Toxicology in Vitro 36 (2016) 114–119

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Rapamycin increases RSV RNA levels and survival of RSV-infected dendritic cell depending on T cell contact

Deise do Nascimento de Freitas [a], Rodrigo Benedetti Gassen [a,b], Tiago Fazolo [a,b], Ana Paula Duarte de Souza [a,c,*]

[a] Laboratório de Imunologia Clinica e Experimental; Centro Infant, Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil
[b] Laboratório de Imunologia Celular e Molecular, PUCRS, Brazil
[c] Faculdade de Farmácia, PUCRS, Brazil

ARTICLE INFO

Article history:
Received 6 March 2016
Received in revised form 19 June 2016
Accepted 22 July 2016
Available online 25 July 2016

Keywords:
Respiratory syncytial virus
Rapamycin
Dendritic cells
CD8 T cells

ABSTRACT

The macrolide rapamycin inhibits mTOR (mechanist target of rapamycin) function and has been broadly used to unveil the role of mTOR in immune responses. Inhibition of mTOR on dendritic cells (DC) can influence cellular immune response and the survival of DC. RSV is the most common cause of hospitalization in infants and is a high priority candidate to vaccine development. In this study we showed that rapamycin treatment on RSV-infected murine bone marrow-derived DC (BMDC) decreases the frequency of $CD8^+CD44^{high}$ T cells. However, inhibition of mTOR on RSV-infected BMDC did not modify the activation phenotype of these cells. RSV-RNA levels increase when infected BMDC were treated with rapamycin. Moreover, we observed that rapamycin diminishes apoptosis cell death of RSV-infected BMDC co-culture with T cells and this effect was abolished when the cells were co-cultured in a transwell system that prevents cell-to-cell contact or migration. Taken together, these data indicate that rapamycin treatment present a toxic effect on RSV-infected BMDC increasing RSV-RNA levels, affecting partially CD8 T cell differentiation and also increasing BMDC survival in a mechanism dependent on T cell contact.

1. Introduction

Rapamycin, the established inhibitor of mTOR (mechanist target of rapamycin), was discovered in soil samples from Easter Island (Rapa Nui) in the 1970s and found to have antifungal activity (Li et al., 2014). mTOR is a serine-threonine protein kinase, which plays a central role in regulating cell growth, metabolism and survival (Wullschleger et al., 2006). Since 1999, rapamycin is approved for acute renal allograft rejection. In 2007 and 2009 FDA approved two derivate of rapamycin, temsirolimus and everolimus, respectively for advanced renal cancer carcinoma treatment. Nowadays, it is known that pharmacological inhibition of mTOR by rapamycin has a wide range of clinical effects and future clinical applications (Hasty, 2010).

Dendritic cells (DC) are important antigen presenting cells that recognize, process and present antigen to T cells and regulate the immune response (Banchereau et al., 2000; Condon et al., 2011). These cells are essential for initiation T cell immunity against virus (Banchereau and Steinman, 1998). In result to rapamycin treatment a lot have been learned about the role of mTOR on DC. mTORC inhibition hampers murine bone marrow derived DC maturation (Hackstein et al., 2003) and endocytosis (Hackstein et al., 2002) impairing T cell differentiation (Turnquist et al., 2010). In contrast, inhibition of mTOR on DC during activation was related to the increase in the lifespan of these cells, the extension of co-stimulatory molecules expression and the induction of an effective and strong specific $CD8^+$ T cells response (Amiel et al., 2012).

Human Respiratory syncytial virus (RSV), an omnipresent virus, is the main cause of lower respiratory tract infection in newborn babies and very young children (Nair et al., 2010; Hall et al., 2009). Hospitalization numbers due to RSV infection in children are increasing each year and global annual death of 200,000 children is estimated in consequence of infection (Nair et al., 2010). Palivizumab, indicated to prevent severe infection in high-risk children, is a very expensive treatment (F. the A.A. of Pediatrics, 2009). Currently, there is no efficient RSV vaccine available, although there are promising approaches being tested in mouse model (Bueno et al., 2008; Anderson et al., 2013) and in clinical trials (Green et al., 2015a; Green et al., 2015b). RSV infection can impair murine (González et al., 2008; Ptaschinski et al., 2015) and human (Gupta et al., 2013) DC function preventing specific T cells activation. In addition, RSV infection induced limited plasmacytoid DC responses in mice (Cormier et al., 2014) and in human (Weng et al., 2014).

We recently demonstrated that RSV-infected infants present elevated mTOR expression comparing to uninfected ones (de Souza et al., 2016). In addition, rapamycin treatment on T cells enhances the frequency of mouse RSV-specific CD8 T cells memory precursors (de Souza et al., 2016). However, so far there is no information about mTOR inhibition on DC during RSV infection. Therefore, the main

Abbreviations: RSV, respiratory syncytial virus; BMDC, bone marrow derived dendritic cells; DC, dendritic cells; mTOR, mechanist target of rapamycin.

* Corresponding author at: Av. Ipiranga, 6690, 2° andar, Sala 31, Hospital São Lucas da PUCRS, Porto Alegre, RS 90610-000, Brazil.
E-mail address: ana.duarte@pucrs.br (A.P.D. Souza).

http://dx.doi.org/10.1016/j.tiv.2016.07.016
0887-2333/

objective of this study was to investigate the role of rapamycin treatment on DC during RSV infection.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice ranging from 8 to 12 weeks old were purchased from Centro de Modelos Biológicos e Experimentais (Cembe) - PUCRS. Mice were housed at the animal facility of Cembe with water and food ad libitum. All animal procedures were performed in accordance with protocols approved by the University ethics Committee (CEUA Protocol number 14/00391).

