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Differential *in vitro* effect of endogenous and exogenous nitric oxide on replication of the persistent respiratory syncytial virus genome

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Summary. – Synthesis of nitric oxide (NO) is induced as an early response to viral challenges. Here, we studied effects of endogenous and exogenous NO on respiratory syncytial virus (RSV) genome replication, using a persistently RSV infected macrophage-like cell line. NO was evaluated indirectly by nitrites accumulation and it was increased in infected macrophages with respect to non-infected cells. Phagocytosis of bacteria by persistently RSV infected macrophages increased nitrites production, and under such conditions the number of RSV-genome copies decreased up to 8.7-fold, whereas chemical inhibition of the inducible-NO synthase enzyme increased viral replication 2.7-fold. Since phagocytosis activates many signaling pathways, which could contribute to viral control, we explored the individual effect of NO by using the NO donor SNAP. Intriguingly, even though SNAP raised nitrites levels up to 3-fold, the number of RSV genome copies augmented 2.3-fold. This enhancement was associated with lengthening of the G0/G1 cell cycle phase mediated by the NO donor, as evaluated by BrdU/7-AAD incorporation through flow cytometry; this phase of the cell cycle was favorable for an increased RSV genome replication. Thus, NO produced endogenously during RSV persistence was not enough to control virus replication, although macrophage activation through phagocytosis inhibited replication of the persistent viral genome. In contrast, the NO donor SNAP increased viral genome replication, at least partially by altering the cell cycle, indicating that both sources of NO were not bioequivalent.

Keywords: cell cycle; endogenous nitric oxide; exogenous nitric oxide; nitric oxide donor; respiratory syncytial virus; viral persistence

Introduction

Nitric oxide (NO) is a free radical with antiviral properties, although it also affects cell proliferation by inducing expression of cell cycle-associated genes, such as the transcription factor E2F1 and the cyclin-dependent kinase inhibitor p21, avoiding cell cycle progression, at least from the G1 phase up to the S phase (Cui *et al*., 2005; Villalobo, 2006). The role of NO as an antiviral agent has been assessed by its capacity to reduce viral genome replication and such an effect has been observed during infection with viruses belonging to a variety of families (e.g. *Arenaviridae*, *Coronaviridae* and *Orthomyxoviridae*) (Åkerström *et al*., 2009; Pozner *et al*., 2008; Rimmelzwaan *et al*., 1999). NO synthesis has also been observed during acute RSV infection of epithelial cells, interfering with virus production (Ali-Ahmad *et al*., 2003); however, effect of NO during RSV persistence is still largely unknown. RSV is an enveloped, negative-sense RNA virus from the *Pneumoviridae* family and is the most important cause of bronchiolitis and pneumonia in children younger than 5 years (Nair *et al*., 2010). In a high percentage of infants hospitalized because of severe RSV disease (up to 40%), asthma-like symptoms are recurrent in the first decade of life (Cassi-

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Abbreviations: iNOS = inducible-NO synthase; NO = nitric oxide; NTHi = non-typeable *Haemophilus influenzae*; RSV = respiratory syncytial virus; SNAP = NO donor

mos *et al*., 2008; Sigurs *et al*., 2000; Zomer-Kooijker *et al*., 2014) and there exists circumstantial evidence suggesting that RSV can establish persistent infections in humans, which could be at least one mechanism associated with such respiratory sequelae (Mejías *et al*., 2005; Piedimonte and Perez, 2014; Wu and Hartert, 2011). Animal models and cell lines support the premise that RSV can persist in its hosts (Bramley *et al*., 1999; Martínez *et al*., 2009; Sarmiento *et al*., 2002; Schwarze *et al*., 2004). A comprehensive analysis of the mechanisms and consequences of persistent RSV infection in the host-cell biology is necessary for a better understanding of RSV pathogenesis.

We previously reported in an *in vitro* model consisting of a mouse macrophage cell line persistently infected with RSV, that NO is produced at lower level than that in acute RSV-infected macrophages. Such deficient NO synthesis was associated with constitutive expression of the enzyme arginase-I along with a preserved enzymatic activity of the inducible-NO synthase (iNOS), implying that arginase-I restricts L-arginine availability as a substrate for iNOS to synthesize NO, contributing to maintenance of the persistent RSV genome in the host cell (Santiago-Olivares *et al*., 2019). Considering that endogenous NO production is low during RSV persistence, its effect on virus genome replication has not been determined. Accordingly, we induced overproduction of both endogenous NO by a phagocytic stimulus and exogenous NO by treatment with a NO donor, and then we evaluated their effects on replication of the persistent viral genome.

Materials and Methods

Cell lines and virus. The mouse macrophage-like cell line P388D1 (ATCC, TIB 63; USA) persistently infected with RSV strain Long (ATCC, VR-26), was established by our group as previously described (Sarmiento *et al*., 2002); it is frequently monitored by direct immunofluorescence and RT-PCR to confirm virus persistence (Gaona *et al*., 2014; Rivera-Toledo *et al*., 2015). This persistently RSV-infected cell line (MΦP) was propagated in cell-culture medium (RPMI, Gibco/BRL, USA) supplemented with 5% fetal bovine serum (Biowest, Mexico), 1% penicillin-streptomycin (Invitro S.A., Mexico) and 1 µM 2-mercaptoethanol (Sigma, USA). Experiments were performed with MΦP from passages 75 to 87, with 90–95% of cells expressing RSV proteins. The original P388D1 cell line was cultured as a non-infected control cell line (MΦN) under conditions similar to MΦP.

