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GTPase Rab5 and Rab7 proteins are modulated by Corynebacterium pseudotuberculosis *in murine macrophages*

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Abstract

Introduction: *Corynebacterium pseudotuberculosis* is an intracellular facultative bacterium that affects macrophages, causing *caseous lymphadenitis in goats and sheep. This disease is characterised by the formation of granulomas and results in significant economic losses for farmers of goats and sheep around the world. Phagosome maturation is essential for the death of intracellular pathogens, involving the fusion of the phagosome with early and late endocytic organelles, a process partially regulated by GTPase Rab5 and Rab7 proteins, respectively.* ***Methodology:*** *we evaluated the ability of an attenuated T1 strain and a wild-type C57 strain of C. pseudotuberculosis to alter Rab5 and Rab7 protein expression in peritoneal CBA mouse macrophages following in vitro infection.* ***Results:*** *Rab5 and Rab7 expression decreased in macrophages infected with the C57 strain compared to uninfected macrophages, while a significant reduction only in Rab7 expression was observed in cells infected with T1. Nonetheless, Rab5 and Rab7 expression was higher in macrophages infected with T1 than in cells infected with the wild-type C57 strain.* ***Conclusions****: our studies indicate a greater degree of modulation of the endosomal proteins directly involved in phagosome maturation by Corynebacterium pseudotuberculosis wild type.*
Keywords: *Corynebacterium pseudotuberculosis; macrophages; phagolysosome; Rab proteins.*

Resumo

Introdução: *Corynebacterium pseudotuberculosis é uma bactéria intracelular facultativa que acomete macrófagos, causando linfadenite caseosa em caprinos e ovinos. Esta doença é caracterizada pela formação de granulomas e resulta em perdas econômicas significativas para criadores de caprinos e ovinos em todo o mundo. A maturação do fagossomo é essencial para a morte de patógenos intracelulares, envolvendo a fusão do fagossomo com organelas endocíticas precoces e tardias, um processo parcialmente regulado pelas proteínas GTPase Rab5 e Rab7, respectivamente.* **Metodologia:** *avaliamos a capacidade de uma cepa T1 atenuada e uma cepa C57 de tipo selvagem de C. pseudotuberculosisto alterar a expressão da proteína Rab5 e Rab7 em macrófagos peritoneais de camundongos CBA após infecção in vitro.* **Resultados:** *a expressão de Rab5 e Rab7 diminuiu em macrófagos infectados com a cepa C57 em comparação com macrófagos não infectados, enquanto uma redução significativa apenas na expressão de Rab7 foi observada em células infectadas com T1. No entanto, tanto a expressão de Rab5 como de Rab7 foram superiores em macrófagos infectados com T1 em comparação com células infectadas com a estirpe C57 de tipo selvagem.* **Conclusões:** *nossos estudos indicam um maior grau de modulação das proteínas endossomais diretamente envolvidas na maturação do fagossomo por Corynebacterium pseudotuberculosis tipo selvagem.*
Palavras-chave: *Corynebacterium pseudotuberculosis; macrófagos; fagolisossomos; proteínas Rab.*

INTRODUCTION

Corynebacterium pseudotuberculosis*, a facultative, intracellular, gram-positive bacterium that grows within macrophages, is the etiologic agent of caseous lymphadenitis (CL). This chronic infectious disease affects small ruminants, including goats and sheep. CL is characterised by the formation of granulomas in the lymph nodes or internal organs, which leads to significant economic losses for breeders due to a progressive debilitation of infected animals that reduces the productivity (wool, meat, milk) and reproductive efficiency of livestock herds[1-4].*

Phagocytosis, the first line of defence against infection by intracellular pathogens, is fundamental to microbial death and antigen presentation[5-7]. Following phagocytosis, intracellular bacteria initially reside in a vacuole known as a phagosome. During the normal cycle of maturation, the phagosome containing the pathogen undergoes a series of fusions with endocytic vesicles, culminating in phagosome-lysosome fusion[8-10].

Members of the Rab family of proteins are important regulators of the phagosome maturation process[11,12]. The Rab family is part of the Ras superfamily of small GTPases and is directly involved in regulating different stages of intracellular vesicle trafficking and controlling vesicle formation, movement, and fusion[13-15].

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Rab5 and Rab7 proteins play specific and coordinated roles in the sequential phagosome maturation[16]. Rab5, localised in early endosomes, is associated with the phagosome after its closure and is essential for the recruitment of Rab7, located in late endosomes, which is necessary for phagolysosome fusion[17,18]. The phagolysosome is an extremely acidic and hydrolytic compartment capable of degrading most bacteria[8,19]. This maturation process is highly complex and is more easily defined by the loss of the GTPase Rab5 and the subsequent recruitment of GTPase Rab7[20,21].

Many intracellular pathogens can avoid intracellular killing by lysosomal enzymes by modulating host cell machinery. This allows some bacteria to avoid phagosomal acidification and interaction with the endosomal-lysosomal pathway by creating an intracellular niche favouring pathogen survival and replication[14,22,23].

Considering how little is known about the mechanisms utilised by C. pseudotuberculosis *to persist within macrophages and the important roles of Rab proteins in the phagosome maturation process. The present study aimed to evaluate the ability of two strains of* C. pseudotuberculosis*, attenuated T1 and wild-type C57, to alter the expression of endosomal proteins GTPase Rab 5 and Rab 7 in murine macrophages. In addition, by studying the interaction of the attenuated and wild variants of* C. pseudotuberculosis *within host cells, we hope to gain insight into the relevant aspects of this microorganism's pathogenesis and virulence in the initial phases of infection.*

METHODOLOGY

Murine macrophages were infected with the attenuated T1 and wild-type C57 of C. pseudotuberculosis, and the expression of GTPase Rab 5 and Rab 7 in infected macrophages was analysed by flow cytometry. The present study aimed to evaluate the ability of C. pseudotuberculosis to alter the expression of these endosomal proteins important in the maturation of the phagolysosome.

