyens, P. Deloron, F. Peyron, Congenital parasitic infections: a review, Acta Trop 121(2) (2012) 55-70.

[81] A.L. Bittencourt, Possible risk factors for vertical transmission of Chagas' disease, Rev Inst Med Trop Sao Paulo 34(5) (1992) 403-8.

[82] R. Siriano Lda, A.O. Luquetti, J.B. Avelar, N.L. Marra, A.M. de Castro, Chagas disease: increased parasitemia during pregnancy detected by hemoculture, Am J Trop Med Hyg 84(4) (2011) 569-74.

[83] K. Racicot, J.Y. Kwon, P. Aldo, M. Silasi, G. Mor, Understanding the complexity of the immune system during pregnancy, Am J Reprod Immunol 72(2) (2014) 107-16.

[84] V.R. Rendell, R.H. Gilman, E. Valencia, G. Galdos-Cardenas, M. Verastegui, L. Sanchez, J. Acosta, G. Sanchez, L. Ferrufino, C. LaFuente, C. Abastoflor Mdel, R. Colanzi, C. Bern, *Trypanosoma cruzi*-infected pregnant women without vector exposure have higher parasitemia levels: implications for congenital transmission risk, PLoS One 10(3) (2015) e0119527.

[85] O. Sanchez Negrette, M.C. Mora, M.A. Basombrio, High prevalence of congenital *Trypanosoma cruzi* infection and family clustering in Salta, Argentina, Pediatrics 115(6) (2005) e668-72.

[86] E. Moretti, B. Basso, I. Castro, M. Carrizo Paez, M. Chaul, G. Barbieri, D. Canal Feijoo, M.J. Sartori, R. Carrizo Paez, Chagas' disease: study of congenital transmission in cases of acute maternal infection, Rev Soc Bras Med Trop 38(1) (2005) 53-5.

[87] E. Hermann, C. Truyens, C. Alonso-Vega, P. Rodriguez, A. Berthe, F. Torrico, Y. Carlier, Congenital transmission of *Trypanosoma cruzi* is associated with maternal enhanced parasitemia and decreased production of interferon-gamma in response to parasite antigens, J Infect Dis 189(7) (2004) 1274-81.

[88] M.M. Garcia, A.M. De Rissio, X. Villalonga, E. Mengoni, R.L. Cardoni, Soluble tumor necrosis factor (TNF) receptors (sTNF-R1 and -R2) in pregnant women chronically infected with *Trypanosoma cruzi* and their children, Am J Trop Med Hyg 78(3) (2008) 499-503.

[89] N.A. Salas, M. Cot, D. Schneider, B. Mendoza, J.A. Santalla, J. Postigo, J.P. Chippaux, L. Brutus, Risk factors and consequences of congenital Chagas disease in Yacuiba, south Bolivia, Trop Med Int Health 12(12) (2007) 1498-505.

[90] B.J. Volta, P.L. Bustos, R.L. Cardoni, A.M. De Rissio, S.A. Laucella, J. Bua, Serum cytokines as biomarkers of early *Trypanosoma cruzi* infection by congenital exposure, J Immunol 196(11) (2016) 4596-602.

[91] E. Hermann, A. Berthe, C. Truyens, C. Alonso-Vega, R. Parrado, F. Torrico, Y. Carlier, V.M. Braud, Killer cell immunoglobulin-like receptor expression induction on neonatal CD8(+) T cells in vitro and following congenital infection with *Trypanosoma cruzi*, Immunology 129(3) (2010) 418-26.

[92] J.A. Marin-Neto, E. Cunha-Neto, B.C. Maciel, M.V. Simoes, Pathogenesis of chronic Chagas heart disease, Circulation 115(9) (2007) 1109-23.

[93] F.R. Gutierrez, P.M. Guedes, R.T. Gazzinelli, J.S. Silva, The role of parasite persistence in pathogenesis of Chagas heart disease, Parasite Immunol 31(11) (2009) 673-85.

[94] A.R.L. Teixeira, M.M. Hecht, M.C. Guimaro, A.O. Sousa, N. Nitz, Pathogenesis of Chagas' disease: parasite persistence and autoimmunity, Clinical Microbiology Reviews 24(3) (2011) 592-630.

[95] E. De Bona, K.C.F. Lidani, L. Bavia, Z. Omidian, L.H. Gremski, T.L. Sandri, I.J. de Messias Reason, Autoimmunity in chronic Chagas disease: a road of multiple pathways to cardiomyopathy?, Front Immunol 9 (2018) 1842.

[96] N. Garg, Mitochondrial disorders in chagasic cardiomyopathy, Front Biosci 10 (2005) 1341-54.

