ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 11, pp. 60202-60207, November, 2022
https://doi.org/10.37118/ijdr.25772.11.2022

RESEARCH ARTICLE OPEN ACCESS

GAP JUNCTIONS IN THE INFECTIOUS PROCESS OF *Trypanosoma cruzi*: A COMPARATIVE STUDY

Souza, D. R.[1,2], Santos-Júnior, J.A.[2], Mesquita-Santos, F.P.[2], Seabra, S.H.[4] and *Fortes, F.S.A[1,2,3]

[1]Postgraduate Program in Translational Biomedicine (BIOTRANS) - UERJ, UNIGRANRIO, InMETRO - Rio de Janeiro, RJ, Brazil; [2]Laboratory of Cellular and Molecular Therapy and Physiology Prof. Antonio Carlos Campos de Carvalho; FCBS, State University of Rio de Janeiro – UERJ; Rio de Janeiro, RJ, Brazil; [3]Postgraduate Program in Physiological Sciences, Department of Physiological Sciences, Institute of Biology, Federal Rural University of Rio de Janeiro - UFRRJ, Seropédica, RJ, Brazil; [4]Laboratory of Cellular and Tissue Biology, State University of Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

ARTICLE INFO

Article History:
Received 06th September, 2022
Received in revised form
16th September, 2022
Accepted 20th October, 2022
Published online 30th November, 2022

KeyWords:
Trypanosoma cruzi, Gap junctions, Cardiomyocyte, Adipocyte, Macrophage.

Corresponding author:
Fortes, F.S.A

ABSTRACT

Chagas disease is a pathology caused by the protozoan *Trypanosoma cruzi*. It is known that such infection is capable of causing damage to the gap junctions, but its engines are not completely described. Therefore, the objective of the work is to review the literature as interactions of the protozoan Trypanossoma crossed in some cell lines, such as: cardiomyocytes, macrophages and adipocytes, with a focus on the changes caused in the class of connexins, especially in the connexin 43, and with the following threats in the intercellular communication of these strains. We have also a made quantitative survey was carried out of the studies selected for each strain. The methodology used was revisional, based on the databases: Portal *Scopus* CAPES, *PubMed*, *SciELO* and Google Scholar, in addition to epidemiological studies from the WHO and Fiocruz databases. The main results were those that show that the proposed cell types differ in how they present their changes in the face of *T. cruzi* infection, showing that these mechanisms are not well understood. It concludes that, there are not enough studies on the infection by *T. cruzi* and its effects on the communicating junctions in the different types of cells of the human body. Studies that could better clarify what are the roles of gap junctions and their mechanisms associated with this type of infection, which affects different systems and presents different modulations depending on the type of infected cell.

Citation: Souza, D. R., Santos-Júnior, J.A., Mesquita-Santos, F.P., Seabra, S.H. and Fortes, F.S.A. 2022. *"*Gap junctions in the infectious process of *Trypanosoma cruzi:* A comparative study*", International Journal of Development Research, 12, (11), 60202-60207.*

INTRODUCTION

Chagas disease or American Trypanosomiasis is one of the main neglected diseases, considered endemic in low-income population regions. It remains a critical illness disease, mainly due to underreporting problems. According to the World Health Organization (WHO), there are 21 endemic countries in Latin America, with about 8 million people infected worldwide. There are 10,000 deaths/year and it is estimated that about 25 million people are at risk of diseasecontracting, surpassing Malaria cases (WHO, 2019). This pathological condition is caused by the protozoan *Trypanosoma cruzi (T.cruzi)* which has an invertebrate insect as its vector, known in Brazil as "barbeiro". It`s a triatominae, belonging to the subfamily *Triatominae* (*Hemiptera*: *Heteroptera*: *Reduviidae*) (LENKO; PAPAVERO, 1979; LENT; WYGODZINSKY 1979). *T. cruzi*is a unicelular flagellate protozoan that belongs to*Trypanossomatidae* family, *Kinetoplastidae* order, and was described as the etiological agent causing Chagas disease (CHAGAS, 1909). It has a single flagellate originated from an invagination, known as a flagellar pocket, and a kinetoplast DNA, able to self-replicate, situated within a single mitochondrion (CLAYTON, 2002). Thisprotozoa assumes different evolutionary forms throughout its biological cycle: amastigote, epimastigote (in triatominae) and trypomastigote. These forms are morphologically differentiated by the general appearance of the cell, the position of the kinetoplast in relation to the nucleus and the region where the flagellum appears (BRENER, 1973; DE SOUZA, 2002; REY, 2013). Genetic diversity of this parasite has been associated with the pathogenesis, distribution and clinical features of the disease (BRISSE *et al*., 2000; ZINGALES *et al*., 2009; MARCILI, 2009).*T. cruzi* presents 6 discrete typing units (DTUs) subdivided at TcI – TcVI, in addition to another genotype, TcBat, recently isolated from bats of the mostly insectivorous species: *Myotis spp.* (*Vespertilionidae*) (9 isolated), *Noctilio albiventris* (*Noctylionidae*) (2 isolated) and 1 of *Thyroptera tricolor* (*Tryropteridae*). Three isolates of these species are fructivorous/insectivorous *Carollia perspicillata* (*Phyllostomidae*), captured in the Amazon, central and southeastern regions of Brazil. In 2014, this new genotype was also isolated from an infected 5-year-old in northwest Colombia (RAMÍREZ *et al*., 2014). Interactions that