Nitrites quantitation and iNOS inhibition. NO synthesis was evaluated indirectly by nitrites production in MΦN and MΦP culture supernatants through the Griess reaction, by using the Griess reagent system (Promega, USA), according to the manufacturer's instructions. Chemical inhibition of iNOS was performed with 200 µM N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma) for 24 h; enzymatic inhibition was evaluated through nitrites production.

Phagocytosis assay and Western blot analysis. The non-typeable *Haemophilus influenzae* (NTHi) strain was provided by Dr. Michael Apicella (University of Iowa, College of Medicine, Department of Microbiology) and cultured as previously described (Campagnari *et al*., 1987). NTHi inactivation was achieved at 70°C, 1 h, in water bath. Then, phagocytosis assay was performed by co-culturing MΦP (1×10^6) and heat-inactivated NTHi (MΦP+NTHi) at a ratio of 1:2 for 24 h in 6-well plates (37°C; 5% CO_2). Nitrites were quantified by Griess reaction in supernatants after time of incubation, and RSV genome copy number was determined from total RNA recovered from the cell monolayer, as described in section *RSV genome quantitation*. Western blot analysis was performed to evaluate iNOS in MΦP+NTHi and MΦP cultures, by using anti-NOS2 antibody (Santa Cruz Biotechnology, USA) and anti-glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH; Santa Cruz Biotechnology, USA) as loading control. Total protein extracts were obtained with the lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS), supplemented with EDTA-free complete protease inhibitor cocktail (Roche, USA).

S-nitroso-N-acetyl-DL-penicillamine treatment and cell viability. MΦP seeded in 6-well plates (1×10^6 /well) were treated for 12 h with 0, 50 and 100 µM NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP; Sigma), prepared in supplemented RPMI. Then, nitrites and the number of RSV genome copies were quantified. Cell viability was analyzed through the tetrazolium reduction assay by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Amresco, USA). MΦP plated in 96-well plates (5×10^4 /well) were treated for 3 h with 200 µl of MTT (0.5 mg/ml in supplemented RPMI). Then, dimethyl sulfoxide was added to solubilize formazan, which was evaluated by measuring absorbance at 570 nm. The percentage of viable cells was calculated as: %viability = [OD (optical density) of SNAP treated cells / OD of control cells] ×100.

RSV genome quantitation. Total RNA was extracted with the Hybrid-R miniprep kit (GeneAll Biotechnology, Korea), according to the manufacturer's instructions. RSV genome copies were measured from total RNA (100 ng/reaction) through one-step quantitative RT-PCR (qRT-PCR) by using the Genesig standard kit for quantification of respiratory syncytial virus type A (Primerdesign Ltd, UK), according to manufacturer's instructions. Samples were analyzed in triplicate in each independent experiment and the number of RSV genome copies was interpolated from the standard curve.

Cell cycle analysis. Cell cycle analysis in MΦN and MΦP treated or untreated with SNAP was performed through flow cytometry by using the APC BrdU flow kit (BD Pharmingen, USA), according to manufacturer's instructions. Acquisition of 15,000 events from each sample was performed in a FACScalibur (BD Pharmingen) and percentages of cells in S, G2/M, and G0/