[97] M.A. Zacks, J.J. Wen, G. Vyatkina, V. Bhatia, N. Garg, An overview of chagasic cardiomyopathy: pathogenic importance of oxidative stress, An Acad Bras Cienc 77(4) (2005) 695-715.

[98] S. Gupta, V. Bhatia, J.-J. Wen, Y. Wu, M.-H. Huang, N.J. Garg, *Trypanosoma cruzi* infection disturbs mitochondrial membrane potential and ROS production rate in cardiomyocytes, Free Radic Biol Med 47(10) (2009) 1414-21.

[99] J.J. Wen, N.J. Garg, Mitochondrial generation of reactive oxygen species is enhanced at the Q(o) site of the complex III in the myocardium of *Trypanosoma cruzi*-infected mice: beneficial effects of an antioxidant, J Bioenerg Biomembr 40(6) (2008) 587-98.

[100] J.J. Wen, N.J. Garg, Mitochondrial complex III defects contribute to inefficient respiration and ATP synthesis in the myocardium of *Trypanosoma cruzi*-infected mice, Antioxid Redox Signal 12(1) (2010) 27-37.

[101] S. Gupta, J.J. Wen, N.J. Garg, Oxidative stress in Chagas disease, Interdiscip Perspect Infect Dis 2009(Article ID: 190354) (2009) 8.

[102] M. Lopez, H.B. Tanowitz, N.J. Garg, Pathogenesis of chronic Chagas disease: macrophages, mitochondria, and oxidative Stress, Curr Clin Microbiol Rep 5(1) (2018) 45-54.

[103] J.J. Wen, G. Vyatkina, N. Garg, Oxidative damage during chagasic cardiomyopathy development: role of mitochondrial oxidant release and inefficient antioxidant defense, Free Radic Biol Med 37(11) (2004) 1821-33.

[104] J.J. Wen, M. Dhiman, E.B. Whorton, N.J. Garg, Tissue-specific oxidative imbalance and mitochondrial dysfunction during *Trypanosoma cruzi* infection in mice, Microbes Infect 10(10-11) (2008) 1201-9.

[105] M. Dhiman, M.P. Zago, S. Nunez, A. Amoroso, H. Rementeria, P. Dousset, F. Nunez Burgos, N.J. Garg, Cardiac-oxidized antigens are targets of immune recognition by antibodies and potential molecular determinants in Chagas disease pathogenesis, PLoS One 7(1) (2012) e28449.

[106] J.J. Wen, P.C. Yachelini, A. Sembaj, R.E. Manzur, N.J. Garg, Increased oxidative stress is correlated with mitochondrial dysfunction in chagasic patients, Free Radic Biol Med 41(2) (2006) 270-6.

[107] T.B. de Oliveira, R.C. Pedrosa, D.W. Filho, Oxidative stress in chronic cardiopathy associated with Chagas disease, Int J Cardiol 116(3) (2007) 357-63.

[108] M. Dhiman, E.S. Nakayasu, Y.H. Madaiah, B.K. Reynolds, J.J. Wen, I.C. Almeida, N.J. Garg, Enhanced nitrosative stress during *Trypanosoma cruzi* infection causes nitrotyrosine modification of host proteins: implications in Chagas' disease, Am J Pathol 173(3) (2008) 728-40.

[109] M. Dhiman, J.G. Estrada-Franco, J.M. Pando, F.J. Ramirez-Aguilar, H. Spratt, S. Vazquez-Corzo, G. Perez-Molina, R. Gallegos-Sandoval, R. Moreno, N.J. Garg, Increased myeloperoxidase activity and protein nitration are indicators of inflammation in patients with Chagas' disease, Clin Vaccine Immunol 16(5) (2009) 660-666.

[110] J.J. Wen, V. Bhatia, V.L. Popov, N.J. Garg, Phenyl-alpha-tert-butyl nitrone reverses mitochondrial decay in acute Chagas' disease, Am J Pathol 169(6) (2006) 1953-64.

[111] R. Perez-Fuentes, J.F. Guegan, C. Barnabe, A. Lopez-Colombo, H. Salgado-Rosas, E. Torres-Rasgado, B. Briones, M. Romero-Diaz, J. Ramos-Jimenez, C. Sanchez-Guillen Mdel, Severity of chronic Chagas disease is associated with cytokine/antioxidant imbalance in chronically infected individuals, Int J Parasitol 33(3) (2003) 293-9.

[112] J.J. Wen, C. Porter, N.J. Garg, Inhibition of NFE2L2-antioxidant response element pathway by mitochondrial reactive oxygen species contributes to development of cardiomyopathy and left ventricular dysfunction in Chagas Disease, Antioxid Redox Signal 27(9) (2017) 550-566.

