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Simvastatin attenuates inflammatory process on LPS-induced acute lung injury in mice

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ABSTRACT

Acute lung injury (ALI) is a disease of high prevalence and is characterized by the excessive production of inflammatory mediators in the lungs of people sick. Inflammation is the major characteristic of ALI and studies report that inhibition of inflammatory cytokines could be an alternative treatment. Statins such as Simvastatin (SV) are known to their use for cholesterol reduction but also for inflammatory and immunoregulatory processes. In this study, we evaluated the effects of SV on LPS-induced alveolar macrophages and in ALI mice model. Our study has demonstrated the protective effects of SV on LPS-activated alveolar macrophages RAW 264.7 and LPS-induced ALI in mice. SV treatment significantly inhibited the alveolar macrophages activation by decreasing the iNOS, IL-1β, and IL-6 gene expression in vitro and in vivo. The treatment also decreased the inflammatory cells migration and the cytokines gene expression. Our findings suggest that SV can act as an anti-inflammatory agent for acute lung injury.

1. Introduction

Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI), are characterized by severe inflammatory processes causing diffuse alveolar damage and resulting in a variable degree of ventilation perfusion mismatch, poor lung compliance, and severe hypoxemia (Saguil and Fargo, 2020). Morbidity and mortality remain high and early recognition of patients is a vital step in providing appropriate care (Saguil and Fargo, 2020). About 150,000 individuals receive an ARDS diagnosis in the United States each year (Butt et al., 2016). A systematic review suggests that the mortality for ARDS is between 36% and 44% (Butt et al., 2016; Phua et al., 2009).

ALI/ARDS is characterized by excessive and uncontrolled systemic inflammatory responses (Huang et al., 2018). Pattern recognition receptors, like Toll-like receptors (TLRs), can initiate inflammatory signaling cascades and the release of proinflammatory cytokines such as tumor necrosis factor a (TNF-a), IL-1β and IL-8. Also, stimulate autophagy or apoptosis and induce the production of antibacterial molecules (Takeuchi and Akira, 2010; Jiang et al., 2017; Lin et al., 2018). Excessive cytokines and chemokines production, oxidative stress, and presence of neutrophils in the lung are the most common consequences observed in ADRS/ALI patients (Matthay et al., 2019). Moreover, the overwhelming inflammatory process during the acute phase of ARDS causes damage to the epithelial/endothelial barrier, increasing pulmonary edema and the

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breakdown of this epithelial barrier exposes the underlying basement membrane, predisposing to bacteremia and sepsis (Dushianthan et al., 2011).

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are widely used to treat hypercholesterolemia and prevent cardiac events (Montecucco et al., 2009). Statins also have anti-inflammatory effects, including reducing C-reactive protein (CRP) concentrations (Quist-Paulsen, 2010; Kim et al., 2019). The addition of statins to human hepatocytes reduces the levels of C-reactive protein induced by circulating interleukin 6 (IL-6), suggesting that the anti-inflammatory effects of statins are hepatic in nature (Mayer et al., 2007; Link et al., 2006; Albert et al., 2001). Furthermore, statins reduce tumor necrosis factor alpha (TNF-α) and interferon gamma (IFNγ) production in stimulated T-lymphocytes and inhibit the T helper cell (Th-1) immune response (Link et al., 2006). In animal models, it has also been shown to be effective in the treatment of septic mice, showing an effect on animal mortality and the pulmonary damage caused by sepsis (Ando et al., 2000; Rosch et al., 2010; Boyd et al., 2012).

Currently, there are no effective pharmacological therapies for ARDS/ALI. The treatment is primarily supportive and focused on the treatment of the underlying condition and bedside care, including mechanical ventilation and corticosteroid administration (Fan et al., 2018). Several studies into ARDS pathogenesis and treatment are concentrated on identifying causative and prognostically important pathways involved in the development of ALI and the progression of ALI to ARDS (Saguil and Fargo, 2020; Matthay et al., 2019). Uncontrolled inflammation is a central issue in this progression and developing new treatment strategies for limiting excessive inflammation is crucial. Thus, the aim of this study was to evaluate the capacity of simvastatin (SV) to immunomodulate this process. Our findings strongly demonstrated that Simvastatin could reduce the expression of inflammatory mediators in a cell lineage of macrophages and ameliorate the LPS-induced acute lung injury in mice, suggesting a potential role for Simvastatin-based therapy to treat clinical ARDS.

