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ALTERATIONS OF THE GAP JUNCTIONS IN THE PROCESS OF INFECTION WITH *Toxoplasma gondii* ON THE INTESTINAL EPITHELIAL CELL

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ABSTRACT

Toxoplasmosis is a disease caused by an obligate intracellular protozoan, *Toxoplasma gondii (T. gondii)*. The gastrointestinal tract is therefore a major route of *T. gondii* infection in most cases. For this reason the intestinal epithelium is a cellular model that provides to study the first line of defense against oral infections. The gap junctions, mediated by connexin 43 (Cx43), that have the role of mediating the interactions between adjacent cells. In the intestinal epithelial cell gap junction formed by Cx43 may be important in modulating the response of the cell to infectious processes, and by allowing the passage of important agents into the body in the process of infection. Although the invasion of parasites into host cells is a known event, the effects of this infection are not yet well established. In view of this, we investigated possible alterations in the positioning and expression of connexin 43 in the intestinal epithelial cell during the infection process of the protozoan *T. gondii*. For this, Immunofluorescence and Westen Blot assays were performed before and after infection, and a statistical analysis of these data was carried out. IEC-6 cells infected with the parasite *Toxoplasma gondii* showed disorganization of F-actin filaments, followed by reduced positioning and expression of connexin 43. This can greatly reduce intercellular communication and consequently the functions of the intestinal epithelium.

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INTRODUCTION

Toxoplasmosis is a high-prevalence zoonotic disease caused by a mandatory intracellular parasite, the protozoan *Toxoplasma gondii* (DUBEY, 2008; RAMÍREZ-FLORES *et al*., 2019). Because it has an asymptomatic character, in immunocompetent individuals, most of the time this parasitemia is not diagnosed (DUBEY, 1991). However, the number of infected is believed to be around one third of the world's population, therefore approximately 2.5 billion people (DUBEY, 2010; HIDE, 2016; YBAÑEZ *et al*., 2020). The serum prevalence of toxoplasmosis is also considered high in Brazil (REMINGTON *et al*., 2006), however the incidence of infection varies according to socioeconomic conditions and geographic location (FORSGREN *et al*., 1991). Studies such as Montoya and Liesenfeld (2004) and Dubey (2005) have shown that the most common way to acquire toxoplasmosis is by consuming water and food contaminated with the oocyst or tissue cyst of the parasite. In some animal species, the protozoan upon reaching the intestine penetrates the enterocytes and rapidly proliferates, promoting inflammation and changes in the physiology and functions of the intestinal epithelium barrier (KHAN & GRIGG, 2017; BURGER *et al*., 2018; YGLESIAS *et al*., 2018). When impaired the function of the intestinal barrier is associated with several intestinal and systemic diseases (MIELE *et al*., 2009). This barrier depends on a variety of structural and mucosal components, and it is known that the integrity and proper functioning of the intestinal epithelial cell layer are maintained by cell junctions, multiprotein structures that form an essential barrier against pathogens and molecules potentially harmful to the body (ODENWALD & TURNER, 2017). The study by Kojima *et al*. (2007), using primary culture of rat hepatocytes showed how cellular communication through the connexins contribute to the maintenance of tight junctions, through the increase in the expression of occlusive proteins. The expression of junction communication in the intestinal

epithelial cell may be involved in modulating the cell response to infectious processes, because it allows the passage of important agents to the body in the infection process (CHEN *et al*., 2014). However, pathological events can partially or completely disrupt the integrity of the intestinal barrier, leading to chronic permeability increase and allowing the entry of microorganisms and harmful substances into the circulation (MIELE *et al*., 2009; MUNIZ, 2012). Although the invasion of parasites in host cells is a known event, the effects of *T. gondii* infection on the intestinal barrier are not yet well established (WEIGHT *et al*., 2015; BRICEÑO *et al*., 2016). A study conducted in 2016 by Trevizan *et al*. showed that acute infection with *T. gondii* caused morphological changes in the intestinal wall of the duodenum in rats. Therefore, in this scenario, it is important to analyze the possible alteration of the morphofunctional positioning of the communicating junction, such as the protein connexin 43, in intestinal epithelial cells during the infectious-inflammatory process with the protozoan *Toxoplasma gondii,* for this it was performed the interaction of the cell with the parasite in order to elucidate important facts about the behavior of the intestinal epithelium and how it responds to such stimuli.