[113] X. Wan, J.J. Wen, S.J. Koo, L.Y. Liang, N.J. Garg, SIRT1-PGC1alpha-NFkappaB pathway of oxidative and inflammatory stress during *Trypanosoma cruzi* infection: benefits of SIRT1-targeted therapy in improving heart function in Chagas disease, PLoS Pathog 12(10) (2016) e1005954.

[114] M. Dhiman, X. Wan, V.L. Popov, G. Vargas, N.J. Garg, MnSOD ^{tg} mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease, J Am Heart Assoc 2(5) (2013) e000302.

[115] X. Ba, S. Gupta, M. Davidson, N.J. Garg, *Trypanosoma cruzi* induces the reactive oxygen species-PARP-1-RelA pathway for up-regulation of cytokine expression in cardiomyocytes, J Biol Chem 285(15) (2010) 11596-606.

[116] I. Chowdhury, S. Koo, L. Yi, S. Gupta, B. Bahar, L. Silla, J. Nunez Burgos, N. Barrientos, M.P. Zago, N.J. Garg, Gene expression profiling and functional characterization of macrophages in response to circulatory microparticles produced during *Trypanosoma cruzi* infection and Chagas disease, J innate Immunity 9(2) (2017) 203-216.

[117] K.M. Bonney, D.J. Luthringer, S.A. Kim, N.J. Garg, D.M. Engman, Pathology and pathogenesis of Chagas heart disease, Annu Rev Pathol 14 (2019) 421-447.

[118] O. Rodriguez-Morales, V. Monteon-Padilla, S.C. Carrillo-Sanchez, M. Rios-Castro, M. Martinez-Cruz, A. Carabarin-Lima, M. Arce-Fonseca, Experimental vaccines against Chagas disease: a journey through history, J Immunol Res 2015(Article ID: 489758) (2015) 8.

[119] J.C. Vazquez-Chagoyan, S. Gupta, N.J. Garg, Vaccine development against *Trypanosoma cruzi* and Chagas disease, Adv Parasitol 75 (2011) 121-46.

[120] M. Arce-Fonseca, M. Rios-Castro, C. Carrillo-Sanchez Sdel, M. Martinez-Cruz, O. Rodriguez-Morales, Prophylactic and therapeutic DNA vaccines against Chagas disease, Parasit Vectors 8 (2015) 121.

[121] N. Khatoon, R. Ojha, A. Mishra, V.K. Prajapati, Examination of antigenic proteins of *Trypanosoma cruzi* to fabricate an epitope-based subunit vaccine by exploiting epitope mapping mechanism, Vaccine 36(42) (2018) 6290-6300.

[122] S. Gupta, C. Smith, S. Auclair, J. Delgadillo Ade, N.J. Garg, Therapeutic efficacy of a subunit vaccine in controlling chronic *Trypanosoma cruzi* infection and Chagas disease is enhanced by glutathione peroxidase over-expression, PLoS One 10(6) (2015) e0130562.

[123] Y. Ma, L.M. Weiss, H. Huang, Strategy for the development of vaccines against Chagas Disease, HSOA Journal of Vaccines Research and Vaccination 1(001) (2015) 8.

[124] N.M. El-Sayed, P.J. Myler, D.C. Bartholomeu, D. Nilsson, G. Aggarwal, A.N. Tran, E. Ghedin, E.A. Worthey, A.L. Delcher, G. Blandin, S.J. Westenberger, E. Caler, G.C. Cerqueira, C. Branche, B. Haas, A. Anupama, E. Arner, L. Aslund, P. Attipoe, E. Bontempi, F. Bringaud, P. Burton, E. Cadag, D.A. Campbell, M. Carrington, J. Crabtree, H. Darban, J.F. da Silveira, P. de Jong, K. Edwards, P.T. Englund, G. Fazelina, T. Feldblyum, M. Ferella, A.C. Frasch, K. Gull, D. Horn, L. Hou, Y. Huang, E. Kindlund, M. Klingbeil, S. Kluge, H. Koo, D. Lacerda, M.J. Levin, H. Lorenzi, T. Louie, C.R. Machado, R. McCulloch, A. McKenna, Y. Mizuno, J.C. Mottram, S. Nelson, S. Ochaya, K. Osoegawa, G. Pai, M. Parsons, M. Pentony, U. Pettersson, M. Pop, J.L. Ramirez, J. Rinta, L. Robertson, S.L. Salzberg, D.O. Sanchez, A. Seyler, R. Sharma, J. Shetty, A.J. Simpson, E. Sisk, M.T. Tammi, R. Tarleton, S. Teixeira, S. Van Aken, C. Vogt, P.N. Ward, B. Wickstead, J. Wortman, O. White, C.M. Fraser, K.D. Stuart, B. Andersson, The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease, Science 309(5733) (2005) 409-15.