2.2. Virus

RSV A strain (line A2) was provided by Fernando Polack, Fundación Infant, Argentina. The virus was grown in Vero cells. Viral plaque forming units (PFU) were identified using an RSV F protein-specific antibody (Millipore, Billerica, MA).

2.3. Murine bone marrow derived dendritic cells cultures

Murine bone marrow derived dendritic cells (BMDC) were grown from bone marrow in AIM-V medium (Gibco, Carlsbad, CA) with GM-CSF and IL-4 (Peprotech, Ribeirao Preto, Brazil), as previously described (Inaba et al., 1992).

2.4. Purification of T cells

Murine T cells were purified from spleen and lymph nodes (inguinal, axillary and brachial) using Pan T cells magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany (order number 130-095-130)), following the manufacturer's instructions.

2.5. Culture of T cells and RSV infected BMDCs

After 6 days of differentiation, 1.5×10^4 BMDCs were treated with 20 ng/mL of rapamycin for 1 h. Cells were washed and infected with 10^2 PFU of RSV for 1 h in RPMI medium without fetal bovine serum (FBS) and then washed to remove the virus. Purified T cells were seeded at concentration of 5.0×10^4/well in 96-well plate in co-culture with BMDC. Cells were co-cultured in RPMI with 5% FBS for 96 h at 37 °C at atmosphere containing 5% CO_2. For some experiments, cells were cultured in a 6.5 mm Transwell® system (CORNING, New York, USA (product number 3470)) with 0.4 μm pore membrane insert to avoid contact between them.

2.6. Flow cytometry

Cells were incubated with FcBlock (supernatant of 2.4G2 cells with 5% of human serum) for 20 min. T cells were stained with anti-CD8a (clone 53,67) and anti-CD44 (clone IM7) (BD Biosciences®) in dark for 30 min. BMDCs were stained with anti-CD11c (clone HC3), anti-CD86 (clone Rit-GL1) and anti-MHCII (clone 2G9) (BD Biosciences®). Samples were acquired on flow cytometer FACS CANTO II (BD Bioscience®). Data were analyzed using FlowJo software (TreeStar).

2.7. Annexin-V apoptosis assay

Apoptosis of BMDC was analyzed with BD Annexin V FITC apoptosis detection kit (BD Bioscience, San Jose, CA) following manufacturer's instruction. Cytometry was operated with a FACS Canto II (BD Bioscience).

2.8. Real-time PCR

Total RNA was extracted from RSV infected BMDC using Viral RNA/DNA Mini Kit (PureLink® - Invitrogen) following the manufacturer's instructions. cDNA was synthetized with random primers using Sensiscript® Reverse Transcription kit (QIAGEN®). The quality of the cDNA for each sample was tested by amplification of β-actin endogenous gene using specific primers and probes from TaqMan Assay (Applied Biosystems®). Samples that did not amplify β-actin gene were excluded. Real time PCR was performed to RSV protein F gene amplification using specific primers and probes (forward 5′-AACAGATGTAAGCAGCTCCGTTATC-3′, reverse 5′-CGATTTTTATTGGATGCTGTACATTT-3′ and probe 5′-FAM/TGCCATAGCATG ACACAATGGCTCCT-TAMRA/-3′). Data were analyzed by cycle threshold (CT). Delta CT value was calculated from the difference of the CT from the RSV protein F gene to the CT of β-actin gene. Fold-change in mRNA abundance was calculated using $2^{(-\Delta\Delta CT)}$ method (Schmittgen and Livak, 2008).

2.9. Statistical analysis

ANOVA test followed by a Bonferroni post-hoc test and *t*-test were applied to parametric data using Graph Pad Prism software (San Diego, CA, USA). Values demonstrated in graph are mean ± standard deviation and a level of significance of $p < 0.05$ was established for the analyses.

3. Results

3.1. Rapamycin treatment on BMDC decreases the frequency of $CD8^+$-$CD44^{high}$ T cells

In order to evaluate if mTOR inhibition on BMDC affects CD8 T cells differentiation during RSV infection, BMDC were treated with 20 ηg/mL of rapamycin before RSV infection and co-cultured with purified T cells. Our results demonstrated that rapamycin treatment on RSV-infected BMDC significantly decreased the frequency of $CD8^+CD44^{high}$ T cells (Fig. 1). Therefore, these data suggest that mTOR on DC is required to CD8 T cell differentiation during RSV infection, since its inhibition by rapamycin decreases the frequency of CD8 T cells with memory phenotype.

Rapamycin is known to diminish murine BMDC maturation, decreasing the expression of CD86, CD40 and MHC class II when these cells are treated during the differentiation process (Hackstein et al., 2003). We hypothesized that rapamycin treatment on RSV-infected BMDC diminishes $CD8^+CD44^{high}$ T cells frequency due to an impairment of BMDC activation. BMDC were differentiated during 6 days, treated with rapamycin, infected with RSV and after 96 h stained to evaluate the maturation markers by flow cytometry. We found that the percentage of $CD11c^+$ cells remains the same with the rapamycin treatment (Fig. 2A and B). In addition, the percentage of $CD11c^+$-$CD86^+MHCII^+$ cells is similar between the RSV-infected BMDC treated or not with rapamycin (Fig. 2C). These data suggest that rapamycin treatment on RSV-infected BMDC, in our conditions, does not affect the activation of BMDC since the expression of co-stimulatory molecule CD86 is preserved.