Animals

Five female CBA mice aged six to eight weeks were provided by the Gonçalo Moniz Institute, Salvador, Bahia-Brazil (FIOCRUZ, Salvador-BA), and housed at the Animal Care Facility of the Health Sciences Institute, Federal University of Bahia (ICS-UFBA). All animals were kept in 30x19.5x12 cm breeding boxes under 21 ± 1°C, 50-60% humidity, and controlled luminosity (12-h daily periods of light and darkness), with rations and water *ad libitum*.

Corynebacterium pseudotuberculosis

T1 and C57 strains of C. pseudotuberculosis *were cultured in brain heart infusion (BHI) for 48 hours at 37ºC for the infection of peritoneal CBA mouse macrophages. The T1 strain was originally isolated from a naturally infected goat in the Santa Luz municipality of the state of Bahia, donated to the collection of the Microbiology Laboratory at the Health Sciences Institute of the Federal University of Bahia (ICS- UFBA) in 2000 by researcher Dr Artur Hage. This strain had been previously identified by the "API coryne" test (BioMérieux AS, Marcy-l'Etoile, France) and, following successive cultures in a specific medium, was considered attenuated due to less intense synergistic hemolysis with* Rhodococcusequiin *comparison to other strains obtained from naturally infected goats. The C57 strain was isolated in 2010 from a naturally infected goat, identified by culturing for* Corynebacterium spp*. in addition to direct multiplex PCR using material collected from granulomas of superficial lymph nodes.*

Bacterial quantification was performed according to a protocol established by Sampaio[24](2019). Briefly, 1 mL of bacterial culture was washed twice in saline under centrifugation for 5 minutes at 5,000 rpm. The supernatant was discarded, and SYBR Safe™ (Invitrogen, Carlsbad, California, USA) was added, followed by incubation for 20 minutes in darkness at room temperature. Further washing was performed to remove the reactants, and the bacterial pellet was resuspended in 1 mL of saline. Next, 300µL of saline, 20µL of the resuspended bacterial pellet and 20µL of fluorescent microspheres (Perfect-Count Microspheres™-Cytognos, Santa Marta de Tormes, Spain) were placed in a test tube and measured by flow cytometry. Bacterial concentrations were calculated as follows: Total bacteria count = number of bacterial events/ numbers of bead events x number of beads per µL (value specified by the manufacturer).

Macrophage harvesting and cultivation

CBA mice were intraperitoneally inoculated with 3mL of 1-month-aged sterile 4% thioglycollate Brewer's medium (HIMEDIA) to stimulate the recruitment of macrophages. After four days of stimulation, all animals were euthanised by cervical dislocation and the peritoneal cavity was exposed under aseptic conditions. Next, 5 mL of sterile refrigerated phosphate buffer saline (PBS) pH 7.2 was injected. After massaging the abdomen, peritoneal cells were recovered and washed twice in PBS under centrifugation at 1500 rpm for 5 minutes at 4°C. Cells were then resuspended in 1 mL of RPMI-1640 (GIBCO) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotic (penicillin and streptomycin - GIBCO), with total cell counts performed at a final volume of 40µL using an automatic cell counter (Hemocytometer CELM cc-530, CELM –Cia Equipadora de LobaratoriosModernos, Barueri-SP). After counting, the concentration was adjusted to $1x10^6$ cells/mL. Cells were then placed in 24-well plates with the supplemented RPMI medium described above and incubated overnight at 37°C under 5% CO_2 for macrophage adherence. After incubation, non-adherent cells were removed by washing the wells with RPMI medium. Finally, RPMI medium supplemented with antibiotic-free SFB was added to the adherent macrophages.

Macrophage infection

Obtained macrophages were infected with the T1 or C57 strain of C. pseudotuberculosis *at a ratio of 1:1 (1 bacterium/macrophage) and incubated for two h at 37ºC under 5% CO_2. Adherent macrophages were cultured only in an RPMI medium with antibiotic-free FBS for controls.*

Expression of Rab 5 and 7 GTPases

To evaluate Rab 5 and Rab 7 expression in uninfected macrophages and cells infected with C. pseudotuberculosis*, adhered cells were first removed by adding 200 µl of trypsin after discarding the culture supernatant and washing well plates with PBS. Cells were then incubated at 37°C under 5% CO2 for 3 min. PBS (800 µL) was added, and cells were collected and then transferred to test tubes, followed by centrifugation for 3 min at 3,250 rpm, after which the supernatant was discarded. After resuspending cells in 100 µL of PBS, the Fc receptor was blocked by adding 1µL of antibody for 30 minutes (CD8a from VMRD INC, Pullman, USA). Cells were then fixed with 50 µL of 3% paraformaldehyde for ten min., washed with chilled PBS containing bovine serum albumin (BSA) (500 for 3 min at 3,500 rpm) and permeabilised with 50µL of solution containing 0.2% BSA and PBS with 0.5% saponin. Cells were subsequently incubated with the specific mouse-derived primary antibody at previously standardised concentrations of 4 µg/mL for Monoclonal Anti-Rab5 (Sigma-Aldrich, St. Louis, USA) and 1 µg/mL for Monoclonal Anti-rab7 (Sigma-Aldrich, St. Louis, USA) for 30 minutes in an ice bath. Next, cells were washed in chilled PBS with 1% BSA (PBS-BSA) pH 7.2 and resuspended in 100 µL PBS-BSA. The Alexa Fluor 488 rabbit anti-mouse IgG (component A) (Molecular Probes, Eugene, USA) was then added at 10 µg/mL concentration and incubated in an ice bath for 30 minutes under dark conditions. Cells were subsequently washed with PBS-BSA, and Alexa Fluor 488 goat anti-rabbit IgG (component B) (Molecular Probes, Eugene, USA) was added at a concentration of 10 µg/mL, followed by incubation in an ice bath for 30 minutes in the dark. Finally, cells were washed with PBS-BSA and resuspended in 100µL of PBS before flow cytometry analysis. Data were acquired and analysed on a FACS Calibur (BD) flow cytometer using Cell Quest Pro software v. 5.2.1. Intracellular levels of Rab 5 and 7 were recorded as geometric means of fluorescence intensity (MFI). For experimental controls, cells were incubated in the absence of the primary antibody or without the primary and secondary antibodies.*