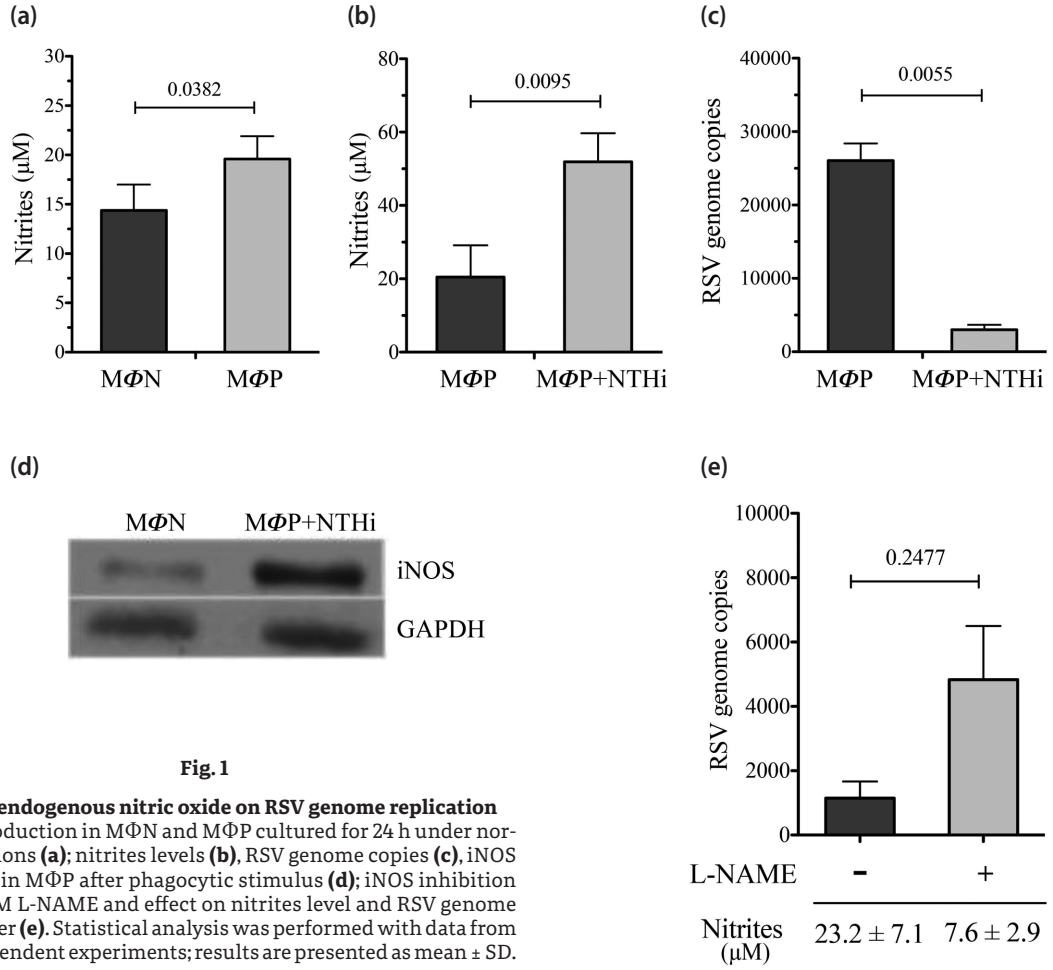

Fig. 1

Effect of endogenous nitric oxide on RSV genome replication
Nitrites production in MΦN and MΦP cultured for 24 h under normal conditions **(a)**; nitrites levels **(b)**, RSV genome copies **(c)**, iNOS expression in MΦP after phagocytic stimulus **(d)**; iNOS inhibition with 200 μM L-NAME and effect on nitrites level and RSV genome copy number **(e)**. Statistical analysis was performed with data from three independent experiments; results are presented as mean ± SD.

G1 were determined according to staining patterns by using the software FCS Express 6 Plus (De Novo Software, USA). For MΦP synchronization in the G0/G1 phase of the cell cycle, cell cultures were incubated for 72 h with RPMI supplemented with 0.2% of fetal bovine serum, instead of 5%.

Statistical analysis. Statistical analysis was performed by using a two-sided, unpaired Student' *t*-test; values of $p < 0.05$ were considered statistically significant.

Results

Endogenous nitric oxide reduced viral genome replication

NO production was evaluated indirectly by the Griess reaction in MΦP and MΦN cultured for 24 h under normal conditions. MΦP and MΦN showed nitrite concentrations of 19.6 ± 1.2 μM and 14.4 ± 1.5 μM, respectively, indicating that chronic RSV infection increased NO synthesis by twenty-five percent ($p = 0.0382$, Fig. 1a). Despite such constitutive, higher NO levels produced by MΦP with respect to MΦN, the RSV genome has remained persistent along cellular passages, suggesting either NO has no antiviral activity against RSV or its concentration was not high enough for viral elimination. To explore such possibilities, we induced overproduction of endogenous NO in MΦP in response to a phagocytic stimulus. Phagocytosis assays were performed by coculturing MΦP and heat inactivated NTHi (MΦP+NTHi) at a ratio of 1:2 for 24 h. Analysis showed 2.5-fold increase in nitrites production (Fig. 1b) after bacterial phagocytosis (20.5 ±5.0 μM *vs.* 51.9±4.5 μM in MΦP and MΦP+NTHi, respectively), besides augmented expression of iNOS enzyme as observed by Western blot analysis (Fig. 1d). Notably, nitrites overproduction after phagocytic stimulus was accompanied by 8.7-fold decrease in the number of RSV genome copies (Fig. 1c). Further treatment of MΦP with the 200 μM iNOS inhibitor L-NAME for 24 h, reduced constitutive production of nitrites 3.0-fold ($p = 0.06$), whereas the number of