3.2. Rapamycin treatment on RSV-infected BMDC increases the RSV-RNA levels

We aimed to investigate whether rapamycin treatment affects RSV replication on BMDC and, consequently, the CD8 T cell differentiation. In order to evaluate that, RSV-RNA levels of infected rapamycin treated BMDC were analyzed by real time PCR after 96 h of infection. Rapamycin treated RSV-infected BMDC presented a significant 9-fold increase on RSV Protein F levels (Fig. 3).

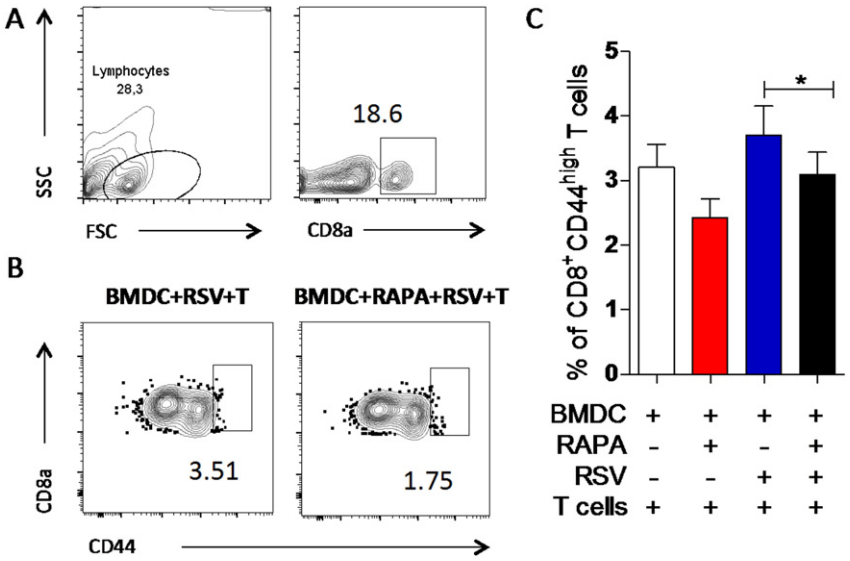


Fig. 1. mTOR inhibition by rapamycin on RSV-infected dendritic cells decreased CD8 T cells differentiation. Murine Bone-marrow-derived dendritic cells (BMDC) were treated or not with 20 ng/mL of rapamycin and infected with 10^2 PFU of RSV and co-culture with T cells during 96 h. Cells were stained with anti-CD8 and anti-CD44 antibodies and were evaluated by flow cytometry. (A) Gate strategy used for flow cytometry analysis of $CD8^+$ T cells population. (B) Dot plots showing the frequency of $CD8^+CD44^{high}$ T cells. (C) Mean frequency of $CD8^+CD44^{high}$ T cells (*$p < 0.05$, one-way ANOVA followed by Bonferroni post-test). The values represent mean ± SD from three independent experiments carried out separately in triplicate.

3.3. Rapamycin decreases apoptosis of RSV-infected BMDC depending on T cell contact

Some viruses can have a negative influence on DC, causing their apoptosis, which prevents active presentation of foreign antigens and T cell activation (Kubicka-sierszen and Grzegorczyk, 2015). Given that rapamycin treatment increases RSV-RNA levels on BMDC, we next asked whether BMDC survival is affected. Accordingly, apoptosis cell death of RSV-infected BMDC treated with rapamycin was evaluated. Our results showed that rapamycin treatment had no effect on the survival of RSV-infected BMDC culture without T cells during 96 h (Fig. 4A). However, when uninfected BMDC and T cells were co-cultured, the rapamycin treatment on BMDC increased the apoptosis of these cells (Fig. 4B). On the contrary, when RSV-infected BMDC and T cells were co-cultured, the rapamycin treatment decreased the apoptosis of BMDC (Fig. 4B). Our results clearly demonstrated that the modulation of rapamycin treatment on BMDC survival was dependent on the presence of T cells in the culture. T cells mediate DC survival secreting soluble factors or interacting with DC by direct cell-to-cell contact (Chen and Wang, 2011). In order to evaluate whether T cells were increasing the survival of rapamycin-treated BMDC by cell-to-cell interaction, BMDC and T cells were co-cultured in a transwell system that prevents cell