METHODS

Intestinal Epithelial Cell Culture (IEC-6): The intestinal epithelial cell lineage (IEC-6) was transferred by the Cell Bank of Rio de Janeiro. Cell plating was performed with an initial density of $1x10^6$cells/mL, in glass coverslips number 1 (Fisherbrand / Fisher Scientific). The cultures were packed in DMEM medium (Dulbecco´s modified Eagle´s medium), supplemented with 10% Bovine Fetal Serum (V/V) (GIBCO), 1Ul/mL insulin (NPH), penicillin 1000UI/mL and 100IU/mL streptomycin (Sigma-Aldrich). The cells were maintained at 37°C in a humid atmosphere at 5% CO_2.

Obtaining Taquizoite from Toxoplasma gondii: Tachyzoite form of *T. gondii* (RH Strain) was provided by the Technology Laboratory in Cell Culture (LTCC) by UERJ, Brazil-RJ. These parasites were obtained through the number of the Ethics Committee of the State University of Northern Fluminense Darcy Ribeiro (ID 124396). Tachyzoites of *T. gondii* (RH strain) were inoculated intraperitoneally into BALB/c mice. After 2-3 days, tachyzoites were harvested using intraperitoneal wash with phosphate buffered saline (PBS; pH 7.3) and then were counted.

Parasit-Cell Interaction: *T. gondii* taquizoite was resuspended in DMEM medium and placed in contact with the cell in the proportion of 10 parasites for each cell (10:1). This was kept 1 hour in a 37°C greenhouse in a humid atmosphere at 5% CO_2. Then, the medium was discarded and replaced by DMEM supplemented with 10% Bovine Fetal Serum, 1Ul/mL of insulin and penicillin 1000IU/mL and streptomycin 100IU/mL and were maintained at 37°C in a humid atmosphere at 5% OF CO_2, in the period of 24, 48 and 72 hours.

Immunofluorescence: The cells were fixed with 4% formaldehyde for 1 h at room temperature. After this stage, the cells were washed with PBS-TRITON X-100R at a concentration of 0.3% for 30 minutes and incubated with bovine serum albumin (BSA Bovine Serum Albumin, SIGMA) free of immunoglobulins, diluted in PBS at 2% for 20 minutes. After blockade, the cells were incubated with incubated with anti-Cx43 polyclonal antibody at a dilution of 1:100 and incubated with secondary antibody ALEXA 488 (at 1:400 dilution (A-11008, invitrogen). Then the lamínulas were incubated with the cytoskeleton marker Faloidine (F-actin) (Alexa Fluor® 546 phalloidin (A22283), Life Technologies) conjugated with fluorochrome ALEXA 546 at a dilution of 1:100 for 2 hours and were marked with DAPI (4',6-diamidine-2- phenylelindo). The markings on the slides were observed in an Epifluorine Microscope AXIOVERT 40 Plus Carl ZEISS (Photo microscope), excited with illumination by high pressure mercury lamp, HBO 50W, (λ = 490 nm) and the emission monitored using a set of emission filters for fluorescence fluoresceína (λ = 525nm). The experiments were submitted to observation and simple analysis or sections in plane Z (three-dimensional reconstruction) using the confocal microscope LSM 710 QUASAR (Carl ZEISS, Oberkochen, Germany). The data were plotted in the GraphPad Prisma program for the acquisition of the markup distribution analysis graph.