2. Materials and methods

2.1. Cell culture

Alveolar macrophage murine cell line (RAW 264.7; ATCC Bank - USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Life-technologies, USA) with 10% fetal bovine serum (FBS; Gibco, Life-technologies, USA) and 1% antibiotics (penicillin/streptomycin; Gibco, Life Technologies, USA) at 37°C in a humidified incubator at 5% CO_2.

2.2. Simvastatin treatment

RAW 264.7 cells were plated for 24 h and treated with SV solution (SV was diluted in absolute ethyl alcohol – nuclear Brazil) at concentrations of 0.5 and 1.0 μM for a period of 1 h. Then, they were treated for another 24 h with 1 μg/mL of LPS (Escherichia coli 026: B6; Sigma-Aldrich, USA). Control and LPS groups were treated with absolute ethyl alcohol for 1 h and after that the LPS group received the bacterial toxin and both groups left for 24 h.

2.3. Cell viability assay

Cell viability was performed by the MTT assay (Life Technologies, USA). The culture medium was removed, and the cells treated with MTT for 3 h. After discarding the excess reagent, the formazan crystals were dissolved with DMSO and the solution was read in a spectrophotometer (EZ Read 400; Biochrom, USA) at a wavelength of 570/620 nm.

2.4. Animals

The animals (Male C57BL/6 mice) weighed 23–30 g and were kept in individual cages at the university's experimental model center with a 12 h light-dark cycle. The project was approved by the university's ethics committee under number 16/00495.

2.5. LPS-induced ALI and animal groups

The animals were divided into 3 groups: 1) Control group that received 50 μL of saline solution by inhalation and 30 min later, received vehicle of SV (absolute ethyl alcohol I.P.). LPS group received LPS by inhalation (50 μL-2 mg/kg) and was treated with absolute ethyl alcohol 30 min later, and LPS + SV group That was treated with 0.2 mg/kg I.P. 30 min after LPS inhalation). A man ingests statins in pills that vary from 5 to 80 mg/day, therefore, considering 20 mg/kg with a weight of 80 kg, the dose per kg would be 0.25 mg/kg in humans. In our work, in mice, we used 0.20 mg/Kg of weight, like used in humans. After 12 h of LPS inhalation, the animals were anesthetized and bronchoalveolar lavage (BALF) was collected with 1 mL of PBS.

2.6. Quantitative gene expression

Total RNA was extracted from RAW 264.7 cells or lung tissue using TRIzol reagent (Invitrogen, USA), according to the manufacturer's instructions. Subsequently, cDNA was synthesized using a reverse transcription kit Goscript™ Reverse Transcriptase (Promega, USA) and the real-time PCR reactions were performed using SYBR Green PCR Master Mix (Applied Biosystems, USA). All PCR reactions were performed in duplicate and water was used as negative control. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous control. The primer sequences were as follows: *GAPDH* (forward) 5-GGGGAGCCAAAAGGGTCATC-3, (reverse) 5-GACGCCTGCTTCACCACCTTCTTG-3; *TLR-4* (forward) 5-TTCAGAGCCGTTGGTGTATC-3, (reverse) 5-CTCCCATTCCAGGTAGGTGT-3; *iNOS* 5-CCTCCTCCACCCTACCAAGT-3, (reverse) 5-CACCCAAAGTGCTTCAGTCA-3; *COX-2* (forward) 5-TTGAAGACCAGGAGTACAG C-3, (reverse) 5-GGTACAGTTCCATGACATCG-3, *TNF-α* (forward) 5-ATAGCTCCCAGAAAAGCAAGC-3, (reverse) 5-CACCCCGAAGTTCAGTAGACA-3; *IL-1β* (forward) 5-GCCCATCCTCTG TGACTCAT-3, (reverse) 5-AGGCCACAGGTATTTTGTCG-3; *IL-6* (forward) 5-TGGAGTCACAGAAGGAGTGGCTAAG-3, (reverse) 5-AGGCCACAGGTATTTTGTCG-3.