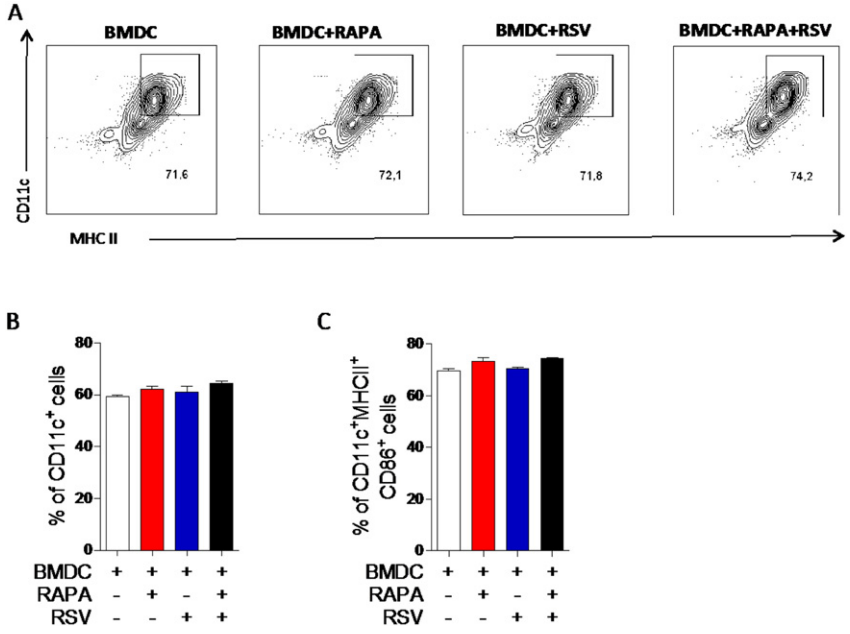


Fig. 2. mTOR inhibition by rapamycin on RSV-infected dendritic cells did not affect the activation of DC. Murine Bone-marrow-derived dendritic cells (BMDC) were treated or not with 20 ng/mL of rapamycin and infected with 10^2 PFU of RSV during 96 h. BMDC were stained with anti-CD11c, anti-CD86 and anti-MHCII. (A) Dot-plots showing the frequency of $CD11c^+MHCII^+CD86^+$ cells. (B) Mean frequency of $CD11c^+$ cells. (C) Mean frequency of $CD11c^+MHCII^+CD86^+$ cells. The values represent mean ± SD from four independent experiments carried out separately in triplicate. The significance of differences between groups was compared using one-way ANOVA followed by Bonferroni post-test.

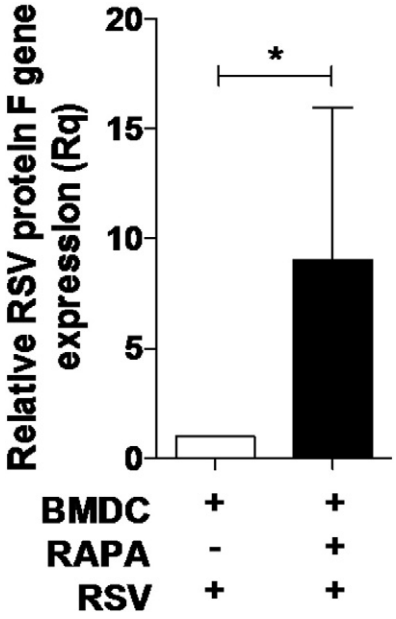


Fig. 3. Rapamycin increases RSV-RNA levels on RSV-infected BMDC. RNA was extracted from RSV-infected bone-marrow derived dendritic cells (BMDC) treated or not with 20 ng/mL of rapamycin after 96 h of infection. RSV protein F gene was amplified with RSV specific primers and probe by real-time PCR. Graph shows the increased-fold of RSV Protein F gene expression on rapamycin treated RSV-infected cells over the RSV infected cells. The values represent mean ± SD from three independent experiments carried out separately in triplicate. The significance of differences between groups was compared using *t*-test (*$p < 0.05$).

contact or migration, but allow the passage of soluble factors. On one hand, when uninfected BMDC and T cells were co-cultured in the transwell system, rapamycin treatment continued to increase the apoptosis of BMDC (Fig. 4C). These data show that this effect occurs in a mechanism independent of T cell contact; probably involving soluble factors secreted by T cells. On the other hand, when RSV-infected BMDC and T cells were co-cultured in the transwell system, the effect of rapamycin treatment on BMDC apoptosis was abolished, displaying a mechanism depending on T cell contact (Fig. 4C). Rapamycin treatment on RSV-infected BMDC had no significant effect on the necrosis/late apoptosis. However, rapamycin treatment on uninfected BMDC increased the percentage of Annexin V^+ PI^+ cells in a mechanism dependent on T cell contact (Supplementary Fig. 1).

4. Discussion

Rapamycin is a drug with many described effects (Li et al., 2014). Besides the anti-fungal and immunosuppressive activity, rapamycin and other mTOR inhibitors present anti-cancer activity in both mice and humans (Meng and Zheng, 2015). mTOR inhibition ameliorates cardiovascular hypertrophy (Xu and Brink, 2016), but chronic rapamycin treatment causes glucose intolerance and hyperlipidemia (Houde et al., 2010). Rapamycin is also described to extend life span in mammals (Ehninger et al., 2014). Furthermore, mTOR inhibition may ameliorate some neurological disorders such as Huntington's, Alzheimer's and Parkinson's disease (Maiese, 2015). Also, rapamycin has been associated with the virus replication control (Brennan et al., 2013). Finally, rapamycin treatment can regulate T cell activation, differentiation and function (Pollizzi and Powell, 2015). In order to better recognize all the potential beneficial and toxic effects of rapamycin treatment, a complete understanding of mTOR activity is important.

Here we investigated rapamycin effects on murine DC during RSV infection. DC are important antigen presenting cells, which recognize, process and present antigen to naïve T cells and regulate the immune response (Banchereau et al., 2000; Condon et al., 2011). During this process DC maturated increasing expression of surface molecules, such as MHC II (Major Histocompatibility Complex class II), co-stimulatory molecules (CD40, CD80 and CD86) and secreting cytokine like IL-12 and interferon type I and III (Banchereau et al., 2000; Banchereau and Steinman, 1998). RSV virus is able to infect and replicate within DC (de Graaff et al., 2005). We found that rapamycin treatment on RSV-infected BMDC slight decreases the frequency of $CD8^+$ $CD44^{high}$ T cells. The role of mTOR inhibition by rapamycin on DC capacity to differentiate T cells is still a contradictory topic. In line with our results, other studies showed that rapamycin-conditioned LPS-stimulated murine and human DC remained poor T-cell differentiators (Turnquist et al., 2010) and these data are similar in human Poly I:C-stimulated monocytes DC (Fekete et al., 2014). In contrast, therapeutic autologous vaccination using murine DC treated with TLR agonists plus rapamycin results in improved CD8 T cells response (Amiel et al., 2012). It is well established that RSV infection on murine DC can impair their function,