Quantitative data and statistical analyses

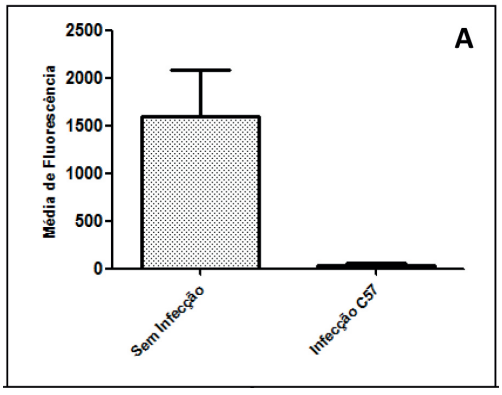
Data analysis was performed using SPSS software (Statistical Package for Social Sciences), version 13.0 for Windows. The Student's T test was used as data distribution was considered normal under Kolmogorov-Smirnov testing. Results were considered statistically when p <0.05. Data are shown as means and standard deviations of three independent experiments. Each experiment was performed three times in triplicate.

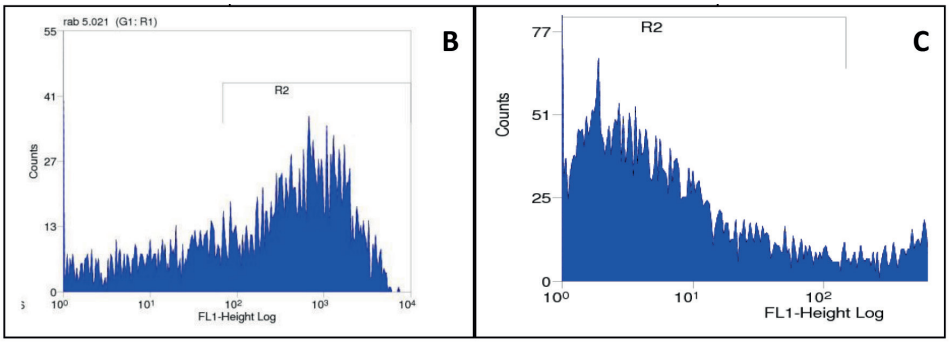
RESULTS

To evaluate C. pseudotuberculosis's ability to regulate GTPase proteins in host cells, Rab 5 and 7 expression was quantified by flow cytometry in uninfected macrophages and cells infected with the attenuated T1 or C57 wild-type strains.

*Figure 1A depicts the mean fluorescence intensity (MFI) of Rab 5 in uninfected macrophages and cells infected with the C57 strain for 2 hours. Figures 1B and 1C contain histograms showing the MFI of Rab 5 expression in control cells and macrophages infected with the C57 strain, respectively. These data demonstrate a statistically significant (p = 0.000) decrease in Rab 5 expression in the C57-infected cells, with a reduction of up to 98% compared to uninfected macrophages (***Figures 1A, 1B, 1C***).*

Figure 1 - *Mean fluorescent intensity (MIF) of Rab5 on uninfected macrophages and on macrophages after 2 hours of infection with the wild-type C57 strain of C. pseudotuberculosis(***A***). Histogram (number of cells vs MIF) of the uninfected macrophages (***B***) and of the macrophages after 2 hours of infection with the wild-type C57 strain(***C***). Data shows the mean and standard deviation of three independent experiments. Student T-test was used. * p=0,000. n=3.*





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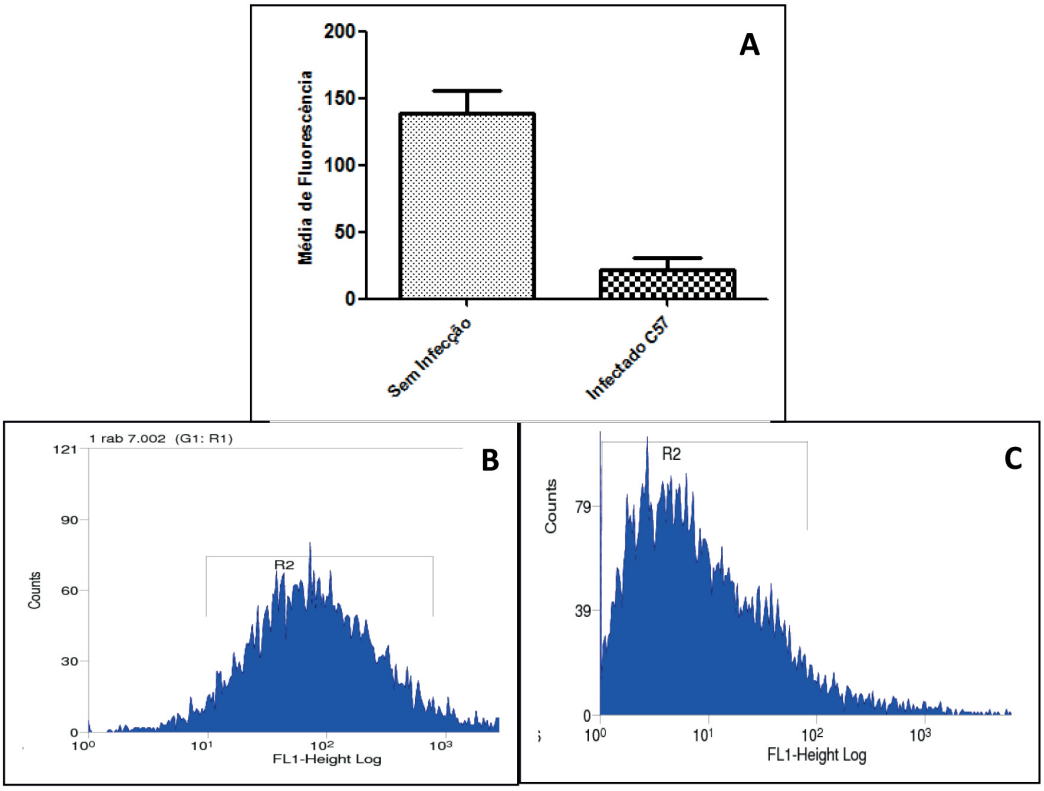
*A statistically significant (p=0.003) decrease in Rab 7 expression was also observed in C57-infected macrophages compared to uninfected cells. Figures 2A and C reveal a significant reduction (84%) in MFI in infected cells compared to uninfected macrophages (***Figures 2A, 2B***).*