occur between the cells in a multicellular organism, the fundamental interactions are those that hold the cells together (HULL, *et al*., 1962). For such interactions occurs, cells have different types of junctions, such as: desmosomes and hemidesmosomes, tight junctions, adherens junctions and gap junctions (GARCIA, *et al*., 2018; MAES, *et al*., 2015). Among these different cell junctions and the different forms of intercellular communication, gap junctions are highlighted, which are characterized by transmembrane channels that allow direct communication between cells and tissues (DHEIN, 1998). These junctions are formed by a multigene family of transmembrane proteins called connexins, which present 21 different isotypes in human species (HERVÉ, *et al*., 2004). Connexins have been described as associated with the reduction of cell communication through gap junctions (DE CARVALHO, *et al*., 1998). Therefore, the aim of the current issue was to review the interactions of *T. cruzi* in different cell lines, emphasizing the changes caused in different class of connexins transmembrane proteinsand to evaluate the consequences associated with changes in cellular communications, resulting from this interaction. In addition, a quantitative survey of the studies described on each strain presented in the literature was carried out.

METHODS

The following databases were used to carry out this work: Portal Scopus CAPES, PubMed, SciELO and Google Scholar. Epidemiological studies were also consulted in WHO (World of Health Organization) and Oswaldo Cruz Foundation (FIOCRUZ) databases. For the inclusion and exclusion criteria, works dating from 1962 to 2022, written in English and German languages, were used. Also, were used keywords: *"Gap Junction", "Connexin 43", "Trypanosoma cruzi", "T. cruzi", "Junctional communication", "Cardiomyocyte", "Adipocyte"* and *"Macrophage"*. For this work, quantitative research was carried out using the keywords, in singular mode and in the English language, to cover a reliable result to the total number of published articles. The search platform of the PubMed site collection was used. Results were found on the October 20th, 2022. The research method consisted of starting with the most generic term and working up to the most specific term, with the aim of analyzing possible combinations related to cell type, gap junctions and *Trypanosoma cruzi*. This allowed reaching a more relevant and accurate quantitative result of the research.

Interaction of T. cruzi in cardiomyocytes and their gap junctions: Gap junctions, which have been described as the main way of cardiac electrical impulse propagation, generate a continuous change in the action potential of adjacent cardiomyocytes in a unilateral direction. Such alteration presents a syncytium behavior, generating a cardiac contraction that behaves like a contraction wave (BARR, *et al*., 1965). Among cardiac connexins, Cx43 is the most abundant gap junction protein in the heart (TEUNISSEN, *et al*., 2004), especially in ventricular myocytes, with intercalated discs as its morphofunctional site under physiological conditions (PETERS, 1996). Cardiomyocytes are the main targets for *Trypanosoma Cruzi* during Chagas disease. Calcium ions plays a fundamental role in muscle contraction and relaxation mechanisms, and are involved in parasite interaction with different cells types (TARDIEUX *et al*., 1994; MORENO *et al*., 1994; YOSHIDA *et al*., 1997; CALER *et al*., 1998). Thapsigargin is a non-competitive inhibitor of Ca^{2+}-ATPase endoplasmic reticulum (SERCA). Meirelles, *et al.* (1999) demonstrated an inhibition of 64 to 68% of the infection of this parasite, when this drug was used during the interaction of *T. Cruzi* with cardiac muscle cells. Gap junctions play an important role in parasitic infections associated studies. Studies of cardiomyocytes alterations during *in vitro* infection with Trypanosoma cruzi, indicated that the parasite was capable of cell host functioning damage through cell-cell communication alterations (DE CARVALHO *et al*., 1992), where synchronous contractions maintenance requires functional gap junctions (DUFFY *et al*., 2006). In 2010, ADESSE *et al*, demonstrated opposite effects in two rodent species, by analysis spontaneous beat rate of rats infected with *T. cruzi* and rat cardiomyocytes (DE CARVALHO *et al*., 1992; BERGDOLT *et al*., 1994). In cells from infected mice, beats were higher, whereas in infected mice myocytes were slower and less rhythmic. Studies in rat cardiomyocytes after *Y* or *T. cruzi Tulahuen* strains infection (DE CARVALHO *et al*., 1992; ADESSE *et al*., 2008) showed that Connexin 43 (Cx43) from infected cells and its permeability was considerably lower after 72 hinfection; coupling between non-parasitized and infected cells was not affected, indicating that secreted factors into the medium are unlikely to be responsible for the decrease in Cx43 (DE CARVALHO *et al*., 1998). This work indicated that alterations in distribution and functionality of Cx43 are related to presence of the parasite in cell regardless of soluble factors related to infection (DE CARVALHO *et al*., 1992). In another study, ADESSE *et al* (2011) demonstrated that, through Cx43 evaluation in rats chagasic hearts, cardiac gap junction proteins and their channels formed are targets of infection. In a population of cardiac myocytes, during acute infection, gap junction abundance and their immunoreactivity with certain antibodies are seriously compromised, as its functional coupling and contraction synchrony. In adjacent uninfected cells, gap junction expression and function are less affected, so there is a cell mosaic that are connected or disconnected to their neighbors, depending on parasitemia presence and extention. In chronic chagasic cardiomyopathy, the number of parasitized cells is low, but circulating factors such as IL-1β and TGF-β are elevated in the myocardium with chronic inflammation, resulting not only in reduced expression of Cx43, but also in structural remodeling due to the fibrosis generated during infection process.