[125] M.S. Cardoso, J.L. Reis-Cunha, D.C. Bartholomeu, Evasion of the immune response by *Trypanosoma cruzi* during acute infection, Front Immunol 6(659) (2015) 15.

[126] B. Wizel, M. Nunes, R.L. Tarleton, Identification of *Trypanosoma cruzi* trans-sialidase family members as targets of protective CD8+ TC1 responses, J Immunol 159(12) (1997) 6120-30.

[127] B. Wizel, M. Palmieri, C. Mendoza, B. Arana, J. Sidney, A. Sette, R. Tarleton, Human infection with *Trypanosoma cruzi* induces parasite antigen-specific cytotoxic T lymphocyte responses, J Clin Invest 102(5) (1998) 1062-71.

[128] V.L. Pereira-Chioccola, S. Schenkman, J.K. Kloetzel, Sera from chronic Chagasic patients and rodents infected with *Trypanosoma cruzi* inhibit trans-sialidase by recognizing its amino-terminal and catalytic domain, Infect Immun 62(7) (1994) 2973-8.

[129] M. Ribeirao, V.L. Pereira-Chioccola, L. Renia, A. Augusto Fragata Filho, S. Schenkman, M.M. Rodrigues, Chagasic patients develop a type 1 immune response to *Trypanosoma cruzi* trans-sialidase, Parasite Immunol 22(1) (2000) 49-53.

[130] M.A. Santos, N. Garg, R.L. Tarleton, The identification and molecular characterization of *Trypanosoma cruzi* amastigote surface protein-1, a member of the trans-sialidase gene super-family, Mol Biochem Parasitol 86(1) (1997) 1-11.

[131] H.P. Low, R.L. Tarleton, Molecular cloning of the gene encoding the 83 kDa amastigote surface protein and its identification as a member of the *Trypanosoma cruzi* sialidase superfamily, Mol Biochem Parasitol 88(1-2) (1997) 137-49.

[132] S.B. Boscardin, S.S. Kinoshita, A.E. Fujimura, M.M. Rodrigues, Immunization with cDNA expressed by amastigotes of *Trypanosoma cruzi* elicits protective immune response against experimental infection, Infect Immun 71(5) (2003) 2744-57.

[133] H.P. Low, M.A. Santos, B. Wizel, R.L. Tarleton, Amastigote surface proteins of *Trypanosoma cruzi* are targets for CD8+ CTL, J Immunol 160(4) (1998) 1817-23.

[134] R.P. Barbosa, B.G. Filho, L.I. Dos Santos, P.A. Junior, P.E. Marques, R.V. Pereira, D.C. Cara, O. Bruna-Romero, M.M. Rodrigues, R.T. Gazzinelli, A.V. Machado, Vaccination using recombinants influenza and adenoviruses encoding amastigote surface protein-2 are highly effective on protection against *Trypanosoma cruzi* infection, PLoS One 8(4) (2013) e61795.

[135] F.A.P. Ribeiro, C. Pontes, R.T. Gazzinelli, O.B. Romero, M.C. Lazzarin, J.F. Dos Santos, F. de Oliveira, L.P. Pisani, J.R.C. de Vasconcelos, D.A. Ribeiro, Therapeutic effects of vaccine derived from amastigote surface protein-2 (ASP-2) against Chagas disease in mouse liver, Cytokine 113 (2019) 285-290.

[136] K.A. Norris, L.M. Galvao, J.E. Schrimpf, J.R. Cancado, A.U. Krettli, Humoral immune response to the *Trypanosoma cruzi* complement regulatory protein as an indicator of parasitologic clearance in human Chagas' disease, Infect Immun 62(9) (1994) 4072-4.

[137] P. Sepulveda, M. Hontebeyrie, P. Liegeard, A. Mascilli, K.A. Norris, DNA-based immunization with *Trypanosoma cruzi* complement regulatory protein elicits complement lytic antibodies and confers protection against *Trypanosoma cruzi* infection, Infect Immun 68(9) (2000) 4986-91.

[138] S.G. Fonseca, H. Moins-Teisserenc, E. Clave, B. Ianni, V.L. Nunes, C. Mady, L.K. Iwai, A. Sette, J. Sidney, M.L. Marin, A.C. Goldberg, L. Guilherme, D. Charron, A. Toubert, J. Kalil, E. Cunha-Neto, Identification of multiple HLA-A*0201-restricted cruzipain and FL-160 CD8+ epitopes recognized by T cells from chronically *Trypanosoma cruzi*-infected patients, Microbes Infect 7(4) (2005) 688-97.