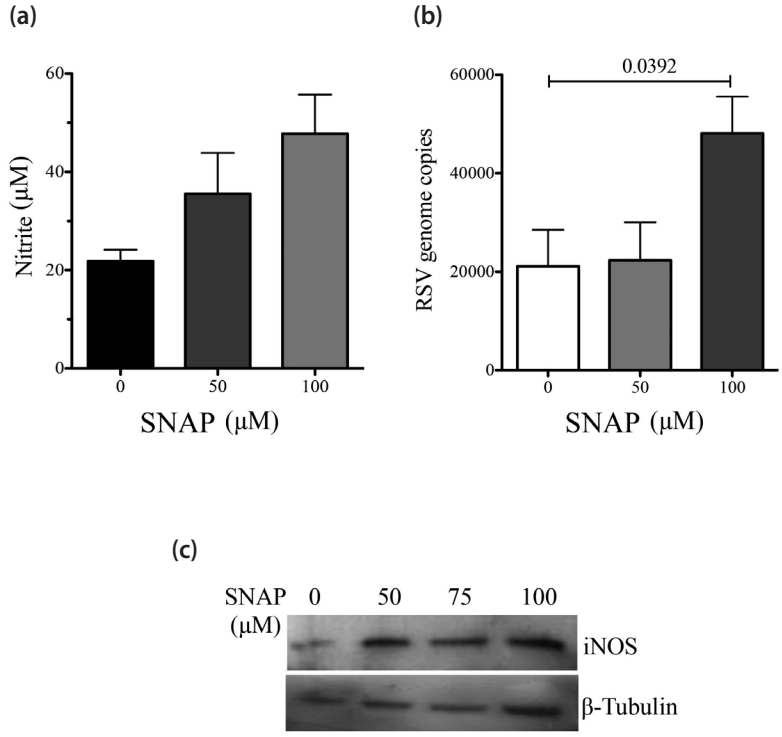

Fig. 2

Effect of exogenous nitric oxide on RSV genome replication

Nitrites **(a)** and RSV genome copies **(b)** were quantified in MΦP treated for 12 h with 0, 50 and 100 μM nitric oxide donor SNAP; four independent experiments were performed. Results are presented as mean ± SD of four independent experiments. Effect of SNAP on iNOS expression was evaluated in MΦP treated with 0, 50, 75 and 100 μM SNAP for 12 h by Western blot analysis **(c)**. Results are representative of three independent experiments.

RSV genome copies showed 4.2-fold increase ($p = 0.24$), suggesting that endogenous NO produced in normal culture conditions controlled at least partially RSV replication; however, no statistical significance was obtained in this enzyme-inhibition assay since results were highly variable between experiments (Fig. 1e). Endogenous NO inhibited RSV genome replication in a concentration dependent manner.

An exogenous source of nitric oxide augmented replication of the persistent RSV genome

It has been reported that phagocytosis does not only induce NO synthesis, but also expression of proinflammatory cytokines such as IL-1, IL-6, TNF-α and IL-8, which could exert in a collective manner antimicrobial effect (Smythies *et al.*, 2005). In order to evaluate a direct antiviral effect of NO, MΦP were treated for 12 h with 0, 50 and 100 μM NO donor SNAP. After treatment, nitrite levels showed a direct correlation with SNAP concentration (Fig. 2a), and at 100 μM concentration we observed similar level of nitrites to that produced in response to phagocytosis of NTHi (Fig. 1b). Also, we observed positive expression of the enzyme iNOS in MΦP treated with SNAP (Fig. 2c), whereas cell viability was 82.6±6.4% and 80.0±6.6 at 50 μM and 100 μM SNAP, respectively. Afterwards, RSV genome copies were quantified and unexpectedly, SNAP did not cause change at 50 μM concentration with respect to mock-treated cells, whereas 100 μM SNAP augmented virus genome copies 2.3-fold, suggesting that the NO donor improved the replication of the persistent RSV genome (Fig. 2b).

Exogenous nitric oxide induced a delay in the G1/S transition associated with improved RSV genome replication

Since NO can block the progression of the cell cycle mainly at the G1/S transition in vascular and myeloid cells (Cui *et al.*, 2005; Napoli *et al.*, 2013), we assessed through flow cytometry whether SNAP was affecting cell cycle progression in MΦP treated with 100 μM SNAP, by using