Western Blot: The cells studied by the Western Blot technique were washed and scraped in a sodium bicarbonate solution, and centrifuged for 10min. 3 mL of Tween 20 was added and homogenized with the aid of a vortex. The material was centrifuged, resuspended and stored at -20ºC in sodium bicarbonate solution, with the protease inhibitor cocktail (Protease inhibitors: PMSF - 50 mM in ethanol; Leupeptin - 5 mg/ml; EDTA - 200 mM; Aprotinin – 10 mg/mL; E-64 – 1 mM; Pepstatin – 1 mg/mL; Antipain – 10 mM; phenanthroline – 200 mM). The separation of the proteins present in the cell was performed using the technique of denaturing polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). The sample with the proteins was added to the running gel (running gel) at a concentration of 10%. The samples were solubilized in sample buffer, and kept at room temperature for about 60 minutes before being applied to the gel. After the electrophoretic run, the gel was placed in contact with the nitrocellulose membrane in a buffer solution and the transfer was carried out under a constant current of 320 mA for 1 hour (BIO-RAD system). Nitrocellulose was incubated in TBS buffer (Tris Buffer Saline) containing 5% kimmed milk powder and 0.5% Tween 20 for 30 min, followed by washing with TBS containing 0.5% Tween 20 (TBS- T) for 5 times for 5 minutes each time. The membrane was incubated for 17 hours at 4ºC under agitation with polyclonal anti-connexin43 antibody (Anti-connexin43/ GJA1 antibody (ab11370)(Abcam)) diluted at a ratio of 1:1000 in TBS-T containing skimmed milk powder a 3%. After washing the nitrocellulose with TBS-T, it was incubated with secondary antibody diluted 1:5000 in TBS-T containing 3% skimmed milk powder, which was linked to horseradish peroxidase for chemiluminescence, for 2 hours. For development, the nitrocellulose was incubated with the ECL (Chemiluminescence Peroxidase Substrate) solution added to Peroxide for analysis in the ChemiDoc MP imaging system BIO-RAD, and evaluated using the Image lab 5.2.1 program.

Statistical analysis: Statistical analysis was performed using the GraphPad Prism 9.3.1 program. The One Way Anova test was used for the inferential analysis at a significance level lowers than 0.05.

RESULTS

IEC-6 Cell Line Immunofluorescence: The intestinal epithelial cell line (IEC-6) was submitted to confocal immunofluorescence microscopy analysis in order to evaluate the profile of junctional communication and morphofunctional positioning of connexin 43 in its microenvironment, as well as in the infectious-inflammatory environment *with Toxoplasma gondii* in the proportion of ten parasites per cell for 24, 48 and 72 hours compared with control cells. In all micrographs the IEC-6 cells scan was incubated with DAPI to mark the cell nucleus in blue, marked with anti-connexin43 antibody to highlight connexin 43 in green, and incubated with faloidine to highlight F-actin filaments in red. When performing the analysis of the experiment, the marking of the control cell (uninfected) was verified, with the nucleus of the cells highlighted with DAPI (Figure 1A), F-actin filaments uniformly organized by all cells, being more evident the marking at the ends of the cell membrane (Figure 1B), and in figure 1C indicates that connexin 43 labelling in its morphofunctional location, with formation of junctional plaque is evident and, although it remains mainly concentrated in the plasma membrane of cells it was also marked in the cytoplasm.

Figure 2 shows infection of the infected cell lineage in the 24-hour period with the same marking described above, seeing in A the marking of the cell nucleus and parasites, however observing the cellular cytoskeleton it is possible to highlight the beginning of the disorganization of F-actin filaments (Figure 2B), and the reduction of the connexin 43 labeling present in the cell membrane (Figure 2C).

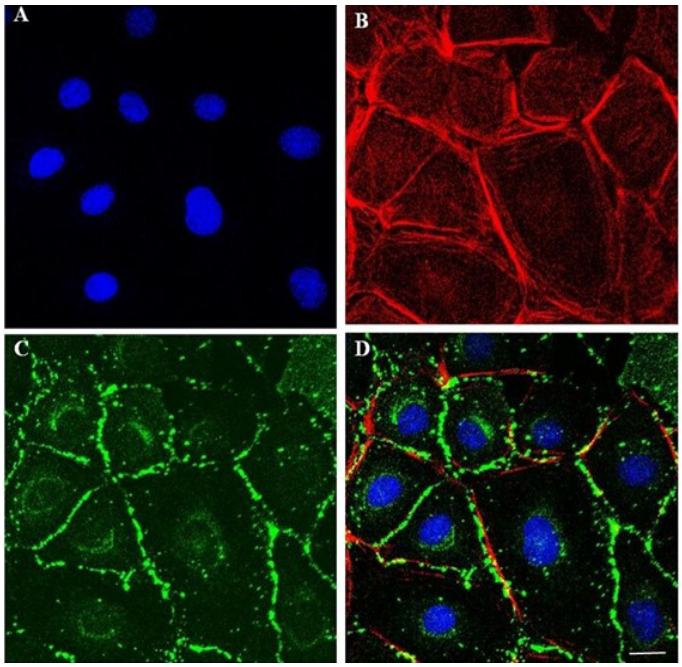

Figure 1. Immunofluorescence of IEC-6 sline control. Control cell immunofluorescence demonstrated by confocal microscopy (LSM710, Zeiss, Germany). We observed that the nucleus of cells marked with DAPI in blue (A) and the marking of F-actin filaments by faloidine in red and connexin 43 in green, located in the plasma membrane and cell cytoplasm (respectively B and C). In micrograph D, overlapping images are demonstrated. Calibration bar: 20µm