Interaction of T. cruzi in macrophages and their gap junctions: Macrophages are cells of the immune system that realize several functions in order to ensure homeostasis, such as phagocytosis, antigen presentation as well as production and secretion of cytokines. These mononuclear phagocytes are present in diferent tissues, receiving specific names such as Kupffer cells in the liver, alveolar macrophages in the lungs and microglia cells in the brain (ABBAS, 2011). In the circulating form, they are called monocytes and have the ability to migrate to tissues, especially during inflammatory reactions. In the tissues, as macrophages, these cells play a special role in the immune response, both for pathogens elimination and in the tissues recovery in a cleaning process, dead cells phagocytosing and in the apoptosis process. The mechanisms of macrophage action are targets of several researches and as technology advances, new discoveries still contribute with important elucidations about the attributions of this vital cell type to the immune system. One of the research targets that is still being investigated concerns the gap junctions of macrophages and their functionality, since there are several divergences found in the literature on this topic. (KANE; BOLS, 1980). The functionality of Cx43 is linked to the presence of a parasite in the cell (DE CARVALHO *et al*., 1992). Gap junctions have important functions in the immune response, including differentiation, neuronal activity, cellular development and synchronization (GOODENOUGH; PAUL, 2003), through cells signaling, interferon (IFN), peptidoglycans, lipopolysaccharides (LPS) and tumor necrosis fator (TNF) could be influence innate and adaptive immune response,through cell recruitment induction and gap junctions can be very important in this process (HANDEL *et al*., 2007). Studies on gap junctions in macrophages are relatively recent. In the 1970s, two studies were able to identify the presence of junctions between macrophages through transmission electron microscopy image. First, in the work of Levy *et al*. (1976), a linear chain orientation of macrophages was observed, which were identified as regions of high contact between cells, named as close junctions. At the end of the same decade, Porvaznik, Macvittie and collaborators (1979) also obtained images, which they identified regions with gap junctions in progenitor cells, originating from dog's bone marrow. In 2004, Fortes *et al*. performed RT-PCR (reverse transcription polymerase chain reaction); western-blot, immunofluorescence, and dye injection assays, in macrophage lineage J774-G8 cells and peritoneal macrophages from *Swiss mice*, demonstrating that these macrophages expressed functional gap junctions. Da Silva *et al*. (2018) evaluated the structural and functional modulation of gap junctions, formed by Cx43 in

macrophage lineages J774-G8, after activation with pro-inflammatory factors (TNF-α and IFN-γ) and in infection with T. cruzi in its trypomastigote form (Y strain). Their results showed that J774-G8 macrophage lineage cells show significant alterations in their intercellular communication profile by gap junctions, when submitted to the microenvironment stimulated with pro-inflammatory factors. Experiments with intracellular dye injections in cultures previously treated with IFN-γ and TNF-α in combination, and in incubations of up to 48h, significantly increased gap junction-mediated communication. Western blot assays showed that there was a significant increase in Cx43 expression. However, when performing connexin immunofluorescence assays, samples infected at intervals of 24, 48 and 72 hours, confocal microscopy analysis revealed that these membrane proteins were not identified in their morphofunctional location. It was observed a progressive disorder, proportional to the time of infection, of F-actin, a protein that composes actin filaments.

Interaction of T. cruzi in adipocytes and their gap junctions: Adipose Tissue (AT), which was once considered inert and static, is now considered the largest endocrine organ specialized in release and storage of lipids, which also releases a large amount of bioactive peptides, proteins, metabolites and signaling lipids (SCHERER, 2016). Most of the fat deposits are white adipocytes (WAT). Brown adipocytes (BAT) are thought to be functional mainly in newborn animals, where mitochondria and lipid particles are abundant and provide a high rate of thermogenesis. However, TAM also has an important metabolic role in adult animals, as shown by positron emission tomography (PET) studies (NEDERGAARD, et. al. 2007). Physiological roles go beyond systemic energy homeostasis. Involved in innate and adaptive responses, TA actively participates in stromal interactions with tumors (PARK, *et al*. 2014). The role of TA in the maintenance of homeostasis of critical organs such as the liver, kidneys and heart, makes its ability to neutralize toxic lipids even more important (STERN, *et al*. 2016). AT is heterogeneous and has a very important cellular variety, in addition to adipocytes, such as: endothelial cells, a variety of immune cells, adipocyte precursor cells, as well as fibroblasts and myofibroblasts, which constitute the stromal vascular fraction of AT. Each of these cell types plays a very important physiological role in TA homeostasis (RUTKOWSKI *et al*. 2015). Dysfunctions in this tissue lead to the impairment and effectiveness of adapting to feeding and fasting conditions, which lead to a range of functional consequences (ASTERHOLM *et al*. 1998; ASTERHOLM *et al*. 2010). In this context, the protozoan *Trypanosoma cruzi* has been founded in this tissue, persisting in the infection (SHOEMAKER *et al*., 1970). Also, was observed, through electron microscopy, *T. cruzi* amastigotes inside adipocytes (ANDRADE, Z.A. 1995) and *T. cruzi* trypomastigotes in TA and adipocytes (COMBS, *et al*. 2005).