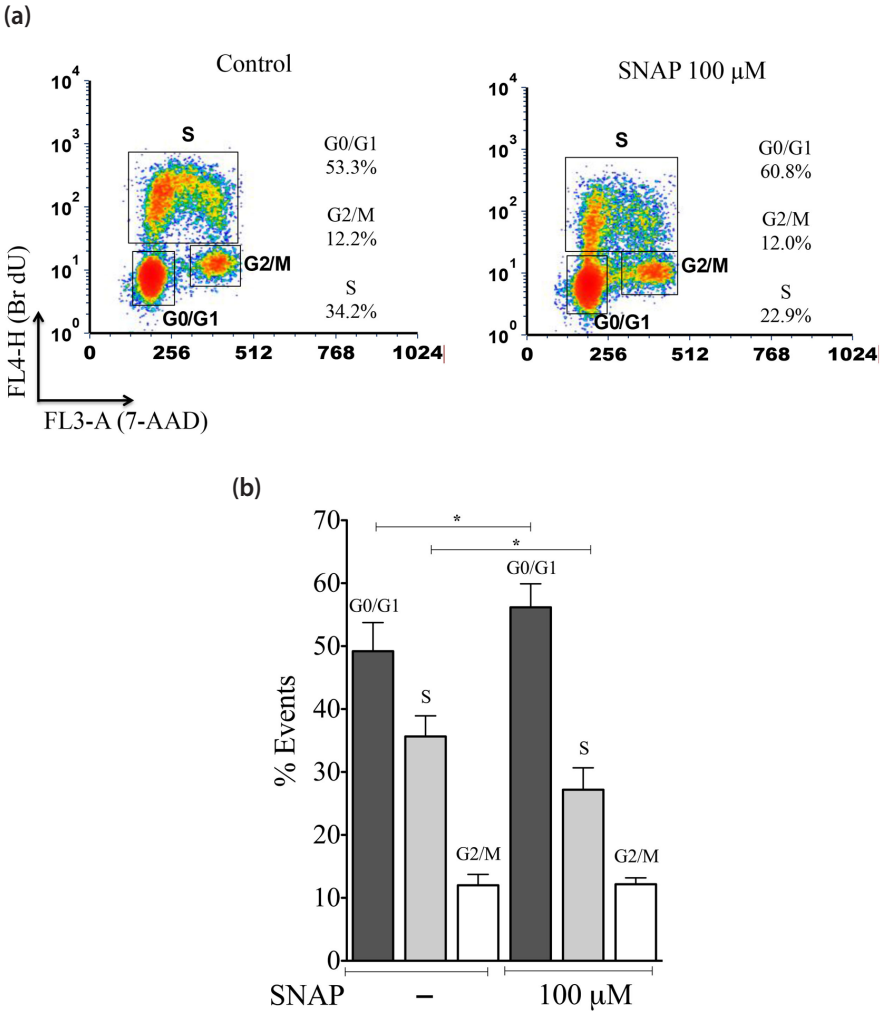

Fig. 3

Exogenous nitric oxide affected G1/S transition of persistently RSV infected macrophages
Representative cell cycle analysis by dot-plots in MΦP treated with 100 μM SNAP, with respect to control cells **(a)**. Percentages of cells in G0/G1, S and G2/M are indicated; bar graph represent the mean ± SD of three independent experiments performed as in a) **(b)**.

BrdU and 7-AAD. A representative experiment was displayed as density-dot plots (Fig. 3a), and average of three independent experiments was represented in a bar graph (Fig. 3b). In standard culture conditions (without SNAP), MΦP cultures showed the following percentages of cells in each cell-cycle phase: 49.2±4.5% in G0/G1, 35.6±3.2% in S and 12.0±1.7% in G2/M. After SNAP treatment, percentage of cells in G0/G1 increased by 13.0%, whereas those in S-phase decreased by 25.0% ($p < 0.05$); cell population in G2/M remained constant. It should be noted that untreated MΦP cultures showed a pattern of cell distribution in S phase, consisting a combination of cells starting DNA synthesis and those with already double content of DNA; in contrast, SNAP changed this pattern to one with higher density of cells recently leaving the G0/G1 phase and starting DNA synthesis, suggesting a delay in the G1/S transition. Thus, if SNAP lengthened the time of MΦP in G0/G1 phase, it might be an advantage for viral genome replication. To prove this hypothesis, we cultured MΦP under fetal bovine serum (FBS) deprivation (with 0.2% FBS) for 72 h, in order to arrest cell culture in the G0/G1 phase (Rosner *et al.*, 2013) and then, the number of RSV genome copies was determined. We observed that FBS starvation significantly increased percentage of cells in G0/G1 phase ($p = 0.04$), whereas percentage of cells in the S phase dramatically decreased ($p = 0.0005$) (Table 1); in contrast, cell population in G2/M phase did not show significant changes. Finally, FBS starvation was associated

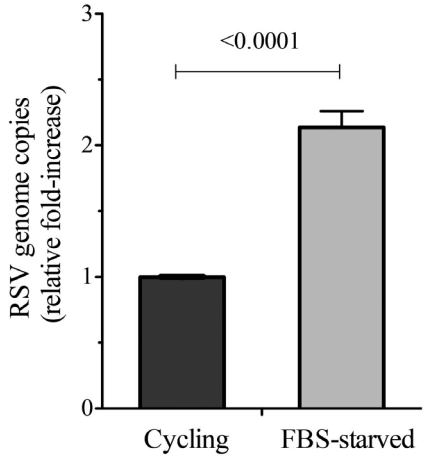

Fig. 4

Synchronization in G0/G1 augmented RSV replication
RSV genome copies were evaluated in MΦP cultured under serum starvation conditions for synchronization in G0/G1 (FBS starved) and in cycling MΦP (cultured with FBS-5%). Viral genome copies were determined as relative fold-increase. Results are presented as mean ± SD from three independent experiments.

Table 1. Increased percentage of cells in G0/G1 phase by effect of serum starvation

Cell cycle phase	Cycling (%)	FBS starved (%)
G0/G1	82.2±2.2	89±3.3
S	10.6±1.4	0.6±0.7
G2/M	5.2±4.6	8.9±3.2

with at least 2-fold increase in the number of RSV genome copies (Fig. 4), suggesting that, persistent RSV genome replicated with higher efficiency when most of cells were in the G0/G1 phase of the cell cycle.