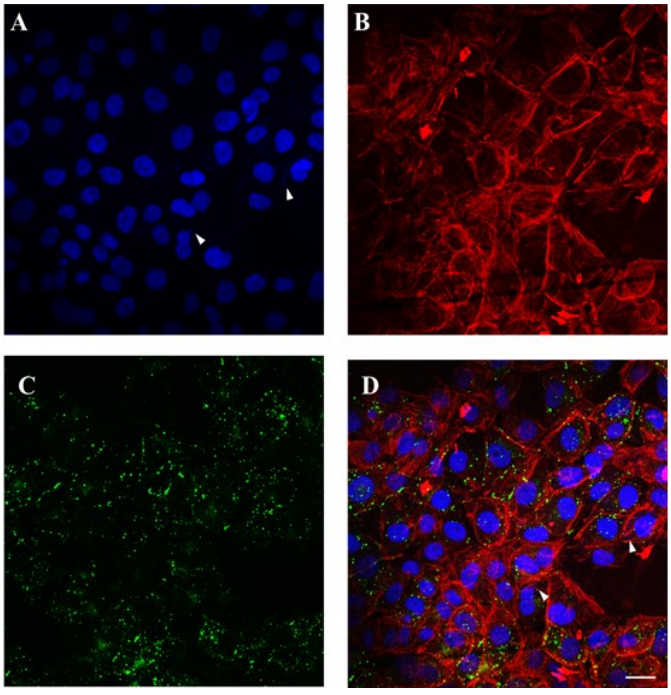

Figure 2. Immunofluorescence of the IEC-6 scan with 24h of infection with *Toxoplasma gondii*. Immunofluorescence demonstrated by confocal microscopy (LSM710, Zeiss, Germany), indicating the marking for the protein connexin 43 and faloidine (marker of F-actin filaments) in the cell line IEC-6 infected with *Toxoplasma gondii,* in the ratio 10:1 (ten parasites to a cell) after 24 hours. In arrows, A and D showed infection by the parasite. In this experiment, connexin 43 was reduced from the plasma membrane of IEC-6 cells after infection (C) when compared to control. In micrograph D, the interposition of images is demonstrated. Calibration bar: 50µm

In the period of 48 hours of infection, (Figure 3) the nucleus of the cell lineage and parasites are marked by DAPI (Figure 3A) and the cell cytoskeleton presents a greater disorganization when compared to 24-hour control and infection (figure 3B), but the marking of connexin 43 on the cell membrane and cytoplasm is present (Figure 3C). In Figure 4, the infection process has 72 hours of progression, and the marking of the cell nucleus and the abundance of the nucleus of the parasites inside the cell (figure 4A) are clear. The marking of the cellular cytoskeleton points to the disorganization of F-actin filaments and the cytoskeleton marking of protozoa inside the cell (Figure 4B), finally, the marking of connexin 43 present in the cell membrane is significantly reduced in the membrane and almost nonexistent in the cellular cytoplasm (Figure 4C).