[139] P.M. Ferrão, C.M. d'Avila-Levy, T.C. Araujo-Jorge, W.M. Degrave, S. Gonçalves Ada, L.R. Garzoni, A.P. Lima, J.J. Feige, S. Bailly, L. Mendonça-Lima, M.C. Waghabi, Cruzipain activates latent TGF-β from host cells during *T. cruzi* invasion, PLoS One 10(5) (2015) e0124832.

[140] L.M. Godsel, D.M. Engman, Flagellar protein localization mediated by a calcium-myristoyl/palmitoyl switch mechanism, Embo j 18(8) (1999) 2057-65.

[141] A.B. Biter, S. Weltje, E.M. Hudspeth, C.A. Seid, C.P. McAtee, W.H. Chen, J.B. Pollet, U. Strych, P.J. Hotez, M.E. Bottazzi, Characterization and stability of *Trypanosoma cruzi* 24-C4 (Tc24-

C4), a candidate antigen for a therapeutic vaccine against Chagas disease, J Pharm Sci 107(5) (2018) 1468-1473.

[142] N. Yoshida, J.E. Araya, J.F. da Silveira, S. Giorgio, *Trypanosoma cruzi*: antibody production and T cell response induced by stage-specific surface glycoproteins purified from metacyclic trypomastigotes, Exp Parasitol 77(4) (1993) 405-13.

[143] J.E. Araya, M.I. Cano, N. Yoshida, J.F. da Silveira, Cloning and characterization of a gene for the stage-specific 82-kDa surface antigen of metacyclic trypomastigotes of *Trypanosoma cruzi*, Mol Biochem Parasitol 65(1) (1994) 161-9.

[144] F.R. Franco, G.S. Paranhos-Bacallà, L.M. Yamauchi, N. Yoshida, J.F. da Silveira, Characterization of a cDNA clone encoding the carboxy-terminal domain of a 90-kilodalton surface antigen of *Trypanosoma cruzi* metacyclic trypomastigotes, Infection and Immunity 61(10) (1993) 4196-4201.

[145] J.P.F. Rodrigues, G.H.T. Sant'ana, M.A. Juliano, N. Yoshida, Inhibition of host cell lysosome spreading by *Trypanosoma cruzi* metacyclic stage-specific surface molecule gp90 downregulates parasite invasion, Infection and Immunity 85(9) (2017) e00302-17.

[146] C. Trujillo, R. Ramirez, I.D. Velez, C. Berberich, The humoral immune response to the kinetoplastid membrane protein-11 in patients with American leishmaniasis and Chagas disease: prevalence of IgG subclasses and mapping of epitopes, Immunol Lett 70(3) (1999) 203-9.

[147] C. Maranon, M.C. Thomas, L. Planelles, M.C. Lopez, The immunization of A2/K(b) transgenic mice with the KMP11-HSP70 fusion protein induces CTL response against human cells expressing the *T. cruzi* KMP11 antigen: identification of A2-restricted epitopes, Mol Immunol 38(4) (2001) 279-87.

[148] I.D. Flechas, A. Cuellar, Z.M. Cucunubá, F. Rosas, V. Velasco, M. Steindel, C. Thomas Mdel, M.C. López, J.M. González, C.J. Puerta, Characterising the KMP-11 and HSP-70 recombinant antigens' humoral immune response profile in chagasic patients, BMC Infect Dis 9 (2009) 186.

[149] B.H. Fralish, R.L. Tarleton, Genetic immunization with LYT1 or a pool of trans-sialidase genes protects mice from lethal *Trypanosoma cruzi* infection, Vaccine 21(21-22) (2003) 3070-80.

[150] R.A. Wrightsman, K.A. Luhrs, D. Fouts, J.E. Manning, Paraflagellar rod protein-specific CD8+ cytotoxic T lymphocytes target *Trypanosoma cruzi*-infected host cells, Parasite Immunol 24(8) (2002) 401-12.

[151] V. Michailowsky, K. Luhrs, M.O. Rocha, D. Fouts, R.T. Gazzinelli, J.E. Manning, Humoral and cellular immune responses to *Trypanosoma cruzi*-derived paraflagellar rod proteins in patients with Chagas' disease, Infect Immun 71(6) (2003) 3165-71.

[152] A. Egui, M.C. Thomas, M. Morell, C. Maranon, B. Carrilero, M. Segovia, C.J. Puerta, M.J. Pinazo, F. Rosas, J. Gascon, M.C. Lopez, *Trypanosoma cruzi* paraflagellar rod proteins 2 and 3 contain immunodominant CD8(+) T-cell epitopes that are recognized by cytotoxic T cells from Chagas disease patients, Mol Immunol 52(3-4) (2012) 289-98.