Figure 2 -*(***A***) Mean fluorescent intensity (MIF) of Rab7 on uninfected macrophages and on macrophages after 2 hours of infection with wild-type C57 strain of C. pseudotuberculosis(***A***). Histogram (number of cells vs MIF) of the uninfected macrophages (***B***) and of the macrophages after 2 hours of infection with the wild-type C57 strain(***C***). Data shows the mean and standard deviation of three independent experiments. Student T-test was used. * p=0,003. n=3.*



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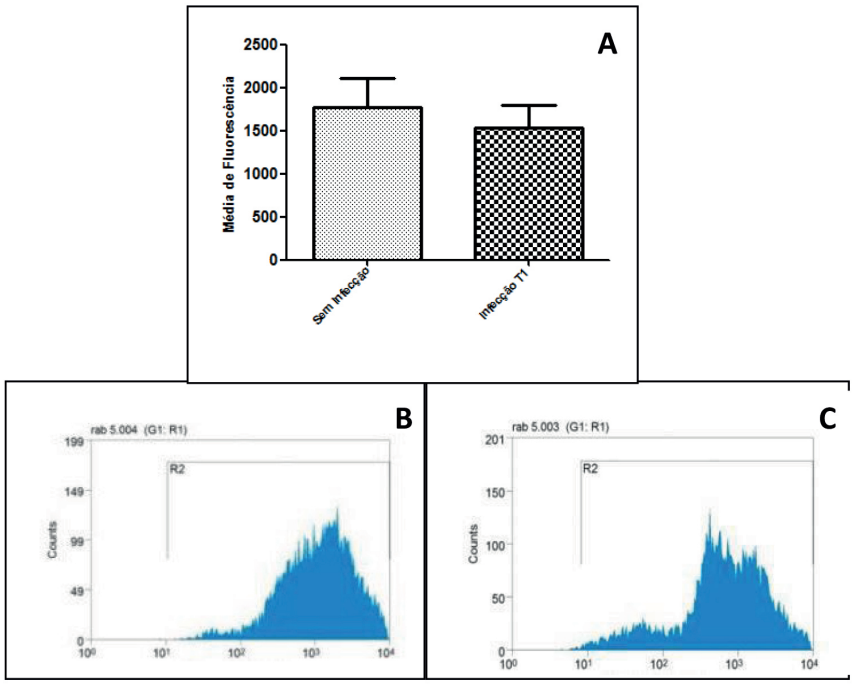
*With respect to Rab 5 expression in macrophages infected with the attenuated T1 strain, no significant differences were observed among cells infected for 2 hours compared to infected macrophages, as similar MFI levels were detected in each group (***Figures 3A, 3B, 3C***).*

Figure 3- *Mean fluorescent intensity (MIF) of Rab5 on uninfected macrophages and on macrophages after 2 hours of infection with attenuatedT1 strain of C. pseudotuberculosis(***A***). Histogram (number of cells vs MIF) of the uninfected macrophages (***B***) and of the macrophages after 2 hours of infection with attenuated T1 strain (***C***). Data shows the mean and standard deviation of three independent experiments. Student T-test was used. There was no statistically significant difference. n=3.*



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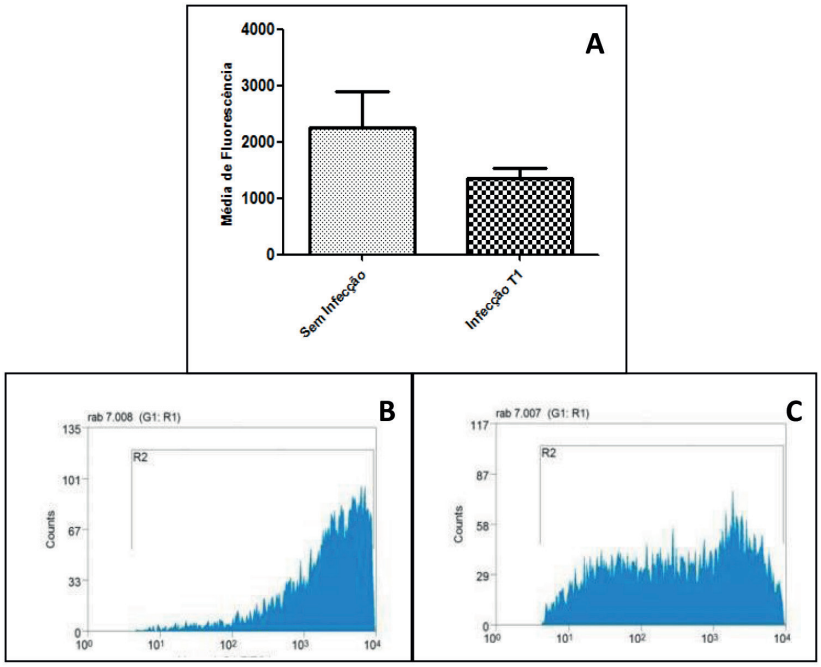
*By contrast, a significant decrease in Rab 7 expression (40%) was seen in the T1-infected macrophages when compared to control cells (p=0.043) (***Figures 4A, 4B, 4C***).*