The parasite resides in the extracellular environment and some ways for it entrance in the cell's parenchyma can be explained, some of them are: through the blood vessels or both inside and outside the tissues. However, only the TA provides an appropriate environment for parasite development and allows it replicate itselves or prevent being eliminated by the immune system. Another way may be by specific entrance, involving receptor recognition with large endothelial expression. This is a process that has not been fully examined, although in the TA endothelium it can be specifically targeted (KOLONIN *et al*., 2004). In addition, adipose tissue is an organ that has vascularity in constant remodeling, more than any other organ and this may also be another possibility, because it was observed that the TA is more susceptible to angiotensin inhibitors in the absence of a mass. tumor (RUPNICK. *et al*., 2002). According to MICHON *et. al.* (2005), gap junctions connect most glandular tissues.Connexins and/or the intercellular diffusion of molecules through junctional channels play very important roles in the optimization and secretion of endocrine and exocrine glands. BURKE *et. al.* (2014) performed experiments with TAM and TAB, staining them to measure the effects of Cx43 expression, in the acute and chronic phases of Chagas disease – 30 days post infection (DPI) and 90 DPI, respectively. Was demonstrated, that 30 DPI, the expression of Cx43 in the BAT of infected mice decreased (80%) compared to the control group (uninfected) which is naturally high. In contrast, in white adipocytes there was a significant increase in the expression of Cx43 (50%). In chronic infection, at 90 DPI, there was a 50% decrease in BAT and a 25% increase in WAT. In both cases, the changes were statistically significant. Many tissues are affected by the parasite; however, white adipocytes are the main targets. This can be observed due to a higher and continuous lipolytic rate in WAT than in TAM (NAGAJYOTHI et. al., 2012). Considering that the function and expression of Cx43 is very important for the adipogenesis and plasticity of white adipocytes (YANAGIYA et. al., 2007; COUSIN *et al*., 1992; LEE et. al., 2014), an increase occurs in the expression of Cx43 in these infected adipocytes, could lead to a pathological remodeling of that tissue. It has been speculated that Cx43 interferes with adipogenesis differentiation (YEGANEH et. al., 2012; CHAO et. al., 2008) and that adipose tissue, as well as adipocytes, are regulated by inflammatory mediators, in the face of *T. cruzi* infection (COMBS *et al*., 2005; TANOWITZ *et al*., 2011). One of the main consequences that this infection brings to the AT is the induction of inflammation. Depending on the tissue, this can increase or decrease Cx43 expression, through the cytokine IL-1B, responsible for regulating this protein (BROSNAN *et al*., 2001; NIGER *et al*., 2010). In experiments with rodents, *T. cruzi* infection has been shown to result in increased expression of IL-1B among other pro-inflammatory cytokines and chemokines (COMBS *et al*., 2005; NAGAJYOTHI et. al., 2012).

Quantitative survey of searched terms: The term *"gap junction"* had a total of 21,136 results comprising years of publication ranging from 1951 to 2022, while the term *"Trypanosoma cruzi"* had a total of 71,381 results comprising years of publication ranging from 1938 to 2020. the keywords corresponding to the terms *"cardiomyocyte"*, *"macrophage"* and *"adipocyte"* obtained total results from 84,854 publications, 374,385 publications and 54,866 publications, comprising years of publication ranging from 1945, 1914 and 1951 to 2022, respectively. Within the terms related to cardiomyocytes, the combinations *"cardiomyocyte gap junction"*, *"cardiomyocyte Trypanosoma cruzi"* and *"cardiomyocyte trypanosoma cruzi gap junction"* were searched and were obtained 1396 publications, 258 publications and 5 publications, comprising the publication years ranging from 1961 to 2022, 1986 to 2022 and from 1992 to 2011 respectively. *"Gap junction studies in cardiomyocytes during T. cruzi infection"* published on the PubMed platform represent 1,93% of published articles involving *T. cruzi* infection in this cell type. Regarding the expressions associated with macrophages, the combinations of *"macrophage gap junction"*, *"macrophage Trypanosoma cruzi"* and *"macrophage Trypanosoma cruzi gap junction"* were searched and were obtaining as results 298 publications, 1,208 publications and no publication, comprising the publcation years ranging from 1975 to 2022 for the terms *"macrophage gap junction"* and from 1958 to 2020 for the terms *"macrophage trypanosoma cruzi"*, while for the terms *"macrophage trypanosoma cruzi gap junction"* only one master's thesis at University of Grande Rio (UNIGRANRIO) collection. As there are no articles on gap junctions in macrophages during T. cruzi infection published on the PubMed platform, then there is a total of 0% of articles published involving T. cruzi infection in this cell type. Already in relation with the terms associated with adipocytes, the combinations of *"adipocyte gap junction"*, *"adipocyte Trypanosoma cruzi"* and *"adipocyte Trypanosoma cruzi gap junction"* were searched, obtaining as results 40 publications, 19 publications and one publication, respectively, comprising the years of publication, ranging from 1982 to 2020 for the terms *"adipocyte gap junction"*, 1995 to 2022 for the terms *"adipocyte Trypanosoma cruzi"*, while for the terms *"adipocyte trypanosoma cruzi gap junction"* there is only one article published in 2014. The study of gap junctions in adipocytes during *T. cruzi* infection published on the PubMed platform represents a total of 5,26% of published articles involving *T. cruzi* infection in this cell type.