Discussion

Considering that the persistent viral genome has been preserved in MΦP cultures over 80 passages, our results indicated that the NO produced by the host cell is not sufficient to clear the virus. Addition of SNAP was an experimental strategy to expose MΦP to higher levels of NO than that produced endogenously. This strategy has been applied to infections with other RNA viruses resulting in inhibition of virus replication (Åkerström *et al*., 2009; Pozner *et al*., 2008; Rimmelzwaan *et al*., 1999; Simon *et al*., 2006). However, two exceptions have been reported, one with HIV and other with RSV, both describing how exogenous NO increased virus replication (Hobson and Everard, 2008; Jiménez *et al*., 2001). In the former case, cells infected with X4 or X4R5 strains of HIV-1 showed stimulation of virus replication by treatment with SNAP or sodium nitroprusside at concentrations ≤100 μM, apparently by a mechanism involving activation of nuclear factor-κB that, in turn, stimulated long terminal repeat-mediated transcription (Jiménez *et al*., 2001). In the second case, RSV-infection of monocyte-derived dendritic cells was maintained *in vitro* at low-to-undetectable levels for prolonged periods of time; however, exposure to $NO_{(gas)}$, 100 μM SNAP or an inhibitor of the enzyme iNOS, elicited higher levels of RSV replication, implying the NO produced endogenously had antiviral activity, whereas the NO from the exogenous sources had the opposite effect (Hobson and Everard, 2008). In agreement with such report, when we treated MΦP with L-NAME, RSV genome copies showed a tendency to increase (not statistically significant), suggesting that endogenous NO was controlling at least partially the replication of the persistent RSV genome. Although the mechanisms associated with improvement of RSV genome replication by effect of NO donors were not determined by the authors (Hobson and Everard, 2008), they suggested that exogenous NO inhibited iNOS expression in the persistently RSV-infected dendritic cells and as a consequence, low levels of endogenous NO allowed viral replication. NO functions as a negative feedback modulator when it is produced at a threshold level, triggering iNOS down-regulation (Chang *et al*., 2004; Morin *et al*., 1994). It has been described in hepatocytes stimulated with cytokines that iNOS mRNA decreased by treatment with SNAP, in a concentration dependent manner (Chen *et al*., 2001); such effect was observed with SNAP concentrations above 250 μM.

In the present study, iNOS expression was not inhibited in MΦP either by treatment with 100 μM SNAP or by phagocytic stimulus (both conditions generated similar amounts of nitrites), suggesting that NO levels were not high enough to down-regulate the enzyme in RSV-infected macrophages; consequently, threshold or effective NO concentration for iNOS down-regulation might be different in macrophages and dendritic cells. Besides, it has been described that a NO-mediated positive feedback regulation of iNOS in human cells, involving S-nitrosylation of Ras protein, which initiates a kinase cascade that results in Akt phosphorylation along with mammalian target of rapamycin (mTOR) activation and subsequent translation of iNOS mRNA, increasing the enzyme levels (Lee and Choy, 2013). Such paradoxical effect of NO on iNOS expression probably depends on the intracellular concentration of NO, with high levels being inhibitory and lower levels stimulatory (Lee and Choy,

2013). Considering that we observed an increase of iNOS protein in MΦP treated with SNAP, we do not rule out this positive feedback regulation might be active; such hypothesis warrants further research to analyze feedback mechanisms regulating iNOS expression in mouse macrophages (since they are widely used *in vitro* models) and its role on viral replication.

Thereby cellular responses to NO are different depending on whether it is produced endogenously by the iNOS enzyme or exogenously by NO donors, because site of production, dynamics of diffusion through membranes, concentration and interaction with other radicals are determinant in its capacity to triggering cell processes (Gansauge *et al*., 1997; Napoli *et al*., 2013; Nedeianu *et al*., 2004; Shah and MacCarthy, 2000). In order to understand the role of SNAP in RSV genome replication we studied its effect on cell cycle progression, since it has been reported that NO participates in cell survival and proliferation in a concentration dependent manner (Napoli *et al*., 2013). Interestingly, SNAP altered cell cycle progression in MΦP by lengthening the G0/G1 phase, where the RSV genome replicated at higher levels. Previous studies have shown that acute RSV infection induces cell cycle arrest in G0/G1 and G2/M phases in primary human bronchial epithelial cells (PHBE) (Gibbs *et al*., 2009; Wu *et al*., 2011), by degradation of cyclin D1 and cyclin D2, whereas arrest in the G0/G1 phase occurs in A549 cells (Gibbs *et al*., 2009); in both cases, virus progeny was amplified.

Here, we observed during persistent RSV infection that SNAP did not change percentage of cells in the G2/M phase, suggesting this cell-cycle phase may be important for replication of the persistent viral genome, in such a way that it was accurately well preserved. We cannot rule out that exogenous NO had targeted some cellular or viral proteins (e.g., cellular transcription factors or the viral polymerase) inducing on them post-translational modifications (e.g., S-nitrosylation) and altering their function or enzymatic activity to enhance genome replication; nevertheless, S-nitrosylation of viral proteins has been normally associated with inhibition of viral replication (Chaturvedi and Nagar, 2009).

In summary, our work showed that the NO produced endogenously and that released by a NO donor are not bioequivalent, since the former controlled replication of the persistent RSV genome in a concentration-dependent manner, while SNAP produced a paradoxical effect, possibly by inducing a delay in the G1/S transition that consequently improved the RSV genome replication, justifying the use of NO donors to induce RSV replication in samples with suspected RSV persistence (Fonceca *et al*., 2017). Although NO synthesis is down regulated in MΦP during RSV persistence (Santiago-Olivares *et al*., 2019), we showed here that macrophage phagocytosis increases NO production and it was associated with significant drop in virus genome replication.