2.7. Total and differential cell analysis in BALF

After 12 h of induction, pleural fluid was collected with the addition of 1 mL of PBS. Cell concentration was evaluated using a Neubauer chamber. After, the BALF was centrifuged (1000 x g for 10 min), and the pellet stained with hematoxylin/eosin for differential cell count (Olympus microscope).

2.8. Protein quantification

BALF total protein was quantified using the NanoDrop spectrophotometer (Model 1000, Thermo Scientific).

2.9. Histological analysis

The lung was perfused with 10% buffered formalin. After, the superior lobe of the right lung was embedded in paraffin, cut in 4 mm sections and stained with hematoxylin and eosin (H&E) (Panótico Rápido; Laborclin, Brazil).

2.10. Quantification of cytokines in lung tissue

the lung was homogenized in PBS (weight/volume 1:2), centrifuged

at 13,000g and IL 6 and TNF alpha were measured in the supernatant using a Magpix equipment (Merck). Results are presented in pg/mg of protein.

2.11. Statistical analysis

The statistics analysis of variance was performed with ANOVA and Tukey was used as a post roc test. Data were expressed as mean ± standard error of the mean (SEM).

3. RESULTS

3.1. Simvastatin reduces the expression of pro-inflammatory genes in RAW 264.7

Resident alveolar macrophages (AMs) and recruited macrophages from the blood are key factors in the pathogenesis of ALI/ARDS (Huang et al., 2018; Lomas-Neira et al., 2006). To assess whether SV could regulate the inflammatory process, we cultivated a mouse macrophage cell line (RAW 264.7) stimulated with LPS and treated with SV. First, we analyzed the toxicity of two concentrations of SV (0.5 and 1 μM) and we did not found significant differences between the groups analyzed (Fig. 1 A). After, we performed an inflammatory stimulus with LPS in cells previously treated with SV for 1 h. LPS activated cell proliferation and SV was unable to reverse this response (Fig. 1B). To identify whether SV was able to immunomodulate the LPS-induced inflammatory response, we evaluated some pro-inflammatory genes. Gene expression profiling demonstrates no significant differences in TLR-4, COX-2, and TNF-α mRNA expression between groups (Fig. 1 C). However, SV treatment tends to decrease the iNOS ($p < 0.078$) and IL-1β ($p < 0.09$), and IL-6 gene expression significantly decreased when compared to LPS group (Fig. 1 C). Altogether, these results demonstrate that SV has low cytotoxicity and can immunomodulate the inflammatory response induced by LPS.