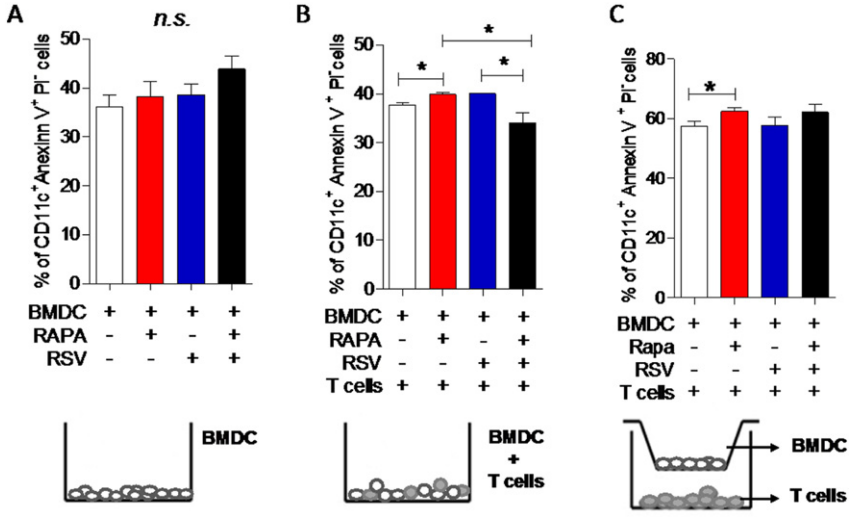
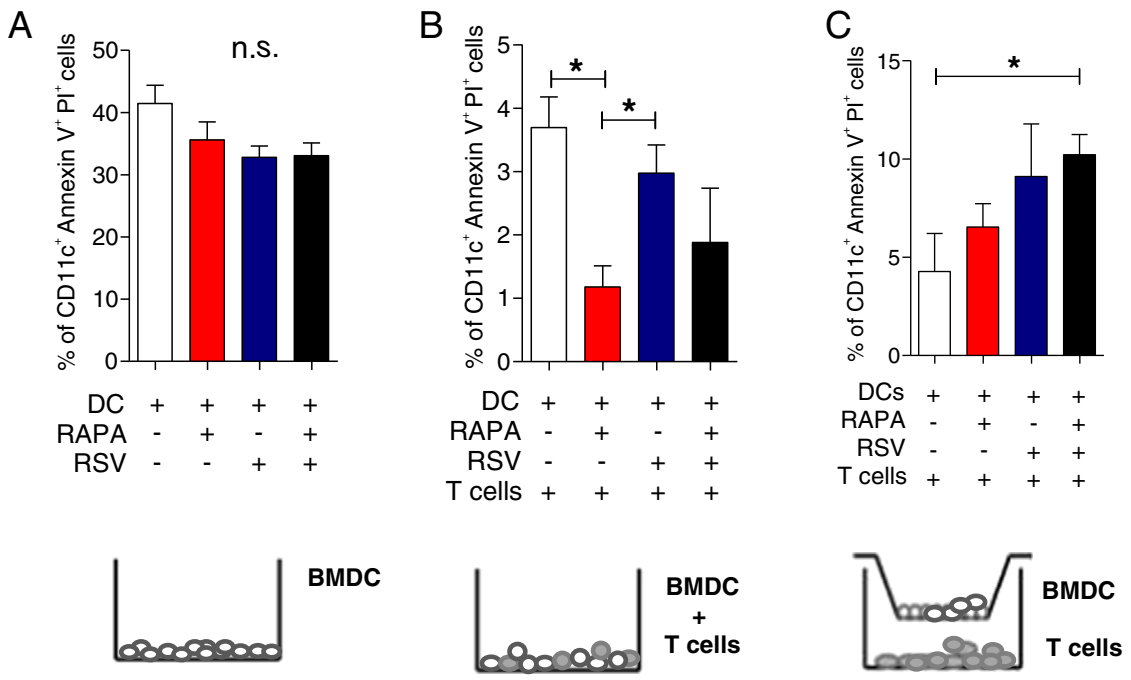


Fig. 4. Rapamycin treatment increases the survival of RSV-infected BMDC dependent on T cell contact. Murine bone-marrow-derived dendritic cells (BMDC) were treated or not with 20 ng/mL of rapamycin and infected with 10^2 PFU of RSV during 96 h. (A) Mean frequency of $CD11c^+$ $AnnexinV^+$ PI^- cells. (B) In the next experiment BMDC in the same conditions were co-cultured with purified T cells. (C) Alternatively, BMDC were co-cultured with T cells in transwell system to avoid direct contact between cells. The values represent mean ± SD from three independent experiments carried out separately in triplicate. The significance of differences between groups was compared using *t*-test one-way ANOVA followed by Bonferroni post-test (*$p < 0.05$, n.s no significance).