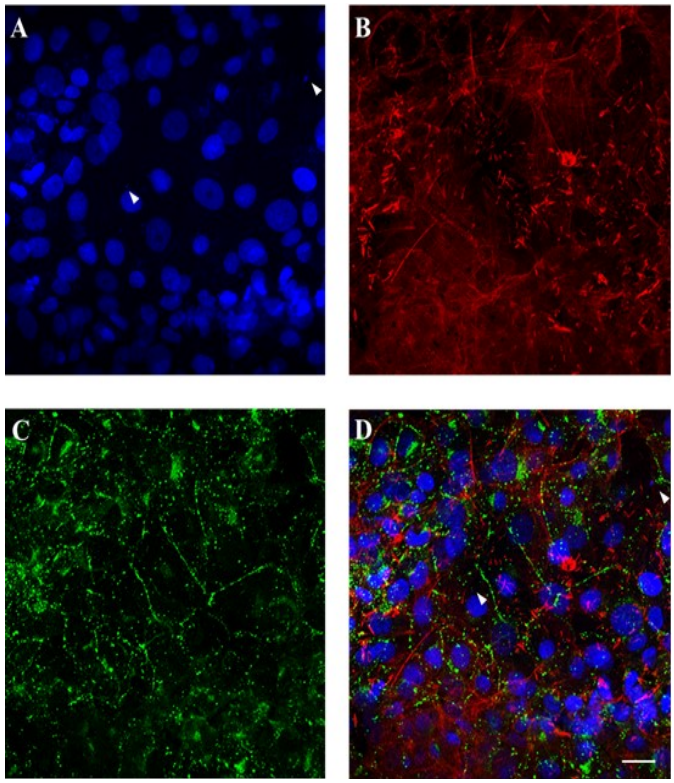

Figure 3. Immunofluorescence of the IEC-6 scan with 48h of infection with Toxoplasma gondii. Immunofluorescence demonstrated by confocal microscopy (LSM710, Zeiss, Germany), indicating the marking for the protein connexin 43 and faloidine (marker of F-actin filaments) in the cell line IEC-6 infected with *Toxoplasma gondii,* in the ratio 10:1 (ten parasites to a cell) after 48 hours. In arrows, A and D demonstrated infection by the parasite. In this experiment, faloidine (B) shows the disorganization of F-actin filaments of the cellular cytoskeleton and connexin 43 (C) was increased from the plasma membrane of IEC-6 cells after infection when compared to control. In micrograph D, the interposition of images is demonstrated. Calibration bar: 50µm

Infection with *Toxoplasma gondii*: Expression of Connexin 43: Through immunoelectrotransfer assays (Western Blot), using IEC-6 cells, the expression of protein levels of connexin 43 in control and infected cells (*T. gondii* 1:1) at 24, 48 and 72 hours was analyzed, as highlighted in figure 6A. In view of the findings, the band representing the sample in which there was interaction with the parasite for an incubation period of 24 hours showed a higher expression of Cx43, when compared to control cells (uninfected) and cells infected at other times. With 72 hours of infection with T. gondii, the expression of protein Cx43 also showed higher values than those found in control cells, however with lower values than those found in 24 hours of infection.

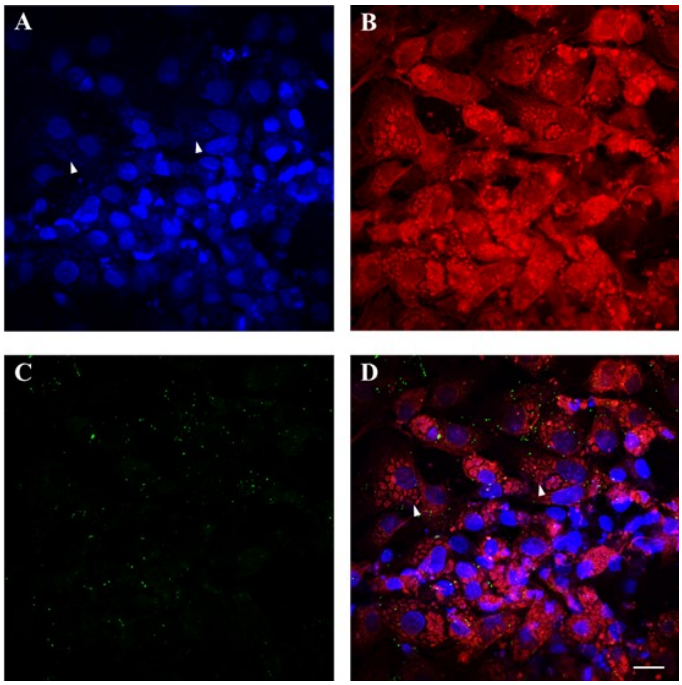

Figure 4. Immunofluorescence of the IEC-6 scan with 72h of infection with *Toxoplasma gondii*. Immunofluorescence demonstrated by confocal microscopy (LSM710, Zeiss, Germany), indicating the marking for the protein connexin 43 and faloidine (marker of F-actin filaments) in the cell line IEC-6 infected with *Toxoplasma gondii,* in the ratio 10:1 (ten parasites for a cell) after 72 hours. In arrows, A and D showed infection by the parasite. In this experiment, the marking of connexin 43 and F-actin were interrupted by infection of the plasma membrane of IEC-6 cells (B, C). In micrograph D, interposed images are demonstrated. Calibration bar: 50µm