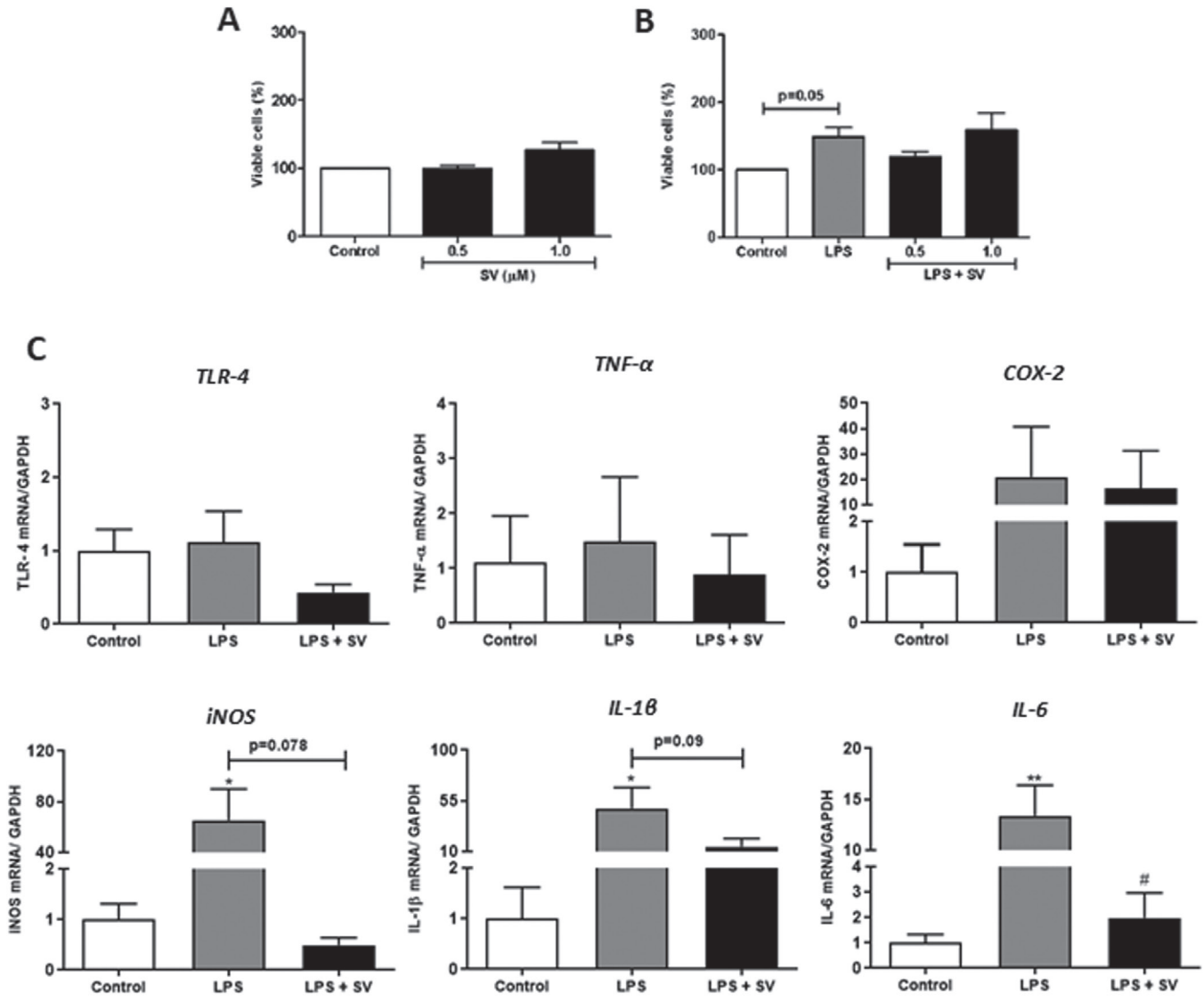

Fig. 1. Simvastatin affects pro-inflammatory genes expression in RAW 264.7. (A) Response dose curve of SV (0.5–1.2 μM). (B) The cells were treated with the indicated concentrations of SV (0.5 and 1 μM) for 24 h and then stimulated or not with LPS (1 μg/mL) for 24 h. The cell viability was assessed using an MTT assay and all experiments were performed in triplicate. Results are expressed as viable cells (%) and the data represent the mean ± SEM of five independent experiments ($n = 5$). (C) mRNA expression levels were measured by RT-qPCR in RAW 264.7 cells. The experiment was normalized to GAPDH expression. Data are expressed as mean ± SEM. Significant differences are relative to Control group, $^{*}p < 0.05$, $^{**}p < 0.01$. Significant differences are relative to LPS group, $^{\#}p < 0.05$.

3.2. Simvastatin reduces inflammatory cell infiltrate in LPS-induced ALI mouse model

We observe that SV could regulate the inflammatory stimulus in LPS-induced model in vitro (decrease of IL-6), so, we hypothesized that SV levels could have a positive effect in a murine acute lung injury model. We used an LPS-induced ALI model, a well-recognized methodology for inducing ALI in mice (Fig. 2A). The LPS binds to TLR-4 receptor, activates the NF-KB pathway, and triggers a cascade of inflammatory mediators (Perros et al., 2011).

With the established model, we analyzed the total and differential cell count in the bronchoalveolar lavage (BALF). The LPS group significantly increased the total inflammatory cell count in BALF when compared with the control group, and intranasal administration of SV significantly decreased the total cell count when compared with the LPS group (Fig. 2B). No differences in cell count of macrophages were found in the different groups analyzed (Fig. 2C). On the other hand, cell counts of neutrophils were significantly increased in the LPS group when compared with the control group and SV treatment significantly decreased this effect when compared with the LPS group (Fig. 2D). We also performed a BALF H&E staining to observe this alteration. Representative images in the different groups evaluated are shown in Fig. 2E. The arrows indicate an increase of neutrophils inflammatory cells in the BALF when compared to the LPS group with control and LPS + SV groups, the same profile found in cell count.