Final Considerations

At the beginning of the research of this work, it was found that there was no comparative study approaching the different cell types

discussed here and their possible alterations in their respective junctional complexes, through infection by *Trypanosoma cruzi*. As well as no surveys were made on the number of publications of the referent studies. Therefore, the objective of this work was to investigate the different interactions of *T. cruzi* in the gap junctions of cardiomyocytes, macrophages and adipocytes, in order to compare the changes in their junctional profiles and to survey the publications on these subjects in the literature. Thus, this would allow analyzing the abundance – or absence – of these studies in the scientific environment, in view of the importance of gap junctions in studies related to parasitic infections. Studies carried out on cardiomyocytes during *T. cruzi* infection demonstrated that the parasite could impair the functioning of the host cell through changes on its junctional profile. These studies described that both in the acute and chronic phases, there is a reduction in the expression and immunoreactivity of Cx43, that was capable to cause problems in the contraction of these cardiomyocytes. The hypothesis raised about these results says that they can be explained due to a change in the transport of Cx43 to the plasma membrane or due to conformational changes in the protein, masking the labeling and recognition of the antibody. Contrasting with the hypothesis raised about the alterations in the gap junctions of cardiomyocytes caused by the parasite, the data from the experiments carried out in macrophages demonstrate that there was a significant increase in the expression of Cx43 in these cells. But in the confocal microscopy evaluation of these macrophages, it was found that despite of connexin expression increase, they were not positioned in their morphofunctional site. The same microscopy assays were performed for F-actin, demonstrating a progressive disorder proportional to the time of infection. These findings indicated that Connexin 43 may be being retained in the cytoplasm, quite possibly due to the damage that *T. cruzi* infection causes to the cell cytoskeleton. In the analysis performed on adipocytes, it was found that both in acute and chronic infections by *T. cruzi*, there was a considerable increase in Cx43 expression in white adipocytes. However, in contrast to the results obtained in this type of adipocyte, in brown adipocytes, submitted to the same experimental conditions, there was a significant decrease in the expression of this same connexin both in acute and chronic infections. This modulation of the adipocytes junctional profile is closely related to the inflammatory process parasitemia induced, but the mechanisms that cause these conflicting results in the Cx43 expression in both types of adipocytes are not yet properly described in the literature. The results of the quantitative survey demonstrated that the studies on the modulation of gap junctions in *Trypanosoma cruzi* infectious process, in different cell types, are recent, especially in macrophages and adipocytes models.In cardiomyocytes, although there are older records on these studies, these are the oldest compared to the two previous cell types.There was also a very large discrepancy in the publications numbers on studies of gap junctions in "*T. cruzi* infection in cardiomyocytes", when compared to the same studies published on "macrophages and adipocytes". It is also worth mentioning that, even so, all the values found in the search for the terms combining "gap junction", "*Trypanosoma cruzi*" and the respective cell types in this study correspond to a very small value when comparing studies involving only "*Trypanosoma cruzi*" or "gap junction" combined with the same cell types. At last, adipocytes represent a much smaller number when compared to studies involving gap junctions and/or *T. cruzi* infection. Therefore, it is possible to verify that still there are many studies to be done on *T. cruzi* infection and its effects on gap junctions both *in vitro* and *in vivo*, in different cell types of human body different systems.

REFERENCES

ABBAS, Abul K.; LICHTMAN, Andrew HH; PILLAI, Shiv. *Cellular and Molecular Immunology.*7ªEd. Elsevier, 2011.

ADESSE, Daniel *et al*. Gap junctions and chagas disease. In: Advances in parasitology. *Academic Press*, 2011. p. 63-81.

ADESSE, Daniel *et al*. Transcriptomic signatures of alterations in a myoblast cell line infected with four distinct strains of *Trypanosoma cruzi*. *The American journal of tropical medicine and hygiene,* v. 82, n. 5, p. 846-854, 2010.

ADESSE, Daniel *et al*. *Trypanosoma cruzi* induces changes in cardiac connexin43 expression. *Microbes and infection*, v. 10, n. 1, p. 21-28, 2008.

ALBAREDA, María Cecilia *et al*. *Trypanosoma cruzi* modulates the profile of memory CD8+ T cells in chronic Chagas' disease patients. *International immunology*, v. 18, n. 3, p. 465-471, 2006.