[153] M. Morell, M.C. Thomas, T. Caballero, C. Alonso, M.C. Lopez, The genetic immunization with paraflagellar rod protein-2 fused to the HSP70 confers protection against late *Trypanosoma cruzi* infection, Vaccine 24(49-50) (2006) 7046-55.

[154] R. Fernandez-Gomez, S. Esteban, R. Gomez-Corvera, K. Zoulika, A. Ouaissi, *Trypanosoma cruzi*: Tc52 released protein-induced increased expression of nitric oxide synthase and nitric oxide production by macrophages, J Immunol 160(7) (1998) 3471-9.

[155] S. Portillo, B.G. Zepeda, E. Iniguez, J.J. Olivas, N.H. Karimi, O.C. Moreira, A.F. Marques, K. Michael, R.A. Maldonado, I.C. Almeida, A prophylactic α-Gal-based glycovaccine effectively protects against murine acute Chagas disease, npj Vaccines 4(1) (2019) 13.

[156] N. Garg, R.L. Tarleton, Genetic immunization elicits antigen-specific protective immune responses and decreases disease severity in *Trypanosoma cruzi* infection, Infect Immun 70(10) (2002) 5547-55.

[157] N.J. Garg, R.L. Tarleton, Elicitation of protective cellular and humoral immune responses to *Trypanosoma cruzi* infection using DNA vaccines can be augmented with cytokines., Proceedings 10th International Congress of Immunology, Bologna, Italy : Monduzzi Editore, International Proceedings Division, ©1998., New Delhi, India, 1998, pp. 1421-1426.

[158] L.E. Rios, J.C. Vázquez-Chagoyán, A.O. Pacheco, M.P. Zago, N.J. Garg, Immunity and vaccine development efforts against *Trypanosoma cruzi*, Acta Tropica (2019) 105168.

[159] C. Teh-Poot, E. Tzec-Arjona, P. Martinez-Vega, M.J. Ramirez-Sierra, M. Rosado-Vallado, E. Dumonteil, From genome screening to creation of vaccine against *Trypanosoma cruzi* by use of immunoinformatics, J Infect Dis 211(2) (2015) 258-66.

[160] V. Bhatia, M. Sinha, B. Luxon, N. Garg, Utility of the *Trypanosoma cruzi* sequence database for identification of potential vaccine candidates by in silico and in vitro screening, Infect Immun 72(11) (2004) 6245-54.

[161] V. Bhatia, N.J. Garg, Previously unrecognized vaccine candidates control *Trypanosoma cruzi* infection and immunopathology in mice, Clin Vaccine Immunol 15(8) (2008) 1158-64.

[162] S. Gupta, X. Wan, M.P. Zago, V.C. Sellers, T.S. Silva, D. Assiah, M. Dhiman, S. Nunez, J.R. Petersen, J.C. Vazquez-Chagoyan, J.G. Estrada-Franco, N.J. Garg, Antigenicity and diagnostic potential of vaccine candidates in human Chagas disease, PLoS Negl Trop Dis 7(1) (2013) e2018.

[163] J.E. Aparicio-Burgos, L. Ochoa-Garcia, J.A. Zepeda-Escobar, S. Gupta, M. Dhiman, J.S. Martinez, R.M. de Oca-Jimenez, M. Val Arreola, A. Barbabosa-Pliego, J.C. Vazquez-Chagoyan, N.J. Garg, Testing the efficacy of a multi-component DNA-prime/DNA-boost vaccine against *Trypanosoma cruzi* infection in dogs, PLoS Negl Trop Dis 5(5) (2011) e1050.

[164] J.E. Aparicio-Burgos, J.A. Zepeda-Escobar, R.M. de Oca-Jimenez, J.G. Estrada-Franco, A. Barbabosa-Pliego, L. Ochoa-Garcia, R. Alejandre-Aguilar, N. Rivas, G. Penuelas-Rivas, M. Val-Arreola, S. Gupta, F. Salazar-Garcia, N.J. Garg, J.C. Vazquez-Chagoyan, Immune protection against *Trypanosoma cruzi* induced by TcVac4 in a canine model, PLoS Negl Trop Dis 9(4) (2015) e0003625.

[165] S. Gupta, N.J. Garg, Prophylactic efficacy of TcVac2 against *Trypanosoma cruzi* in mice, PLoS Negl Trop Dis 4(8) (2010) e797.

[166] S. Gupta, N.J. Garg, Delivery of antigenic candidates by a DNA/MVA heterologous approach elicits effector CD8+T cell mediated immunity against Trypanosoma cruzi, Vaccine 30(50) (2012) 7179-86.

[167] C.A. Meseda, K.L. Elkins, M.J. Merchlinsky, J.P. Weir, Prime-boost immunization with DNA and modified vaccinia virus ankara vectors expressing herpes simplex virus-2 glycoprotein D elicits greater specific antibody and cytokine responses than DNA vaccine alone, J Infect Dis 186(8) (2002) 1065-73.