Supplementary Fig. 1. Rapamycin treatment increases the survival of RSV-infected BMDC dependent on T cell contact. Murine bone-marrow-derived dendritic cells (BMDC) were treated or not with 20 ng/mL of rapamycin and infected with 10^2 PFU of RSV during 96 h. (A) Mean frequency of $CD11c^+AnnexinV^+PI^+$ cells. (B) In the next experiment BMDC in the same conditions were co-cultured with purified T cells. (C) Alternatively, BMDC were co-cultured with T cells in transwell system to avoid direct contact between cells. The values represent mean ± SD from three independent experiments carried out separately in triplicate. The significance of differences between groups was compared using *t*-test one-way ANOVA followed by Bonferroni post-test (*$p < 0.05$, n.s no significance).

preventing specific T cells response (Openshaw and Chiu, 2013). Here we described that during RSV infection rapamycin treatment on murine DC partially impair their capability to differentiate CD8 T cells, not by decreasing the co-stimulatory molecules but by increasing virus replication.

In accordance with our observation, rapamycin treatment increased Hepatitis E virus (Zhou et al., 2014), Bluetongue virus (Lv et al., 2015) and Herpes simplex virus-1 (Kobayashi et al., 2012) replication. However, other studies have shown that rapamycin may act as an antiviral drug, such as in HIV infection (Heredia et al., 2015). Interestingly, on one hand rapamycin led to a reduction in the ability of B cell lines to undergo Epstein-Barr virus lytic replication. On the other hand, EBV-positive epithelial cell lines undergo higher levels of lytic replication when treated with rapamycin (Adamson et al., 2014). Taken together these data suggest that rapamycin effect on virus replication depends on the virus and cell type used. Our study showed that rapamycin increases virus replication on RSV-infected DC. The effect of rapamycin on RSV replication in lung epithelial cells still needs to be determined.

Rapamycin treatment increases the apoptosis of BMDC when uninfected BMDC and T cells were co-cultured in a mechanism independent of T cell contact; probably involving soluble factors secreted by T cells. Cytokines have been described to decrease the survival of DC, such as IL-10 (Chang et al., 2007) and TGF-β (Ito et al., 2006). IL-10 promote cell death in DC by down-regulating anti-apoptotic molecules, Bcl-2 and Bcl-xL (Chang et al., 2007). In contrast, we show that rapamycin treatment decreased the apoptosis of BMDC when RSV-infected BMDC and T cells were co-cultured, in a mechanism depending on T cell contact. This increase on the BMDC survival might allow the more RSV replication. T cells have been shown to inhibit murine DC apoptosis by direct contact via activation of NF-κβ and FOXO1 (Riol-Blanco et al., 2009). The survival of DC needs to be thoroughly regulated to maintain a balanced and functional immune system. Augment in the survival of DC might be deleterious, for example, disruption of apoptosis in murine DC leads to systemic autoimmunity (Chen et al., 2006; Ohnmacht et al., 2009).

In conclusion, our data demonstrate that rapamycin treatment on murine BMDC during RSV infection present a toxic effect increasing virus replication on these cells and consequently partially impairing their capability to differentiate CD8 T cell. In addition, we show that rapamycin modulates the survival of DC during RSV infection depending on T cell contact. These data contribute to better understand the role of mTOR on DC and future application of rapamycin.

The following is the supplementary data related to this article.

Acknowledgment

This study was supported by Conselho Nacional de Pesquisa (CNPq) (grant number 477359/2013-2). Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS) (grant number 001884-25.51/13.4). Freitas, D. N received master scholarship from CNPq.

References

Adamson, A.L., Le, B.T., Siedenburg, B.D., 2014. Inhibition of mTORC1 inhibits lytic replication of Epstein-Barr virus in a cell-type specific manner. Virol. J. 11, 110. http://dx.doi.org/10.1186/1743-422X-11-110.

Amiel, E., Everts, B., Freitas, T.C., King, I.L., Curtis, J.D., Pearce, E.L.E.J., et al., 2012. Inhibition of mechanistic target of rapamycin promotes dendritic cell activation and enhances therapeutic autologous vaccination in mice. J. Immunol. 189, 2151–2158. http://dx.doi.org/10.4049/jimmunol.1103741.

Anderson, L.J., Dormitzer, P.R., Nokes, D.J., Rappuoli, R., Roca, A., Graham, B.S., 2013. Strategic priorities for respiratory syncytial virus (RSV) vaccine development. Vaccine 31 (Suppl. 2), B209–B215. http://dx.doi.org/10.1016/j.vaccine.2012.11.106.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252. http://dx.doi.org/10.1038/32588.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.J., et al., 2000. Immunobiology of dendritic cells. Annu. Rev. Immunol. 18, 767–811. http://dx.doi.org/10.1146/annurev.immunol.18.1.767.

Brennan, D.C., Aguado, J.M., Potena, L., Jardine, A.G., Legendre, C., Säemann, M.D., et al., 2013. Effect of maintenance immunosuppressive drugs on virus pathobiology: evidence and potential mechanisms. Rev. Med. Virol. 23, 97–125. http://dx.doi.org/10.1002/rmv.1733.

Bueno, S.M., González, P.A., Cautivo, K.M., Mora, J.E., Leiva, E.D., Tobar, H.E., et al., 2008. Protective T cell immunity against respiratory syncytial virus is efficiently induced