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References

Åkerström S, Gunalan V, Keng CT, Tan YJ, Mirazimi A (2009): Dual effect of nitric oxide on SARS-CoV replication: Viral RNA production and palmitoylation of the S protein are affected. Virology 395, 1–9. https://doi.org/10.1016/j.virol.2009.09.007

Ali-Ahmad D, Bonville CA, Rosenberg HF, Domachowske JB (2003): Replication of respiratory syncytial virus is inhibited in target cells generating nitric oxide in situ. Frontiers Biosci. 8, a48–a53. https://doi.org/10.2741/986

Bramley AM, Vitalis TZ, Wiggs BR, Hegele RG (1999): Effects of respiratory syncytial virus persistence on airway responsiveness and inflammation in guinea-pigs. Eur. Respir. J. 14, 1061–1067. https://doi.org/10.1183/09031936.99.14510619

Campagnari AA, Gupta MR, Dudas KC, Murphy TF, Apicella MA (1987): Antigenic diversity of lipooligosaccharides of nontypable Haemophilus influenzae. Infect. Immun. 55, 882–887. https://doi.org/10.1128/IAI.55.4.882-887.1987

Cassimos DC, Tsalkidis A, Tripsianis GA, Stogiannidou A, Anthracopoulos M, Ktenidou-Kartali S et al. (2008): Asthma, lung function and sensitization in school children with a history of bronchiolitis. Pediatr. Int. 50, 51–56. https://doi.org/10.1111/j.1442-200X.2007.02509.x

Chang K, Lee SJ, Cheong I, Billiar TR, Chung HT et al. (2004): Nitric oxide suppresses inducible nitric oxide synthase expression by inhibiting post-translational modification of IκB. Exp. Mol. Med. 36, 311–324. https://doi.org/10.1038/emm.2004.42

Chaturvedi UC, Nagar R (2009): Nitric oxide in dengue and dengue haemorrhagic fever: Necessity or nuisance? FEMS Immunol. Med. Microbiol. 56, 9–24. https://doi.org/10.1111/j.1574-695X.2009.00544.x

Chen JX, Berry LC, Tanner M, Chang M, Myers RP et al. (2001): Nitric oxide donors regulate nitric oxide synthase in bovine pulmonary artery endothelium. J. Cell Physiol. 186, 116–123. https://doi.org/10.1002/1097-4652(200101)186:1<116::AID-JCP1005>3.0.CO;2-X

Cui X, Zhang J, Ma P, Myers DE, Goldberg IG, Sittler KJ et al. (2005): cGMP-independent nitric oxide signaling and regulation of the cell cycle. BMC Genomics 6, 151. https://doi.org/10.1186/1471-2164-6-151

Fonceca AM, Chopra A, Levy A, Noakes PS, Poh MWP, Bear NL et al. (2017): Infective respiratory syncytial virus is present in human cord blood samples and most prevalent during winter months. PLoS ONE 12, 1–10. https://doi.org/10.1371/journal.pone.0173738

Gansauge S, Gansauge F, Nussler AK, Rau B, Poch B, Schoenberg MH et al. (1997): Exogenous, but not endogenous, nitric oxide increases proliferation rates in senescent human fibroblasts. FEBS Letters 410, 160–164. https://doi.org/10.1016/S0014-5793(97)00544-9

Gaona J, Santiago-Olivares C, Ortega E, Gomez B (2014): Respiratory syncytial virus persistence in macrophages upregulates fc-gamma receptors expression. Viruses 6, 624–639. https://doi.org/10.3390/v6020624

Gibbs JD, Ornoff DM, Igo HA, Zeng JY, Imani F (2009): Cell cycle arrest by transforming growth factor beta1 enhances replication of respiratory syncytial virus in lung epithelial cells. J. Virol. 83, 12424–12431. https://doi.org/10.1128/JVI.00806-09

Hobson L, Everard ML (2008): Persistence of respiratory syncytial virus in human dendritic cells and influence of nitric oxide. Clin. Exp. Immunol. 151, 359–366. https://doi.org/10.1111/j.1365-2249.2007.03560.x

Jiménez JL, González-Nicolás J, Alvarez S, Fresno M and Muñoz-Fernández MA (2001): Regulation of human immunodeficiency virus type 1 replication in human T lymphocytes by nitric oxide. J. Virol. 75, 4655–4663. https://doi.org/10.1128/JVI.75.10.4655-4663.2001

Lee M, Choy JC (2013): Positive feedback regulation of human inducible nitric-oxide synthase expression by Ras protein S-nitrosylation. J. Biol. Chem. 288, 15677–15686. https://doi.org/10.1074/jbc.M113.475319

Martínez I, Lombardía L, Herranz C, García-Barreno B, Domínguez O, Melero JA (2009): Cultures of HEp-2 cells persistently infected by human respiratory syncytial virus differ in chemokine expression and resistance to apoptosis as compared to lytic infections of the same cell type. Virology 388, 31–41. https://doi.org/10.1016/j.virol.2009.03.008