Figure 4 - *Mean fluorescent intensity (MIF) of Rab7 on uninfected macrophages and on macrophages after 2 hours of infection with attenuated T1 strain of C. pseudotuberculosis (***A***). Histogram (number of cells vs MIF) of the uninfected macrophages (***B***) and of the macrophages after 2 hours of infection with attenuated T1 strain(***C***). Data shows the mean and standard deviation of three independent experiments. Student T-test was used. * p=0,043. n=3.*



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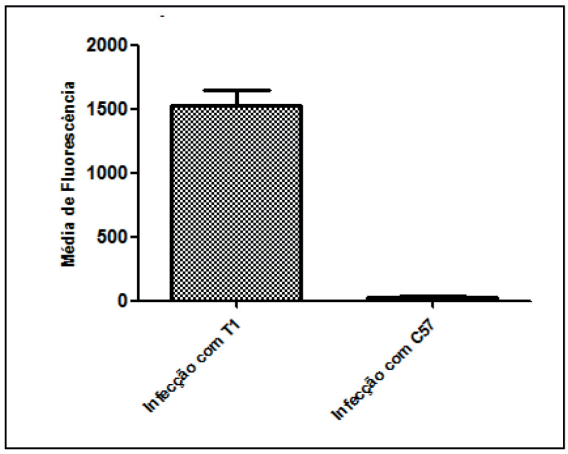
A comparison of Rab 5 expression between macrophages infected with the T1 and C57 strains revealed that C57-infected cells had a significantly lower MFI than those infected with the T1 strain (p=0.000) **(Figure 5)**.

Figure 5- *Mean fluorescent intensity (MIF) of Rab5 on macrophages after 2 hours of infection with the attenuated T1 strain and the wild-type C57 strain of C. pseudotuberculosis. Data shows the mean and standard deviation of three independent experiments. Student T-test was used. *p=0,000. n=3*



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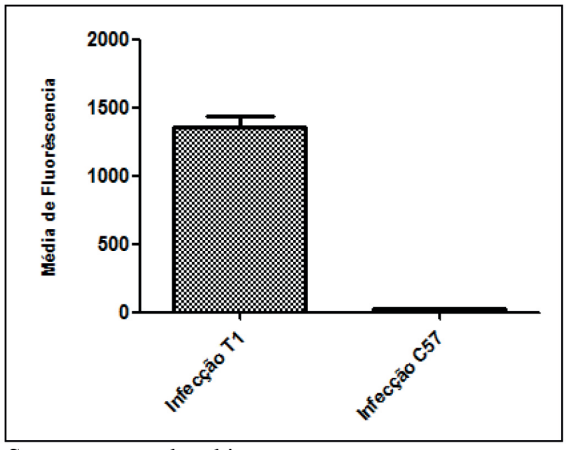
*Rab 7 expression was similarly lower (p=0.000) in C57-infected macrophages than in cells infected with the T1 strain, with decreased MFI observed (***Figure 6***).*

Figure 6- *Mean fluorescent intensity (MIF) of Rab7 on macrophages after 2 hours of infection with the attenuated T1 strain and the wild-type C57 strain of C. pseudotuberculosis. Data shows the mean and standard deviation of three independent experiments. Student T-test was used. *p=0,000. n=3*



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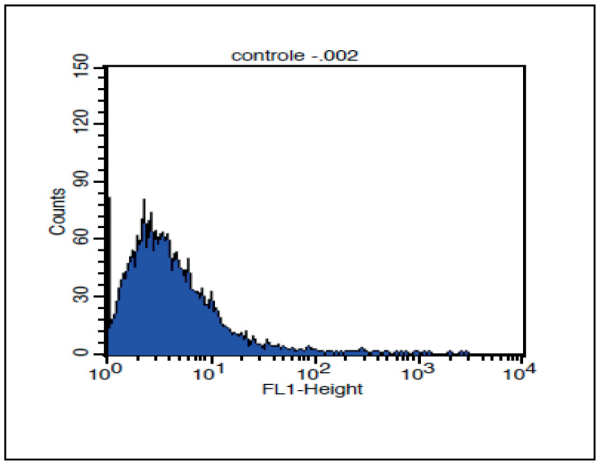
*No fluorescence was observed in experimental control cells, which were incubated in the absence of the primary antibody or without either the primary or secondary antibodies (***Figure 7***).*

Figure 7- *Macrophages cultured without primary and secondary antibodies (negative control).*



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DISCUSSION

Quantitative analysis of mean fluorescence revealed that Rab5 and Rab7 expression decreased by approximately 98% and 84% in the macrophages infected with the wild-type C57 strain of C. pseudotuberculosis compared *to uninfected control cells. A significant decrease in GTPase Rab7 expression was seen in cells infected with the attenuated T1 strain in relation to controls.*

The results obtained herein with respect to the C57 wild strain are partially consistent with other reports published in the literature on intracellular pathogens, such as Mycobacterium bovis, Leishmania donovani *and T. Cruzi, which demonstrate that phagosomes containing these microorganisms are unable to fuse with late endosomes and/or lysosomes yet interact with early endosomes. Our results about the attenuated T1 strain are consistent with the consensus in the literature.*

Via et al.[25] *(1997) showed that J774 macrophage phagosomes infected with* Mycobacterium bovis *BCG acquire and retain Rab5, an early endosomal membrane fusion regulator, but fail to bind to Rab 7, a late endosome specific GTP binding protein. These authors defined the stage between early and late endosomes as the point of defect in the maturation of the phagosome containing mycobacteria.*

In the infection of Leishmania donovani *promastigotes, phagosome maturation was also inhibited due to Rab7 recruitment deficiency, which prolonged the survival of these microorganisms in J774 murine macrophages*[26].