by recombinant BCG. Proc. Natl. Acad. Sci. U. S. A. 105, 20822–20827. http://dx.doi.org/10.1073/pnas.0806244105.
Chang, W.L.W., Baumgarth, N., Eberhardt, M.K., Lee, C.Y.D., Baron, C.A., Gregg, J.P., et al., 2007. Exposure of myeloid dendritic cells to exogenous or endogenous IL-10 during maturation determines their longevity. J. Immunol. 178, 7794–7804. http://dx.doi.org/10.4049/jimmunol.178.12.7794.
Chen, M., Wang, J., 2011. Regulation of immune responses by spontaneous and T cell-mediated dendritic cell death. J. Clin. Cell. Immunol. S3. http://dx.doi.org/10.4172/2155-9899.S3-005.
Chen, M., Wang, Y.-H., Wang, Y., Huang, L., Sandoval, H., Liu, Y.-J., et al., 2006. Dendritic cell apoptosis in the maintenance of immune tolerance. Science 311, 1160–1164. http://dx.doi.org/10.1126/science.1122545.
Condon, T.V., Sawyer, R.T., Fenton, M.J., Riches, D.W., 2011. Lung dendritic cells at the innate-adaptive immune interface. J. Leukoc. Biol. 90, 883–895. http://dx.doi.org/10.1189/jlb.0311134.
Cormier, S.A., Shrestha, B., Saravia, J., Lee, G.I., Shen, L., DeVincenzo, J.P., et al., 2014. Limited type I interferons and plasmacytoid dendritic cells during neonatal respiratory syncytial virus infection permit immunopathogenesis upon reinfection. J. Virol. 88, 9350–9360. http://dx.doi.org/10.1128/JVI.00818-14.
de Graaff, P.M.A., de Jong, E.C., van Capel, T.M., van Dijk, M.E.A., Roholl, P.J.M., Boes, J., et al., 2005. Respiratory syncytial virus infection of monocyte-derived dendritic cells decreases their capacity to activate CD4 T cells. J. Immunol. 175, 5904–5911. http://dx.doi.org/10.4049/jimmunol.175.9.5904.
de Souza, A.P.D., de Freitas, D.N., Antuntes Fernandes, K.E., D'Avila da Cunha, M., Antunes Fernandes, J.L., Benetti Gassen, R., et al., 2016. Respiratory syncytial virus induces phosphorylation of mTOR at ser2448 in CD8 T cells from nasal washes of infected infants. Clin. Exp. Immunol. 183, 248–257. http://dx.doi.org/10.1111/cei.12720.
Ehninger, D., Neff, F., Xie, K., 2014. Longevity, aging and rapamycin. Cell. Mol. Life Sci. 71, 4325–4346. http://dx.doi.org/10.1007/s00018-014-1677-1.
F. the A.A. of Pediatrics, 2009. Policy statements-Modified reccomendations for use of palivizumab for prevention of respiratory syncytial virus infections. Pediatrics 124, 1694–1701.
Fekete, T., Pazmandi, K., Szabo, A., Bacsi, A., Koncz, G., Rajnavölgyi, E., 2014. The antiviral immune response in human conventional dendritic cells is controlled by the mammalian target of rapamycin. J. Leukoc. Biol. 96, 579–589. http://dx.doi.org/10.1189/jlb.2A0114-048RR.
González, P.a., Prado, C.E., Leiva, E.D., Carreño, L.J., Bueno, S.M., Riedel, C.a., et al., 2008. Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. Proc. Natl. Acad. Sci. U. S. A. 105, 14999–15004. http://dx.doi.org/10.1073/pnas.0802555105.
Green, C.A., Scarselli, E., Sande, C.J., Thompson, A.J., de Lara, C.M., Taylor, K.S., et al., 2015a. Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci. Transl. Med. 7, 300ra126. http://dx.doi.org/10.1126/scitranslmed.aac5745.
Green, C.A., Scarselli, E., Voysey, M., Capone, S., Vitelli, A., Nicosia, A., et al., 2015b. Safety and immunogenicity of novel respiratory syncytial virus (RSV) vaccines based on the RSV viral proteins F, N and M2-1 encoded by simian adenovirus (PanAd3-RSV) and MVA (MVA-RSV); protocol for an open-label, dose-escalation, single-centre, phase 1 cl. BMJ Open. 5, e008748. http://dx.doi.org/10.1136/bmjopen-2015-008748.
Gupta, M.R., Kolli, D., Garofalo, R.P., 2013. Differential response of BDCA-1+ and BDCA-3+ myeloid dendritic cells to respiratory syncytial virus infection. Respir. Res. 14, 71. http://dx.doi.org/10.1186/1465-9921-14-71.
Hackstein, H., Taner, T., Logar, A.J., Thomson, A.W., 2002. Rapamycin inhibits macropinocytosis and mannose receptor-mediated endocytosis by bone marrow-derived dendritic cells. Blood 100, 1084–1087 (http://www.ncbi.nlm.nih.gov/pubmed/12130531 (accessed January 26, 2016).
Hackstein, H., Taner, T., Zahorchak, A.F., Morelli, A.E., Logar, A.J., Gessner, A., et al., 2003. Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. Blood 101, 4457–4463. http://dx.doi.org/10.1182/blood-2002-11-3370.
Hall, C.B., Weinberg, G.a., Iwane, M.K., Blumkin, A.K., Edwards, K.M., Staat, M.a., et al., 2009. The burden of respiratory syncytial virus infection in young children. N. Engl. J. Med. 360, 588–598. http://dx.doi.org/10.1056/NEJMoa0804877.
Hasty, P., 2010. Rapamycin: the cure for all that ails. J. Mol. Cell Biol. 2, 17–19. http://dx.doi.org/10.1093/jmcb/mjp033.