ANDRADE, Zilton A.; ROCHA SILVA, H. R. Parasitism of adipocytes by *Trypanosoma cruzi*. Memorias-Instituto Oswaldo Cruz, v. 90, p. 521-522, 1995.

ASTERHOLM, Ingrid Wernstedt *et al*. Lack of "immunological fitness" during fasting in metabolically challenged animals. *Journal of Lipid Research*, v. 53, n. 7, p. 1254-1267, 2012.

ASTERHOLM, Ingrid Wernstedt; SCHERER, Philipp E. Enhanced metabolic flexibility associated with elevated adiponectin levels. *The American journal of pathology*, v. 176, n. 3, p. 1364-1376, 2010.

BARR, L_; DEWEY, M. M.; BERGER, W. Propagation of action potentials and the structure of the nexus in cardiac muscle. The Journal of general physiology, v. 48, n. 5, p. 797-823, 1965.

BERGDOLT, B. A. *et al*. *Trypanosoma cruzi*: effects of infection on receptor mediated chronotropy and Ca2+ mobilization in rat cardiac myocytes. *Experimental parasitology*, v. 78, n. 2, p. 149-160, 1994.

BRENER, Zigman. Biology of *Trypanosoma cruzi*. Annual Reviews in Microbiology, v. 27, n. 1, p. 347-382, 1973.

BRISSE, Sylvain; BARNABÉ, Christian; TIBAYRENC, Michel. Identification of six *Trypanosoma cruzi* phylogenetic lineages by random amplified polymorphic DNA and multilocus enzyme electrophoresis. *International Journal for Parasitology*, v. 30, n. 1, p. 35-44, 2000.

BROSNAN, Celia F.; SCEMES, Eliana; SPRAY, David C. Cytokine regulation of gap junction connectivity: an open-and-shut case or changing partners at the Nexus?. *The American journal of pathology,* v. 158, n. 5, p. 1565, 2001.

BURKE, Shoshana *et al*. Adipocytes in both brown and white adipose tissue of adult mice are functionally connected via gap junctions: implications for Chagas disease. *Microbes and infection*, v. 16, n. 11, p. 893-901, 2014.

CALER, Elisabet V. *et al*. Oligopeptidase B-dependent signaling mediates host cell invasion by *Trypanosoma cruzi*. The EMBO journal, v. 17, n. 17, p. 4975-4986, 1998.

CARDILLO, Fabíola *et al*. NK1. 1+ cells and T-cell activation in euthymic and thymectomized C57Bl/6 mice during acute *Trypanosoma cruzi* infection. *Scandinavian journal of immunology,* v. 55, n. 1, p. 96-104, 2002.

CHAGAS C. Nova tripanossomíase humana: Estudos sobre a morfologia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., agente etiológico de nova entidade mórbida do homem. Mem Inst. Oswaldo Cruz 1909; 1:159-218.

CHAO, Lily C. *et al*. Inhibition of adipocyte differentiation by Nur77, Nurr1, and Nor1. *Molecular endocrinology*, v. 22, n. 12, p. 2596-2608, 2008.

CLAYTON, Christine E. Life without transcriptional control? From fly to man and back again. *The EMBO journal*, v. 21, n. 8, p. 1881-1888, 2002.

COMBS, Terry P. *et al*. The adipocyte as an important target cell for *Trypanosoma cruzi* infection. *Journal of Biological Chemistry*, v. 280, n. 25, p. 24085-24094, 2005.

COUSIN, B. *et al*. Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. Journal of cell science, v. 103, n. 4, p. 931-942, 1992.

DA SILVA, C. M. *et al*. Comunicação juncional no sistema imunológico: expressão das junções comunicantes na infecção com *Trypanosoma cruzi* e no tratamento com fatores pró-imune inflamatórios. Dissertação (Mestrado em biomedicina translacional) - Programa de Pós-graduação em biomedicina translacional, [S. l.], 2018.

DAMOUCHE, Abderaouf *et al*. Adipose tissue is a neglected viral reservoir and an inflammatory site during chronic HIV and SIV infection. PLoS pathogens, v. 11, n. 9, p. e1005153, 2015.

DE CARVALHO, A. Campos *et al*. Gap junction distribution is altered between cardiac myocytes infected with *Trypanosoma cruzi*. Circulation Research, v. 70, n. 4, p. 733-742, 1992.

DE CARVALHO, Antonio C. Campos *et al*. Gap junction disappearance in astrocytes and leptomeningeal cells as a consequence of protozoan infection. *Brain research,* v. 790, n. 1-2, p. 304-314, 1998.

DHEIN, S. Gap junction channels and cellular communication. Recent discoveries of importance in medicine. Deutsche medizinische Wochenschrift (1946), v. 123, n. 30, p. 912, 1998.

DIAS, JCP., and COURA, JR., org. Clínica e terapêutica da doença de Chagas: uma abordagem prática para o clínico geral [online]. Rio de Janeiro: Editora FIOCRUZ, 1997. 486 p. ISBN 85-85676-31-0.

DUFFY, Heather S.; FORT, Alfredo G.; SPRAY, David C. Cardiac connexins: genes to nexus. In: Cardiovascular Gap Junctions. Karger Publishers, 2006. p. 1-17.