3.3. Lung inflammation is attenuated in LPS-induced ALI mice treated with SV

Since the animals treated with SV showed a reduction in the disruption of the alveolar–capillary barrier, observed with a reduction in BALF cell count, we next determined whether SV would reduce the inflammatory process in the lung. Lung sections were subjected to H&E staining 12 h after ALI induction. Histological alterations were observed

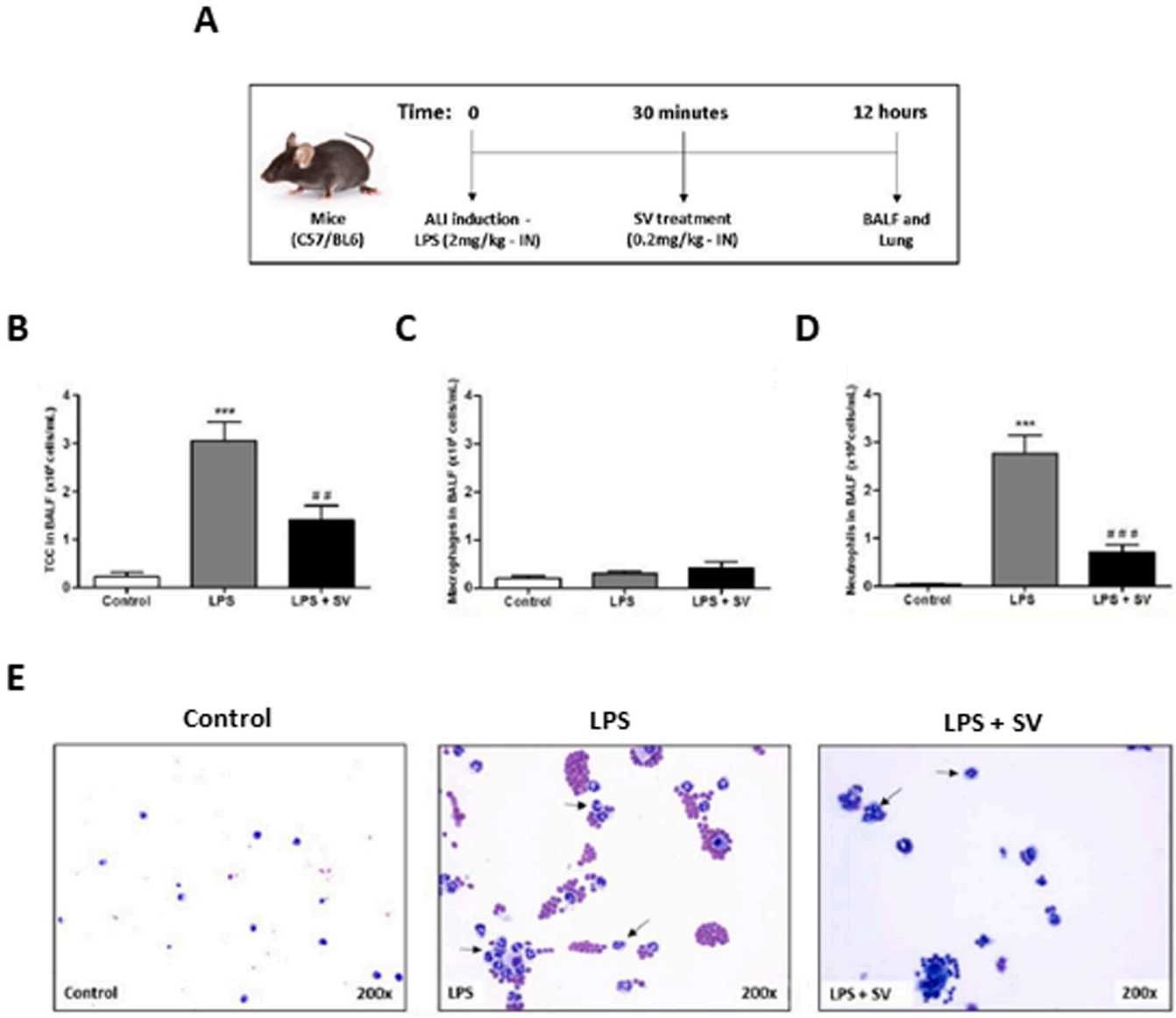

Fig. 2. Simvastatin reduces inflammatory infiltrated cells in BALF. (A) Experimental design of LPS-induced ALI. The timeline is expressed in minutes or hours. LPS: Lipopolysaccharide; ALI: Acute lung injury; IN: intranasal; SV: Simvastatin; BALF: broncho-alveolar lavage fluid. (B) Total cells count (TCC) (n = 6). (C and D) differential cells count (macrophages and neutrophils respectively) quantified 12 h after ALI induction (n = 6). (E) Microscopic observation of H&E staining inflammatory cells presents in the different groups (magnification x 200). The arrows indicate neutrophils inflammatory cells in the BALF. Data are expressed as mean ± SEM. Significant differences are relative to Control group, $^{**}p < 0.01$. Significant differences are relative to LPS group, $^{\#\#\#}p < 0.001$.

in the LPS group which included inflammatory cells infiltration and thickening of the alveolar wall (Fig. 3A). Animals treated with simvastatin showed a reduction in this infiltration when compared to the LPS group. Besides that, animals that received LPS demonstrated a significant increase in lung weight when compared with the control group (Fig. 3B). This increase was not found in the group treated with SV, suggesting that SV could reduce the edema formation.

We also evaluated the concentration of cytokines in the lung. As expected, the LPS group showed a significant increase in the TNF-α and IL-6 concentration when compared to the control group (Fig. 3C). This pro-inflammatory effect caused by LPS was reduced in animals treated with SV, which corroborates the histopathological results found. Furthermore, we analyzed the gene expression of iNOS, IL-1β, and IL-6 in the lung to assess whether we would have the same profile found in macrophages treated with SV and stimulated with LPS. The results demonstrate no significant differences in iNOS gene expression between groups analyzed (Fig. 3D), however, the LPS group significantly increased the IL-1β and IL-6 mRNA expression when compared to the control group, and the treatment with SV decreased not significantly the IL-1β ($p = 0,12$) and significantly the IL-6 mRNA (Fig. 3D). All these results are strong indicators that SV could have a beneficial effect on the acute lung injury process, modulating the pro-inflammatory response.

4. Discussion

As people take simvastatin for the treatment of dyslipidemia, our first idea was to assess whether pre-use (as the people) could be a cellular protective agent against lung inflammation. Our results showed that pre-