Heredia, A., Le, N., Gartenhaus, R.B., Sausville, E., Medina-Moreno, S., Zapata, J.C., et al., 2015. Targeting of mTOR catalytic site inhibits multiple steps of the HIV-1 lifecycle and suppresses HIV-1 viremia in humanized mice. Proc. Natl. Acad. Sci. U. S. A. 112, 9412–9417. http://dx.doi.org/10.1073/pnas.1511144112.
Houde, V.P., Brûlé, S., Festuccia, W.T., Blanchard, P.-G., Bellmann, K., Deshaies, Y., et al., 2010. Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes 59, 1338–1348. http://dx.doi.org/10.2337/db09-1324.
Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., et al., 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 176, 1693–1702. http://dx.doi.org/10.1084/jem.176.6.1693.
Ito, M., Minamiya, Y., Kawai, H., Saito, S., Saito, H., Nakagawa, T., et al., 2006. Tumor-derived TGFbeta-1 induces dendritic cell apoptosis in the sentinel lymph node. J. Immunol. 176, 5637–5643 (http://www.ncbi.nlm.nih.gov/pubmed/16622033 (accessed February 22, 2016).
Kobayashi, M., Wilson, A.C., Chao, M.V., Mohr, I., 2012. Control of viral latency in neurons by axonal mTOR signaling and the 4E-BP translation repressor. Genes Dev. 26, 1527–1532. http://dx.doi.org/10.1101/gad.190157.112.
Kubicka-sierszen, A., Grzegorczyk, J.Ł., 2015. State of the art paper. The Influence of Infectious Factors on Dendritic Cell Apoptosis http://dx.doi.org/10.5114/aoms.2015.54860.
Li, J., Kim, S.G., Blenis, J., 2014. Rapamycin: one drug, many effects. Cell Metab. 19, 373–379. http://dx.doi.org/10.1016/j.cmet.2014.01.001.
Lv, S., Xu, Q., Sun, E., Yang, T., Li, J., Feng, Y., et al., 2015. Autophagy activated by Bluetongue virus infection plays a positive role in its replication. Viruses 7, 4657–4675. http://dx.doi.org/10.3390/v7082838.
Maiese, K., 2015. Targeting molecules to medicine with mTOR, autophagy, and neurodegenerative disorders. Br. J. Clin. Pharmacol. http://dx.doi.org/10.1111/bcp.12804.
Meng, L.-H., Zheng, X.S., 2015. Toward rapamycin analog (rapalog)-based precision cancer therapy. Acta Pharmacol. Sin. 36, 1163–1169. http://dx.doi.org/10.1038/aps.2015.68.
Nair, H., Nokes, D.J., Gessner, B.D., Dherani, M., Madhi, S.A., Singleton, R.J., et al., 2010. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 375, 1545–1555. http://dx.doi.org/10.1016/S0140-6736(10)60206-1.
Ohnmacht, C., Pullner, A., King, S.B.S., Drexler, I., Meier, S., Brocker, T., et al., 2009. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. J. Exp. Med. 206, 549–559. http://dx.doi.org/10.1084/jem.20082394.
Openshaw, P.J., Chiu, C., 2013. Protective and dysregulated T cell immunity in RSV infection. Curr. Opin. Virol. 3, 468–474. http://dx.doi.org/10.1016/j.coviro.2013.05.005.
Pollizzi, K.N., Powell, J.D., 2015. Regulation of T cells by mTOR: the known knowns and the known unknowns. Trends Immunol. 36, 13–20. http://dx.doi.org/10.1016/j.it.2014.11.005.
Ptaschinski, C., Mukherjee, S., Moore, M.L., Albert, M., Helin, K., Kunkel, S.L., et al., 2015. RSV-induced H3K4 demethylase KDM5B leads to regulation of dendritic cell-derived innate cytokines and exacerbates pathogenesis in vivo. PLoS Pathog. 11, e1004978. http://dx.doi.org/10.1371/journal.ppat.1004978.
Riol-Blanco, L., Delgado-Martín, C., Sánchez-Sánchez, N., Alonso-C, L.M., Gutiérrez-López, M.D., Del Hoyo, G.M., et al., 2009. Immunological synapse formation inhibits, via NF-kappaB and FOXO1, the apoptosis of dendritic cells. Nat. Immunol. 10, 753–760. http://dx.doi.org/10.1038/ni.1750.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108. http://dx.doi.org/10.1038/nprot.2008.73.
Turnquist, H.R., Cardinal, J., Macedo, C., Rosborough, B.R., Sumpter, T.L., Geller, D.A., et al., 2010. mTOR and GSK-3 shape the CD4+ T-cell stimulatory and differentiation capacity of myeloid DCs after exposure to LPS. Blood 115, 4758–4769. http://dx.doi.org/10.1182/blood-2009-10-251488.
Weng, K., Zhang, J., Mei, X., Wu, A., Zhang, B., Cai, M., et al., 2014. Lower number of plasmacytoid dendritic cells in peripheral blood of children with bronchiolitis following respiratory syncytial virus infection. Influenza Other Respir. Viruses 8, 469–473. http://dx.doi.org/10.1111/irv.12242.
Wullschleger, S., Loewith, R., Hall, M.N., 2006. TOR signaling in growth and metabolism. Cell 124, 471–484. http://dx.doi.org/10.1016/j.cell.2006.01.016.
Xu, L., Brink, M., 2016. mTOR, cardiomyocytes and inflammation in cardiac hypertrophy. Biochim. Biophys. Acta http://dx.doi.org/10.1016/j.bbamcr.2016.01.003.
Zhou, X., Wang, Y., Janssen, H.L.A., Van der laan, L.J.W., Metselaar, H.J., Peppelenbosch, M.P., et al., 2014. O160 rapamycin and everolimus facilitate hepatitis e virus replication revealing a hepatocyte-specific PI3K–AKT–mTOR-dependent defense mechanism. J. Hepatol. 60, S66. http://dx.doi.org/10.1016/S0168-8278(14)60162-8.