A previous study by Batista et al.[27] *(2006) employing both laser confocal microscopy and flow cytometry showed that Rab7 and Rab11 expression, but not Rab5, were regulated during T. cruzi infection in murine cardio-*

myocytes, with an approximate MFI reduction of 30% and 39%, respectively, in comparison to uninfected cardiac cultures. In this study, alterations in GTPase Rab expression during T. cruzi infection correlated with a deficiency in endocytosis.

The results obtained herein suggest that wild-type C57 C. pseudotuberculosis *modulates the maturation of the phagosome from the beginning to the end of the maturation process by regulating Rab5 and Rab7. In addition, these results suggest that the C57 strain remains in nascent phagosomes that do not evolve into early or late phagosomes. It is possible that bacterial components (virulence factors, secreted and somatic proteins) are responsible for the observed decreases in Rab 5 and Rab 7 expression. Accordingly, insufficient recruitment of these endosome GTPases to phagosomes probably occurs. This inhibition consequently affects fusion with lysosomes containing acidic enzymes, preventing the bacterium's death. In this way, bacteria can escape the host's innate and impair the subsequent adaptive response, thereby persisting as intracellular parasites in the non-acidified environment of nascent phagosomes.*

According to Cardoso, Jordão, and Vieira[28] (2010), GTPase Rab10 transiently binds with phagosomes at very early stages and is necessary for phagosome maturation. The expression of the constitutively active mutant of Rab10 increases the percentage of Rab5-positive phagosomes containing M. bovis *BCG. Consequently, levels of active Rab10 (bound to GTP) can regulate the transition from nascent phagosomes to initial phagosomes[28]. Based on this information, it is plausible to believe that wild-type C57* C. pseudotuberculosis could also modulate the expression of Rab10 during the *initial stages of infection, i.e. the lack of Rab 10 may consequently impede the recruitment of Rab5. In addition, the C57 strain could affect Rab5 intracellular trafficking by limiting binding to the macrophage recognition receptor.*

Hard[29] (1975) viewed an intact lipid layer of C. pseudotuberculosis *in peritoneal mouse macrophages on electron microscopy and believed this to be associated with the persistence of viable bacteria in phagocytes. Mckean, Daviesjk, Moore[30] (2007) showed that phospholipase D (PLD) of* C. pseudotuberculosis *contributed to reducing the viability of J774 macrophages. However, the literature contains no studies to date regarding the interference of these virulence factors on intracellular trafficking pathways, especially on the phagosome maturation pathway.*

The results presented here with respect to the attenuated T1 strain of C. pseudotuberculosis *suggest that although this strain remains in early phagosomes that acquire Rab 5, these recruit lessRab7. This bacterial strain likely secretes components different from those of the virulent strain, i.e., these components do not affect the acquisition of Rab5 by the phagosome. Although the T1 strain acquires Rab5, decreased recruitment of Rab7 is significant in that it also leads to reduced recruitment of the Rab7-effector protein RILP (RabInteracting Lysosomal Protein), which is responsible for phagosome-lysosome fusion[31-32-33].*

Pathogen-based manipulation of the mechanism regulating the active state (GTP-linked) versus inactive state (linked to GDP) of Rab proteins is very efficient at subverting intracellular traffic[34,35]. In addition, direct degradation of the Rab proteins is an effective strategy for pathogens to control intracellular transport[36].

Sun et al.[37] (2007), using RAW 264.7 macrophages, demonstrated that inactive Rab7 (GDP-bound) predominated in viable M. bovis *BCG-infected cells and that the culture supernatant of* M. bovis *BCG contained a factor that catalysed the change from GTP to GDP in Rab7-recombinant molecules. Another study by Sun et al.[38] (2010) showed that a nucleoside diphosphate kinase (NdkA) of* M. tuberculosis *acted directly on GTP bound to Rab5 or Rab7.*

Since Rab proteins' functioning is regulated by protein expression, membrane binding, and activation[23,39], it is possible that C. pseudotuberculosis *modulates the activation state of Rab proteins. This would significantly decrease the accumulation/activation of Rabs5 and 7 in cells infected with C57 while reducing the accumulation/activation of Rab7 in T1-infected cells.*

Nevertheless, further study is needed to clarify how the expression of these Rab proteins is modulated in macrophages and any possible molecules involved in the decrease/inhibition of expression/recruitment of Rab5 and Rab7 in T1 and C57 C. pseudotuberculosis *infection.*

A comparison of the modulation of Rab expression in macrophages infected by these two different C. pseudotuberculosis *strains showed lower Rab5 and Rab7 MFI in the macrophages infected with the wild-type C57 strain compared to attenuated T1.*

These findings are in accordance with a previous study by Bugalhão[40] (2013), which demonstrated that the avirulent H37Ra and virulent H37Rv strains of M. tuberculosis *exhibit different capabilities to manipulate host vesicular trafficking pathways in THP-1 macrophages silenced for RabGTPases. Decreased Rab7 and Rab34 protein expression significantly increased the intracellular survival capacity of H37Ra*M. Tuberculosis*, yet this was not affected in the H37Rv strain. Both Rab7 and Rab34 are important for the functioning and positioning of lysosomes.*

The results obtained herein indicate that more Rab 5 and 7 are recruited by the phagosomes containing the attenuated T1 strain, and although both strains (T1 and C57) significantly interfere with Rab7 binding, modulation by C57 is more pronounced. As previously mentioned, each strain appears to survive in a distinct compartment within macrophages, i.e. C57 in nascent phagosomes and T1 in early phagosomes that recruit Rab5. These results further suggest that the T1 and C57 strains probably produce different molecules, thereby involving a variety of recognition receptors and the induction of different signalling cascades that divergently affect endocytic intracellular trafficking and phagosome maturation. It is also possible that the attenuated T1 strain produces less

PLD, an important virulence factor, in relation to wild-type C57. Future studies should be conducted to elucidate these potential differences in the context of the present findings.