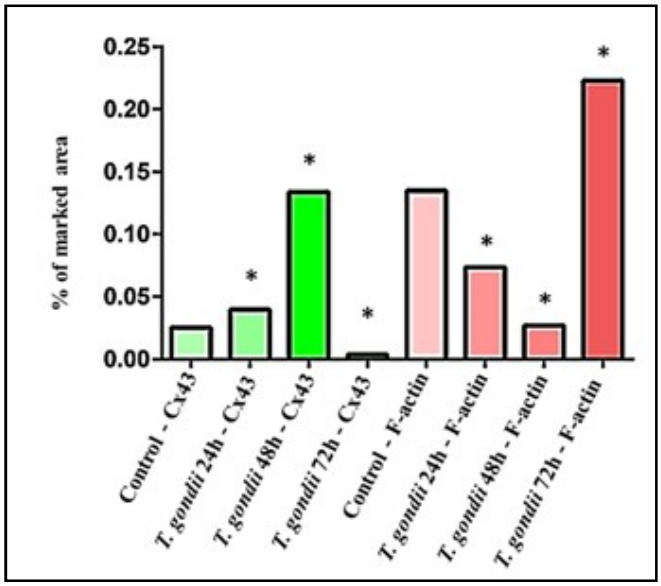

Figure 5. Distribution analysis. Graph of confocal immunofluorescence analysis showing the percentage of the area marked by anti-connexin 43 (green) and faloidine (red) antibodies over the total area of the image. It is possible to observe that in 48h of infection with the protozoan *Toxoplasma gondii* there is an increase in the area marked with anti-Cx43 antibody and this marking decreases significantly after 72 h of infection. When analyzing the marking of F-actin filaments by faloidine, it is notorious the decrease in structure according to the increase in parasitemia, however in 72h of infection it is possible to visualize the significant increase of this marking due to the large cellular damage, causing the cytoskeleton of the protozoan to also be marked. P<0,05.

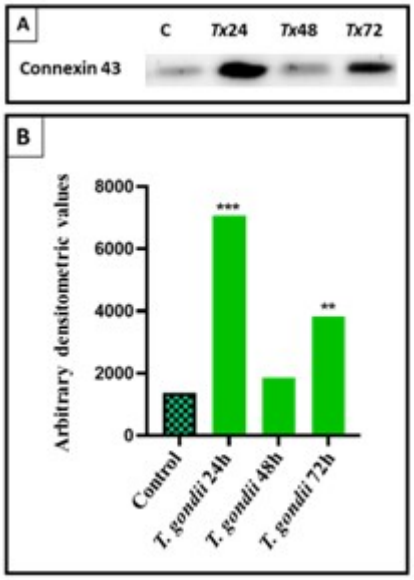

Figure 6. Western Blot of the IEC-6 strain infected with *Toxoplasma gondii*. Connexin 43 protein expression levels in A. The wells were organized as follows: Control Cells (C); Cell infected with *Toxoplasma gondii* in the periods of 24, 48 and 72 hours (Tx24, Tx48 and Tx72). The labeling at 43 kDa is a result of the use of the polyclonal antibody to connexin 43. There is an increase in the expression of Cx43 in relation to the control IEC-6 cells, in relation to the infected IEC-6 cells, especially when analyzing the period of 24 hours of infection. Graph (B) represents the densitometry made from the markings of the bands observed in the Western blot image of IEC-6 cell cultures. In B demonstrates that at 24h and 72h of infection there was a statistically significant increase in connexin 43 expression. P<0.05 (*); P<0.01(**), P<0.001(***); P<0.0001 (****)