FORTES, Fabio SA *et al*. Modulation of intercellular communication in macrophages: possible interactions between GAP junctions and P2 receptors. *Journal of Cell Science*, v. 117, n. 20, p. 4717-4726, 2004.

FRANKE-FAYARD, Blandine *et al*. Murine malaria parasite sequestration: CD36 is the major receptor, but cerebral pathology is unlinked to sequestration. Proceedings of the National Academy of Sciences, v. 102, n. 32, p. 11468-11473, 2005.

GARCIA, Miguel A.; NELSON, W. James; CHAVEZ, Natalie. Cell–cell junctions organize structural and signaling networks. *Cold Spring Harbor perspectives in biology*, v. 10, n. 4, p. a029181, 2018.

GOODENOUGH, Daniel A.; PAUL, David L. Beyond the gap: functions of unpaired connexon channels. Nature reviews Molecular cell biology, v. 4, n. 4, p. 285-295,2003.

GUIMARÃES, Marco Cesar Cunegundes; DA GAMA FILHO, Reubes Valério. Diferenciação de Monócitos-macrófagos de Gallus gallus: uma abordagem morfológica. Perspectivas Online 2007-2011, v. 3, n. 9, 2009.

HANDEL, Andreas *et al*. Gap junction-mediated antigen transport in immune responses. *Trends in Immunology*, v. 28, n. 11, p. 463-466, 2007.

HERVÉ, Jean-Claude; BOURMEYSTER, Nicolas; SARROUILHE, Denis. Diversity in protein–protein interactions of connexins: emerging roles. *Biochimica et Biophysica Acta (BBA)-Biomembranes,* v. 1662, n. 1-2, p. 22-41, 2004.

HULL, R. N.; CHERRY, W. R.; TRITCH, O. J. Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2 (NCTC-3196) and their utility in virus research. *Journal of Experimental Medicine*, v. 115, n. 5, p. 903-918, 1962.

JAWETZ, E.; MELNICK, J. L.; ADELBERG, E. A. Microbiologia médica, 26ª edição, AMGH, 2014.

JUNQUEIRA LUIZ, C.; CARNEIRO, *J. Histología básica*. Texto e atlas. 2013.

KANE, A. B.; BOLS, N. C. A study of metabolic cooperation with rat peritoneal macrophages. *Journal of cellular physiology,* v. 102, n. 3, p. 385-393, 1980.

KOLONIN, Mikhail G. *et al*. Reversal of obesity by targeted ablation of adipose tissue. Nature medicine, v. 10, n. 6, p. 625-632, 2004.

LEE, Yun-Hee; MOTTILLO, Emilio P.; GRANNEMAN, James G. Adipose tissue plasticity from WAT to BAT and in between. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* v. 1842, n. 3, p. 358-369, 2014.

LENKO, K.; PAPAVERO, N. Insetos no Folclore. Série Conselho Estadual de Artes e Ciências Humanas. São Paulo: Coleções Folclore, 1979; 18.

LENT, H; WYGODZINSKY P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bull Amer Mus. Nat. Hist., 1979; 163: 127-520.

LEVY, J. A. *et al*. Possible communication between murine macrophages oriented in linear chains in tissue culture. Experimental cell research, v. 103, n. 2, p. 375-385, 1976.

LINCK, G. *et al*. Demonstration of gap junctions or nexus by lanthanum in the brown fat of the European hamster (Cricetus cricetus). Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles, v. 278, n. 1, p. 87, 1974.

MAES, Michaël *et al*. Connexin and pannexin signaling in gastrointestinal and liver disease. Translational Research, v. 166, n. 4, p. 332-343, 2015.

MARCILI, A. *et al*. A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and Histone H2B genes and genotyping based on ITS1 rDNA. Parasitology, v. 136, n. 6, p. 641-655, 2009.

MEIRELLES, Maria de Nazareth Leal de *et al*. Trypanosoma cruzi-cardiomyocytes: new contributions regarding a better understanding of this interaction. Memórias do Instituto Oswaldo Cruz, v. 94, p. 149-152, 1999.

MICHON, Laetitia *et al*. Involvement of gap junctional communication in secretion. *Biochimica et Biophysica Acta (BBA)-Biomembranes,* v. 1719, n. 1-2, p. 82-101, 2005.

MORENO, S. N. *et al*. Cytosolic-free calcium elevation in *Trypanosoma cruzi* is required for cell invasion. *The Journal of experimental medicine,* v. 180, n. 4, p. 1535-1540, 1994.

NAGAJYOTHI, Fnu *et al*. Response of adipose tissue to early infection with *Trypanosoma cruzi* (Brazil strain). *Journal of Infectious Diseases*, v. 205, n. 5, p. 830-840, 2012.

NEDERGAARD, Jan; BENGTSSON, Tore; CANNON, Barbara. Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology-Endocrinology and Metabolism,* v. 293, n. 2, p. E444-E452, 2007.

NIGER, Corinne; HOWELL, Floyd D.; STAINS, Joseph P. Interleukin-1 β increases gap junctional communication among synovial fibroblasts via the extracellular-signal-regulated kinase pathway. Biology of the Cell, v. 102, n. 1, p. 37-49, 2010.