Mejías A, Chávez-Bueno S, Ramilo O (2005): Respiratory syncytial virus pneumonia: mechanisms of inflammation and prolonged airway hyperresponsiveness. Curr. Opin. Infect. Dis. 18, 199–204. https://doi.org/10.1097/01.qco.0000168378.07110.72

Morin C, Fessi H, Devissaguet JP, Puisieux F, Barratt G (1994): Factors influencing macrophage activation by muramyl peptides: inhibition of NO synthase activity by high levels of NO. Biochim. Biophys. Acta 1224, 427–432. https://doi.org/10.1016/0167-4889(94)90278-X

Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ et al. (2010): Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. The Lancet 375, 1545–1555. https://doi.org/10.1016/S0140-6736(10)60206-1

Napoli C, Paolisso G, Casamassimi A, Al-Omran M, Barbieri M, Sommese L et al. (2013): Effects of nitric oxide on cell proliferation: Novel insights. J. Am. Coll. Cardiol. 62, 89–95. https://doi.org/10.1016/j.jacc.2013.03.070

Nedeianu S, Pali T, Marsh D (2004): Membrane penetration of nitric oxide and its donor S-nitroso-N-acetylpenicillamine: a spin-label electron paramagnetic resonance spectroscopic study. Biochim. Biophys. Acta 1661, 135–143. https://doi.org/10.1016/j.bbamem.2003.12.008

Piedimonte G, Perez MK (2014): Alternative mechanisms for respiratory syncytial virus (RSV) infection and persistence: Could RSV be transmitted through the placenta and persist into developing fetal lungs? Curr. Opin. Pharmacol. 16, 82–88. https://doi.org/10.1016/j.coph.2014.03.008

Pozner RG, Collado S, de Giusti CJ, Ure AE, Biedma ME, Romanowski V et al. (2008): Astrocyte response to Junín virus infection. Neurosci. Lett. 445, 31–35. https://doi.org/10.1016/j.neulet.2008.08.059

Rimmelzwaan GF, Baars MM, de Lijster P, Fouchier RA, Osterhaus AD (1999): Inhibition of influenza virus replication by nitric oxide. J. Virol. 73, 8880–8883. https://doi.org/10.1128/JVI.73.10.8880-8883.1999

Rivera-Toledo E, Torres-González L, Gómez B (2015): Respiratory syncytial virus persistence in murine macrophages impairs IFN-β response but not synthesis. Viruses 7, 5361–5374. https://doi.org/10.3390/v7102879

Rosner M, Schipany K, Hengstschläger M (2013): Merging high-quality biochemical fractionation with a refined flow cytometry approach to monitor nucleocytoplasmic protein expression throughout the unperturbed mammalian cell cycle. Nat. Protoc. 8, 602–626. https://doi.org/10.1038/nprot.2013.011

Santiago-Olivares C, Rivera-Toledo E, Beatriz G (2019): Nitric oxide production is downregulated during respiratory syncytial virus persistence by constitutive expression of arginase-1. Arch. Virol. 164, 2231–2241. https://doi.org/10.1007/s00705-019-04259-0

Sarmiento RE, Tirado R, Gómez B (2002): Characteristics of a respiratory syncytial virus persistently infected macrophage-like culture. Vir. Res. 84, 45–58. https://doi.org/10.1016/S0168-1702(01)00420-8

Schwarze J, O'Donnell DR, Rohwedder A, Openshaw PJM (2004): Latency and persistence of respiratory syncytial virus despite T cell immunity. Am. J. Res. Crit. Med. 169, 801–805. https://doi.org/10.1164/rccm.200308-1203OC

Shah AM, MacCarthy PA (2000): Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol. Ther. 86, 49–86. https://doi.org/10.1016/S0163-7258(99)00072-8

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B (2000): Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am. J. Respir. Crit Care Med. 161, 1501–1507. https://doi.org/10.1164/ajrccm.161.5.9906076

Simon M, Falk KI, Lundkvist Å, Mirazimi A (2006): Exogenous nitric oxide inhibits Crimean Congo hemorrhagic fever virus. Vir. Res. 120, 184–190. https://doi.org/10.1016/j.virusres.2006.03.005

Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH et al. (2005): Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J. Clin. Invest. 115, 66–75. https://doi.org/10.1172/JCI200519229

Villalobo A (2006): Nitric oxide and cell proliferation. FEBS J. 273, 2329–2344. https://doi.org/10.1111/j.1742-4658.2006.05250.x

Wu P, Hartert T (2011): Evidence for a causal relationship between respiratory syncytial virus infection and asthma. Expert. Rev. Anti. Infect. Ther. 9, 731–745. https://doi.org/10.1586/eri.11.92

Wu W, Munday DC, Howell G, Platt G, Barr JN, Hiscox JA (2011): Characterization of the interaction between human respiratory syncytial virus and the cell cycle in continuous cell culture and primary human airway epithelial cells. J. Virol. 85, 10300–10309. https://doi.org/10.1128/JVI.05164-11

Zomer-Kooijker K, Van Der Ent CK, Ermers MJJ, Uiterwaal CSPM, Rovers MM, Bont LJ (2014): Increased risk of wheeze and decreased lung function after respiratory syncytial virus infection. PLoS ONE 9, 3–8. https://doi.org/10.1371/journal.pone.0087162