DISCUSSION

Connexin43 and F-actin Labelling in *Toxoplasma gondii* Infection: Recently several studies have investigated possible changes caused by *Toxoplasma gondii* in specific proteins of host cells. Proteins related to the modulation of a series of processes such as immune response, cell cycle, metabolism, apoptosis and cytoskeleton organization (ZHOU *et al*., 2011; ELE *et al*., 2016). Research's showed direct relationships of infection with protozoan and changes in cell junctions, and Bricenõ *et al.* (2016) when infecting cells of the Caco-2 singage with the protozoan *Toxoplasma gondii* identified changes in the proteins ZO-1, Claudina-1 and Ocludine, which were concentrated in the cytoplasm of the cell, as a consequence of a reduction in polarity due to decreased transtelial resistance. In addition, the infection promoted changes in the distribution of actin filament, making the microvilli of cells less developed. In the experiments carried out it was possible to observe the evolution of the process of disorganization of the cytoskeleton, as shown in figures 2B, 3B and 4B, corroborating the data of Briceño *et al*. (2016), although they are distinct cell lines, as in the Caco-2 cell, the IEC-6 cell after the *Toxoplasma gondii* evasion process has the organization of the cytoskeleton interrupted which promotes a discontinuous network patterns, resulting in structural changes of the epithelial cell monolayer due to Infection by *T. gondii* in the host microenvironment. It is worth mentioning that, it is through the microtubules that the conexons are transported from the Golgi complex to the plasma membrane of the cell, after synthesizing and oligomerization of the connexins (DHEIN, 1998; GAIETTA *et al*., 2002; SOSINSKY *et al*., 2007). Other proteins that deserve to be highlighted in this process are α-catenin and α-actinin that are part of the cytoskeleton formation and according to Giepmans (2004) these proteins are related to the insertion of the connexin in the membrane. Studies by Fujimoto *et al.* (1997) showed through confocal microscopy assays that at the beginning of junctional plate

formation α-catenins have co-location with Cx43 in the cell plasma membrane, therefore it assumes extreme importance in the insertion of connexin in the plasma membrane. Based on these data, it can be inferred that the reduction of connexin 43 is directly linked to the behavior of the cellular cytoskeleton as seen in Figure 1, 2, 3 and 4 of this study, a similar behavior also reported in the experiments of De Carvalho *et al*. (2021). In the graph of the confocal immunofluorescence analysis, where it is possible to observe the percentage of the area marked by anti-connexin antibodies 43 and faloidine on the total area of the image, it is visualized that with the increase of the disorganization of the cytoskeleton, shown by the disarrangement of f-actin filaments, there were changes in the positioning of the Cx43 protein at its morphofunctional site in the plasma membrane (Figure 2). However, two points worth mentioning are the increase in the marking of connexin 43 in the cytoplasm at 48h (Figure 3C) and the increase in the marking of F-actin filaments at 72h (Figure 4B). The increase in faloidine marking is justified by the fact that the great cellular damage caused by the protozoan makes the protocol for marking F-actin filaments of cell IEC-6 also be able to mark the *Toxoplasma gondii* cytoskeleton inside the cell.

In relation to the increase in Cx43 marking in 48 hours of infection in cell IEC-6 can be inferred to the fact that the cell attempt to position the connexins previously produced in the plasma membrane, however with the increase of the cytoskeleton disarray a portion of the connexin remained in the cytoplasm. It is known that the interaction between cells by the communicating junctions is regulated both by the synthesis of the connexins, as well as by their transport, oligomerization, insertion and removal of the cell membrane. However, the life cycle of connexin is short, research shows that its half life does not exceed 5 hours (LAIRD, 2005). Studies have already shown that connexin 43 interacts with ZO-1 protein in cardiomyocytes, but how this interaction occurs has not yet been well elucidated (TOYOFUKU, 2001). Toyofuku also showed through electrophysiological analysis that the regulation of communicating junctions is directly involved with the tyrosine phosphorylation site and with the ZO-1 binding domain in connexin 43. Other studies such as Laird's (2005) and Hunter's (2003) have related the interaction between these proteins with the size of the junctional plaque. In view of the above, it is possible to infer the direct relationship between the evasion process of *Toxoplasma gondii* with the disorganization of the cytoskeleton, the changes in ZO-1 as reported in the studies by Briceño (2016) and Jones (2017) and the decrease in the positioning of Cx43 in the intestinal epithelial cell as demonstrated in the present study. We highlight then that the protozoan has the ability to reshape the cell cytoskeleton and modify the entire junctional complex of the cell, not only the occlusion junctions but also the communicating junctions mediated by Cx43, this being a possible mechanism of protozoan evasion.