PARK, Jiyoung *et al*. Obesity and cancer—mechanisms underlying tumour progression and recurrence. Nature Reviews Endocrinology, v. 10, n. 8, p. 455, 2014.

PETERS, Nicholas S. New insights into myocardial arrhythmogenesis: distribution of gap-junctional coupling in normal, ischaemic and hypertrophied human hearts. *Clinical science,* v. 90, n. 6, p. 447-452, 1996.

PORVAZNIK, Martin; MACVITTIE, Thomas J. Detection of gap junctions between the progeny of a canine macrophage colony-forming cell in vitro. *The Journal of cell biology*, v. 82, n. 2, p. 555-564, 1979.

RAMÍREZ, J. D. *et al*. First report of human *Trypanosoma cruzi* infection attributed to TcBat genotype. Zoonoses and public health, v. 61, n. 7, p. 477-479, 2014.

REY, L. Protozoários agentes de doenças humanas. In: Coura, J.R. (Ed.), Dinâmica das doenças infecciosas e parasitárias. 2ªed. Rio de Janeiro: Guanabara Koogan, 2013.

RUPNICK, Maria A. *et al*. Adipose tissue mass can be regulated through the vasculature. Proceedings of the National Academy of Sciences, v. 99, n. 16, p. 10730-10735, 2002.

RUTKOWSKI, Joseph M.; STERN, Jennifer H.; SCHERER, Philipp E. The cell biology of fat expansion. Journal of cell biology, v. 208, n. 5, p. 501-512, 2015.

SCHERER, Philipp E. The multifaceted roles of adipose tissue—therapeutic targets for diabetes and beyond: The 2015 Banting Lecture. Diabetes, v. 65, n. 6, p. 1452-1461, 2016.

SCHILLER, Paul C. *et al*. Inhibition of gap-junctional communication induces the trans-differentiation of osteoblasts to an adipocytic phenotype in vitro. *Journal of Biological Chemistry,* v. 276, n. 17, p. 14133-14138, 2001.

SHERIDAN, Judson D. Electrical coupling between fat cells in newt fat body and mouse brown fat. *The Journal of cell biology,* v. 50, n. 3, p. 795-803, 1971.

SHOEMAKER, Jon P.; HOFFMAN JR, Robert V.; HUFFMAN, David G. *Trypanosoma cruzi*: preference for brown adipose tissue in mice by the Tulahuen strain. *Experimental parasitology*, v. 27, n. 3, p. 403-407, 1970.

SILVA, D. T. & MEIRELLES, M. N. L. Expression of the endoplasmic reticulum enzymes in the *Trypanosoma cruzi*

infected and non infected cardiomyocytes. Mem Inst Oswaldo Cruz 93 (Suppl. II): p. 116, 1998.

SOUZA, Wanderley de. Basic cell biology of *Trypanosoma cruzi*. Current pharmaceutical design, v. 8, n. 4, p. 269-285, 2002.

SPRAY, David C. *et al*. Gap junctions and bystander effects: Good Samaritans and executioners. Wiley Interdisciplinary Reviews: Membrane Transport and Signaling, v. 2, n. 1, p. 1-15, 2013.

STERN, Jennifer H.; RUTKOWSKI, Joseph M.; SCHERER, Philipp E. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. Cell metabolism, v. 23, n. 5, p. 770-784, 2016.

TANOWITZ, Herbert B. *et al*. Adipose tissue, diabetes and Chagas disease. In: Advances in parasitology. *Academic Press*, 2011. p. 235-250.

TARDIEUX, I.; NATHANSON, M. H.; ANDREWS, N. W. Role in host cell invasion of *Trypanosoma cruzi*-induced cytosolic-free Ca2+ transients. *The Journal of experimental medicine,* v. 179, n. 3, p. 1017-1022, 1994.

TEUNISSEN, Birgit EJ; BIERHUIZEN, Marti FA. Transcriptional control of myocardial connexins. *Cardiovascular research*, v. 62, n. 2, p. 246-255, 2004.

VIEIRA, Mauricio *et al*. Cellular signaling during the macrophage invasion by *Trypanosoma cruzi*. *Histochemistry and cell biology,* v. 118, n. 6, p. 491-500, 2002.

YANAGIYA, Takahiro; TANABE, Atsushi; HOTTA, Kikuko. Gap-junctional communication is required for mitotic clonal expansion during adipogenesis. Obesity, v. 15, n. 3, p. 572-582, 2007.

YEGANEH, Azadeh *et al*. Connexin 43 phosphorylation and degradation are required for adipogenesis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, v. 1823, n. 10, p. 1731-1744, 2012.

YOSHIDA, Nobuko *et al*. Removal of sialic acid from mucin-like surface molecules of *Trypanosoma cruzi* metacyclic trypomastigotes enhances parasite-host cell interaction. *Molecular and biochemical parasitology,* v. 84, n. 1, p. 57-67, 1997.

ZINGALES, Bianca *et al*. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. *Memorias do Instituto Oswaldo Cruz*, v. 104, n. 7, p. 1051-1054, 2009.

****