CONCLUSIONS

In conclusion, C. pseudotuberculosis *has the ability to alter Rab protein expression in peritoneal mouse macrophages cultured* in vitro*, modulating host cell intracellular trafficking using a strategy similar to that of other intracellular pathogens, i.e. via the regulation of Rab5 and/or Rab7. Decreased Rab 5 and 7 expressions in cells infected with the wild-type C57 strain and of only Rab7 in the attenuated T1-infected macrophages indicates that these strains modulate Rab proteins differently, with more marked modulation by the C57 wild-type with respect to the fundamental factors involved in phagosome-lysosome progression, which act throughout the maturation process. These results offer insight into how* C. pseudotuberculosis *evades initial macrophage response by altering the normal physiology of the host cells, consequently avoiding bacterial destruction and immune recognition mechanisms.*

DECLARATIONS

Ethics approval and consent to participate

The present study received approval from the animal experimentation institutional review board of the Health Sciences Institute (CEUA-ICS), Federal University of Bahia (protocol n°035/2012).

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REFERENCES

1. Santos LM, Stanisic D, Menezes UJ, Mendonça MA, Barral TD, Seyffert N, et al. Biogenic Silver Nanoparticles as a Post-surgical Treatment for Corynebacterium pseudotuberculosis Infection in Small Ruminants. Front Microbiol. 2019;10:824. doi: 10.3389/fmicb.2019.00824

2. De Souza AP, Vale VL, Silva MDA C, Araújo IB, Trindade SC, De Moura-Costa LF, et al. Envolvimento do MAPK na produção de citocinas em resposta à infecção por Corynebacterium pseudotuberculosis. BMC Microbiol. 2014;14:230. doi: 10.1186/S12866-014-0230-6

3. Zerbinati J, Greve IC, Leal RF, Amorin LMPV, Silva DL, Viegas SRAA, et al. Produção e padronização de um antígeno para um teste ELISA indireto no diagnóstico de linfadenite caseosa em soros caprinos. Rev Acad. 2007;5(3):285-93. doi:10.7213/cienciaanimal.v5i3.10116

4. Mckean S, John D, Moore R. Identification of macrophage induced genes of Corynebacterium pseudotuberculosis by differential fluorescence induction. Microbes Infection. 2005. doi:10.1016/j.micinf.2005.05.002

5. Uribe-Querol E, Rosales C. Fagocitose: Nossa Compreensão Atual de um Processo Biológico Universal. Imunol frontal. 2020;11:1066. doi: 10.3389/fimmu.2020.01066

6. Thakur A, Mikkelsen H, Jungersen G. Intracellular Pathogens: Host Immunity and Microbial Persistence Strategies. J Immunol Res. 2019 Apr;2019:1356540. doi: 10.1155/2019/1356540

7. Weiss G, Schaible UE. Mecanismos de defesa de macrófagos contra bactérias intracelulares. Imunol Rev. 2015;264(1):182-203. doi: 10.1111/IMR.12266

8. Lee H-JI, Woo Y, Hahn T-W, Jung YM, Jung Y-M. Formation and Maturation of the Phagosome: A Key Mechanism in InnateImmunityagainstIntracellularBacterialInfection. Microorganisms. 2020;8(9):1298; doi: 10.3390/microorganisms8091298

9. Uribe-Querol E, Rosales C. Controle da fagocitose por patógenos microbianos. Imunol frontal. 2017;8:1368. doi: 10.3389/fimmu.2017.01368

10. Thi EP, Lambertz U, Reiner NE. Dormindo com o inimigo: como os patógenos intracelulares lidam com um estilo de vida de macrófagos. PLoS Pathog. 2012; 8(3):e1002551. doi: 10.1371/journal.ppat.1002551

11. Gutierrez MG. Functional role(s) of phagosomal Rab GTPases. Small GTPases. 2013;4(3):148-58. doi: 10.4161/sgtp.25604

12. Flannagan RS, Cosio G, Grinstein S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. Nat Rev Microbiol. 2009;355-66. doi:10.1038/nrmicro2128

13. Homma Y, Hiragi S, Fukuda M. Rab family of small GTPases: an updated view on their regulation and functions. FEBS J. 2021;288:36-55. doi: 10.1111/febs.15453

14. Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. Physiol Rev. 2011;91(1):119-49. doi: 10.1152/physrev.00059.2009.

15. Hyun HP. Structural Basis of Membrane Trafficking by Rab Family Small G Protein. Int J Mol Sci. 2013;14:8912-23; doi:10.3390/ijms14058912

16. Saito-Nakano Y, Yasuda T, Nakada-Tsukui K, Leippe M, Nozaki T. Os vacúolos associados a Rab5 desempenham um papel único na fagocitose do parasita protozoário entérico Entamoeba histolytica. J Biol Chem. 2004;279(47):49497-507. doi: 10.1074/JBC. M403987200

17. Langemeyer L, Borchers AC, Herrmann E, Füllbrunn N, Han Y, Perz A, et al. A conserved and regulated mechanism drives endosomal Rab transition. Elife. 2020;9:e56090. doi: 10.7554/eLife.56090

18. Poteryaev D, Datta S, Ackema K, Zerial M, Spang A. Identification of the switch in early-to-late endosome transition. Cell. 2010 Apr 30;141(3):497-508. doi: 10.1016/j.cell.2010.03.011

19. Flannagan Rs, Heit B, Heinrichs De. Antimicrobial Mechanisms of Macrophages and the Immune Evasion Strategies of Staphylococcus aureus. Pathogens. 2015;4(4):826-68. doi: 10.3390/pathogens4040826

20. Borchers AC, Langemeyer L, Ungermann C. Who's in control? Principles of Rab GTPase activation in endolysosomal membrane trafficking and beyond. J Cell Biol. 2021;220(9). doi: 10.1083/jcb.202105120.