[168] N. Climent, S. Guerra, F. Garcia, C. Rovira, L. Miralles, C.E. Gomez, N. Pique, C. Gil, J.M. Gatell, M. Esteban, T. Gallart, Dendritic cells exposed to MVA-based HIV-1 vaccine induce highly functional HIV-1-specific CD8(+) T cell responses in HIV-1-infected individuals, PLoS One 6(5) (2011) e19644.

[169] M. Arce-Fonseca, M.A. Ballinas-Verdugo, E.R. Zenteno, D. Suarez-Flores, S.C. Carrillo-Sanchez, R. Alejandre-Aguilar, J.L. Rosales-Encina, P.A. Reyes, O. Rodriguez-Morales, Specific humoral and cellular immunity induced by *Trypanosoma cruzi* DNA immunization in a canine model, Vet Res 44(1) (2013) 15.

[170] E. Dumonteil, J. Escobedo-Ortegon, N. Reyes-Rodriguez, A. Arjona-Torres, M.J. Ramirez-Sierra, Immunotherapy of *Trypanosoma cruzi* infection with DNA vaccines in mice, Infect Immun 72(1) (2004) 46-53.

[171] G. Sanchez-Burgos, R.G. Mezquita-Vega, J. Escobedo-Ortegon, M.J. Ramirez-Sierra, A. Arjona-Torres, A. Ouaissi, M.M. Rodrigues, E. Dumonteil, Comparative evaluation of therapeutic DNA vaccines against *Trypanosoma cruzi* in mice, FEMS Immunol Med Microbiol 50(3) (2007) 333-41.

[172] H. Zapata-Estrella, C. Hummel-Newell, G. Sanchez-Burgos, J. Escobedo-Ortegon, M.J. Ramirez-Sierra, A. Arjona-Torres, E. Dumonteil, Control of *Trypanosoma cruzi* infection and changes in T-cell populations induced by a therapeutic DNA vaccine in mice, Immunol Lett 103(2) (2006) 186-91.

[173] I.A. Quijano-Hernandez, M.E. Bolio-Gonzalez, J.C. Rodriguez-Buenfil, M.J. Ramirez-Sierra, E. Dumonteil, Therapeutic DNA vaccine against *Trypanosoma cruzi* infection in dogs, Ann N Y Acad Sci 1149 (2008) 343-6.

[174] E. Dumonteil, DNA vaccines against protozoan parasites: advances and challenges, J Biomed Biotechnol 2007(6) (2007) 90520.

[175] I.S. Caldas, A. Talvani, S. Caldas, C.M. Carneiro, M. de Lana, P.M. da Matta Guedes, M.T. Bahia, Benznidazole therapy during acute phase of Chagas disease reduces parasite load but does not prevent chronic cardiac lesions, Parasitology Research 103(2) (2008) 413.

[176] J.J. Wen, S. Gupta, Z. Guan, M. Dhiman, D. Condon, C. Lui, N.J. Garg, Phenyl-alpha-tert-butyl-nitrone and Benzonidazole treatment controlled the mitochondrial oxidative stress and evolution of cardiomyopathy in chronic chagasic rats, J Am Coll Cardiol 55(22) (2010) 2499-508.

[177] C. Panis, T.L. Mazzuco, C.Z. Costa, V.J. Victorino, V.L. Tatakihara, L.M. Yamauchi, S.F. Yamada-Ogatta, R. Cecchini, L.V. Rizzo, P. Pinge-Filho, *Trypanosoma cruzi*: effect of the absence of 5-lipoxygenase (5-LO)-derived leukotrienes on levels of cytokines, nitric oxide and iNOS expression in cardiac tissue in the acute phase of infection in mice, Exp Parasitol 127(1) (2011) 58-65.

[178] J.J. Wen, X. Wan, J. Thacker, N.J. Garg, Chemotherapeutic efficacy of phosphodiesterase inhibitors in chagasic cardiomyopathy, JACC Basic Transl Sci 1(4) (2016) 235-250.

[179] J.J. Wen, Y.W. Yin, N.J. Garg, PARP1 depletion improves mitochondrial and heart function in Chagas disease: effects on POLG dependent mtDNA maintenance, PLoS Pathog 14(5) (2018) e1007065.

[180] J.M. Cohen, M. Beddaoui, M.S. Kramer, R.W. Platt, O. Basso, S.R. Kahn, Maternal antioxidant levels in pregnancy and risk of preeclampsia and small for gestational age birth: a systematic review and meta-analysis, PLoS One 10(8) (2015) e0135192.