Protein Expression of Cx43 in *Toxoplasma gondii* Infection: Over the years, numerous studies have been carried out in order to have a better understanding of the changes caused by the protozoan *Toxoplasma gondii* in the human body. *T. gondii* tachyzoites infect and transmigrate between adjacent intestinal epithelial cells and promote the production of specific inflammatory mediators. During the invasion, the *T. gondii* parasite was co-localized with the Occludin protein. This finding inferred that Occludin could be playing some role in the invasion of *T. gondii* in epithelial cells of the small intestine (Weight, *et al*., 2015). Among the several important proteins in this scenario, the connexins, which form gap junctions, involved in the junctional communication between cells, allowing the passage of molecules with molecular weight up to 1kDa, responsible for regulating numerous tissue functions, deserve to be highlighted. In parasitic infections, these proteins undergo considerable functional changes, assuming an important role in altering the infected tissue behavior. (DE CARVALHO *et al*., 1998) Studies on the interaction of cardiomyocytes with the protozoan *Trypanosoma cruzi* demonstrated a decrease in Cx43 and ZO-1 proteins in the plasma membrane (Goldenberg *et al*., 1999). Thus, the present study sought to analyze the levels of protein expression of connexin 43 (Cx43), thus allowing a better understanding of the profile of junctional communication in a microenvironment of acute infection generated by the protozoan. From the results found, it was possible to observe that there was an increase in the protein expression of Cx43 after 24 hours of infection (figure 6A - *Tx*24) when compared with the levels expressed by the control cells (uninfected) (figure 6A - C). This data is in line with the findings of Adesse *et al*. (2008) using cultured cardiomyocytes from Swiss Webster mouse embryos. Their studies showed that during the first hours of infection with *Trypanosoma cruzi*, the cells showed an increase of approximately 32% in the expression of protein Cx43. In this way, it is possible to observe that within 24 hours of infection with the *T. gondii* parasite, the activation of cell signaling pathways that stimulate the intestinal epithelial tissue, in this case represented by the IEC-6 cells, can occur, intensifying their communication profile intercellular and thus promoting a significant increase in Cx43 protein expression. Therefore, it would be possible to increase the formation of gap junctions that could be acting in the process of regulation of the acute infection generated by the parasite. The increase in both expression and immunoreactivity of connexins in pro-inflammatory and injured environments has already been observed in several studies. The altered expression of connexins is also associated with the inflammatory changes of the nasosinusal mucosa that characterize chronic rhinosinusitis (KIM *et al*., 2016). Zou *et al*. (2019) evaluated whether inhibition of Cx43 would attenuate sepsis-induced intestinal injury and were able to demonstrate in their studies that connexin in the intestinal epithelium peaked after 24 h, precisely when the injury was more pronounced. They showed, through Western Blot analysis and Luciferase assay, that with the inhibition of connexin 43, there is an attenuation in the progression of the lesion due to the blocking of the passage of reactive oxygen species through the junctional channel.

However, after 48 hours of parasite-cell interaction, it was possible to identify a significant reduction in the protein expression of Cx43, which can be explained by the imbalance and cellular disorganization to which the infected cells are submitted. Similar data were found in the work by Goldenberg *et al*. (1999) who observed an increase in the number of *T. cruzi* parasites per cell with the infection time in embryonic cultures of cardiac myocytes, while Cx43 specific labeling is proportionally reduced in infected cells and only cells with internalized parasites lose their Cx43 specific marking. Despite the decrease in the levels of connexin expressed in 48h of infection, it was possible to observe that after 72h of interaction of IEC-6 with the protozoan *Toxoplasma gondii*, connexin levels rose again. On the other hand, these data associated with 72h of parasitic infection are controversial when compared to the literature, which demonstrates that kidney epithelial cells (MDCK II cells) when infected with *T. cruzi* for 72h showed a drastic decrease in immunoreactivity. Cx43 in most highly infected cells and a 61% drop in protein levels (ADESE *et al*., 2011). A similar response was found in J774-G8 macrophage cells, where within 48h of infection there is a drastic drop in connexins and with 72h of infection the protein was practically non-existent in the culture (DE CARVALHO *et al*., 2021). The difference between these data can be partly explained by the high concentrations of parasites used in the studies, since the present study used a milder infection rate.

CONCLUSION

In view of the results presented, it is concluded that in the cell line IEC-6 infected with *Toxoplasma gondii* the cytoskeleton protein F-actin is disorganized. An attempt occurred to direct Cx43 to the cell membrane in 48h of infection with *T. gondii* and Cx43 protein in IEC-6 suffered a significant reduction in the plasma membrane after infection with the protozoan *Toxoplasma gondii,* and the interruption of positioning at its morphofunctional site in 72 hours of infection.

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