21. Pei G, Bronietzki M, Gutierrez MG. Immune regulation of Rab proteins expression and intracellular transport. J Leukoc Biol. 2012;92:41-50. doi: 10.1189/jlb.0212076

22. Sachdeva K, Sundaramurthy V. The Interplay of Host Lysosomes and Intracellular Pathogens. Front Cell Infect Microbiol. 2020;10:595502. doi: 10.3389/fcimb.2020.595502

23. Brumell JH, Scidmore MA. Manipulation of rab GTPase function by intracellular bacterial pathogens. Microbiol Mol Biol Rev. 2007;71(4):636-52. doi: 10.1128/MMBR.00023-07

24. Sampaio GP, Vale V, Moura Costa LF, Fraga RE, Santos HH, Sá MC, et al. Padronização de técnicas por citometria de fluxo para avaliar Corynebacterium pseudotuberculosis e células fagocitárias murinas. Pubvet. 2019;13(11). doi:10.31533/pubvet.v13n11a443.1-9

25. Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V. A parada da maturação do fagossomo micobacteriano é causada por um bloqueio na fusão da vesícula entre os estágios controlados por rab5 e rab7. J Biol Chem. 1997;272(20):13326-31. doi: 10.1074/jbc.272.20.13326

26. Scianimanico S, Desrosiers M, Dermine JF, Méresse S, Descoteaux A, Desjardins M. O recrutamento prejudicado da pequena GTPase rab7 se correlaciona com a inibição da maturação do fagossomo por promastigotas de Leishmania donovani. Microbiol celular. 1999;1(1):19-32. doi: 10.1046/j.1462-5822.1999.00002.x

27. Batista DGJ, Silva CF, Mota RA, Costa LC, Meirelles MNL, Meuser-Batista M, Soeiro MNC. Trypanosoma cruzi modulates the expression of rabs and alters the endocytosis in mouse Cardiomyocytes in vitro. J Histochemistry & Cytochemistry. 2006;54(6):604-16. doi:10.1369/jhc.5A6654.2005

28. Cardoso CMP, Jordão L, Vieira OV. Rab10 Regulates Phagosome Maturation and Its Over expression Rescues Mycobacterium-Containing Phagosomes Maturation. Traffic. 2010;11(2):221-35. doi:10.1111/j.1600-0854.2009.01013.x

29. Hard GC. Comparative toxic effect of the surface lipid of Corynebacterium ovis on peritoneal macrophages. Infect Immun. 1975;12(6):1439-49. doi: 10.1128/iai.12.6.1439-1449.1975

30. Mckean SC, Daviesjk, Moore RJ. Probing the heat shock response of Corynebacterium pseudotuberculosis: the major virulence factor, phospholipase D, is downregulated at 43 degrees C. Res Microbiol. 2007;doi:10.1016/j.resmic.2006.12.006

31. Cantalupo G, Alifano P, Roberti V, Bruni CB, Bucci C. Rab-interacting lysosomal protein (RILP): the Rab7 effector required for transport to lysosomes. Embo J. 2001;doi:10.1093/emboj/20.4.683

32. Harrison RE, Bucci C, Vieira OV, Schroer TA, Grinstein S. Phagosomes fuse with late endosomes and/or lysosomes by extension of membrane protrusions along microtubules: role of Rab7 and RILP. Mol Cell Biol. 2003;23(18):6494-506. doi: 10.1128/MCB.23.18.6494-6506.2003

33. Jordens I, Marsman M, Kuijl C, Neefjes J. Rab proteins, connecting transport and vesicle fusion. Traffic. 2005;6(12):1070-7. doi: 10.1111/j.1600-0854.2005.00336.x

34. Ingmundson A, Delprato A, Lambright DG, Roy CR. Legionella pneumophila proteins that regulate Rab1 membrane cycling. Nature. 2007;450(7168):365-9. doi: 10.1038/nature06336

35. Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR. The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. Nat Cell Biol. 2006; doi:10.1038/ncb1463

36. Spano S, Liu X, Galan JE. O direcionamento proteolítico de Rab29 por uma proteína efetora distingue os compartimentos intracelulares de Salmonella adaptada ao homem e de hospedeiro amplo. Proc Natl Acad Sci EUA. 2011. doi:10.1073/pnas.1111959108

37. Sun J, Deghmane AE, Soualhine H, Hong T, Bucci C, Solodkin A, et al. Mycobacterium bovis BCG interrompe a interação de Rab7 com RILP contribuindo para a inibição da maturação do fagossomo. J Leukoc Biol. 2007; 82(6):1437-45. doi: 10.1189/jlb.0507289

38. Sun J, Wang X, Lau A, Liao TY, Bucci C, Hmama Z. A micobactéria nucleosídeo difosfato quinase bloqueia a maturação do fagossomo em macrófagos murinos RAW 264.7. PLoS Um. 2010;5(1):e8769. doi: 10.1371/journal.pone.0008769

39. Stein MP, Dong J, Wandinger-Ness A. Proteínas Rab e tráfego endocítico: alvos potenciais para intervenção terapêutica. Adv Drug Deliv Rev. 2003;55(11):1421-37. doi: 10.1016/j.addr.2003.07.009

40. Bugalhão JMN. Mycobacterium tuberculosis subversion of host vesicular traffic pathways [dissertação]. Lisboa: Faculdade de Ciências, Universidade de Lisboa; 2013.

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