[181] M.H. Schoots, S.J. Gordijn, S.A. Scherjon, H. van Goor, J.-L. Hillebrands, Oxidative stress in placental pathology, Placenta 69 (2018) 153-161.

[182] N. Sobočan, A. Katušić Bojanac, N. Sinčić, M. Himelreich-Perić, J. Krasić, Ž. Majić, G. Jurić-Lekić, L. Šerman, M. Vlahović, D. Ježek, F. Bulić-Jakuš, A free radical scavenger ameliorates teratogenic activity of a DNA hypomethylating hematological therapeutic, Stem Cells and Development 28(11) (2019) 717-733.

[183] A. Rumbold, E. Ota, C. Nagata, S. Shahrook, C.A. Crowther, Vitamin C supplementation in pregnancy, Cochrane Database of Systematic Reviews (9) (2015) CD004072.

[184] A. Rumbold, E. Ota, H. Hori, C. Miyazaki, C.A. Crowther, Vitamin E supplementation in pregnancy, Cochrane Database of Systematic Reviews (9) (2015) CD004069.

[185] J.R.T. Darby, M.H.B. Mohd Dollah, T.R.H. Regnault, M.T. Williams, J.L. Morrison, Systematic review: Impact of resveratrol exposure during pregnancy on maternal and fetal outcomes in animal models of human pregnancy complications—Are we ready for the clinic?, Pharmacological Research 144 (2019) 264-278.

[186] M.-J. Pinazo, J. Muñoz, E. Posada, P. López-Chejade, M. Gállego, E. Ayala, E. del Cacho, D. Soy, J. Gascon, Tolerance of Benznidazole in treatment of Chagas' disease in adults, Antimicrobial Agents and Chemotherapy 54(11) (2010) 4896-4899.

[187] G. Mor, I. Cardenas, The immune system in pregnancy: a unique complexity, Am J Reprod Immunol 63(6) (2010) 425-33.

[188] S. Robertson, M. G. Petroff, J. S. Hunt, Immunology of Pregnancy, 2015, pp. 1835-1874.

[189] L.M. Gaynor, F. Colucci, Uterine natural killer cells: functional distinctions and influence on pregnancy in humans and mice, Front Immunol 8 (2017) 467.

[190] J.K. Riley, W.M. Yokoyama, NK cell tolerance and the maternal-fetal interface, Am J Reprod Immunol 59(5) (2008) 371-87.

[191] H. Wang, M. He, Y. Hou, S. Chen, X. Zhang, M. Zhang, X. Ji, Role of decidual CD14(+) macrophages in the homeostasis of maternal-fetal interface and the differentiation capacity of the cells during pregnancy and parturition, Placenta 38 (2016) 76-83.

[192] M.B. Brown, M. von Chamier, A.B. Allam, L. Reyes, M1/M2 macrophage polarity in normal and complicated pregnancy, Front Immunol 5 (2014) 606.

[193] K.C. Wheeler, M.K. Jena, B.S. Pradhan, N. Nayak, S. Das, C.D. Hsu, D.S. Wheeler, K. Chen, N.R. Nayak, VEGF may contribute to macrophage recruitment and M2 polarization in the decidua, PLoS One 13(1) (2018) e0191040.

[194] P.B. Aldo, K. Racicot, V. Craviero, S. Guller, R. Romero, G. Mor, Trophoblast induces monocyte differentiation into CD14+/CD16+ macrophages, Am J Reprod Immunol 72(3) (2014) 270-84.

[195] R. Zhang, H. Zhang, X. Liu, Q. Fu, X. Xu, X. Hu, The immunoprotective role of interleukin-10 in abnormal pregnancy outcome induced by *Toxoplasma gondii* infection, Gynecol Obstet Invest 73(3) (2012) 223-9.

[196] M. Zhao, R. Zhang, X. Xu, Y. Liu, H. Zhang, X. Zhai, X. Hu, IL-10 reduces levels of apoptosis in *Toxoplasma gondii*-infected trophoblasts, PLoS One 8(2) (2013) e56455.

[197] R.A. Porritt, P.J. Hertzog, Dynamic control of type I IFN signalling by an integrated network of negative regulators, Trends Immunol 36(3) (2015) 150-60.

[198] C.S. Nelson, B.C. Herold, S.R. Permar, A new era in cytomegalovirus vaccinology: considerations for rational design of next-generation vaccines to prevent congenital cytomegalovirus infection, NPJ Vaccines 3 (2018) 38.

[199] L.A. Grohskopf, L.Z. Sokolow, K.R. Broder, E.B. Walter, A.M. Fry, D.B. Jernigan, Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices—United States, 2018–19 influenza season, MMWR Recomm Rep 67(3) (2018) 1-20.