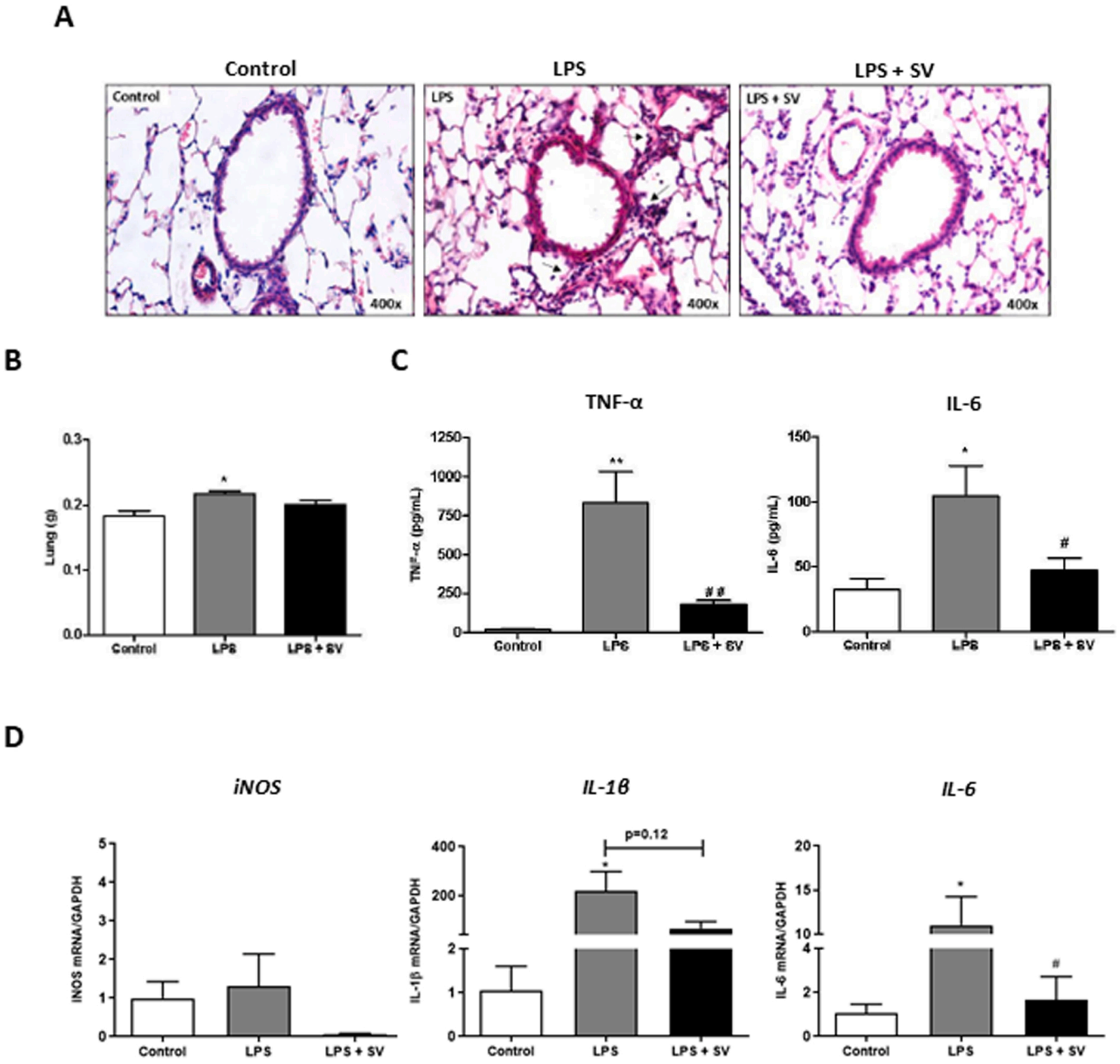

Fig. 3. Simvastatin decreases inflammation in LPS-induced ALI mouse model. (A) Representative histological lung images obtained from mice of different groups (H&E staining magnification x 400). The arrows indicate an increase of inflammatory cells in the lung. (B) Lung weight (n = 6). (C) TNF-α and IL-6 lung levels (n = 6). (D) mRNA expression levels were measured by RT-qPCR. The experiment was normalized to GAPDH expression (n = 6). Data are expressed as mean ± SEM. Significant differences are relative to Control group, *$p < 0.05$, **$p < 0.01$. Significant differences are relative to LPS group, $^{\#}p < 0.05$.

treatment, that is, the use of SV by people for the treatment of dyslipidemia, can protect lung cells from possible bacterial infections. The next step was to assess whether SV could be used as a treatment, and so we performed this "in vivo" study, and we conclude that it can be used in the treatment of LPA.

Our study demonstrated, for the first time, the protective effects of SV on LPS-activated alveolar macrophages RAW 264.7 and on LPS-induced acute lung injury in mice. SV treatment significantly inhibited the alveolar macrophages activation by decreasing the iNOS, IL-1β, and IL-6 expression in vitro. When we apply the SV as a treatment for acute lung injury, the drug administration demonstrated an effect similar to that found in RAW 264.7 cells exposed to LPS. SV treatment decreased lung injury, inflammatory cells migration, and cytokines release and expression. These data suggest that SV treatment can act as an anti-inflammatory agent for acute lung injury.

Statins are suppressors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and act as a catalyst to the rate-determining step in the biosynthesis of cholesterol (Gilbert et al., 2017). Studies demonstrated that SV has a beneficial effect as a treatment for coronary diseases and to decrease the pro-inflammatory process implicated in atherosclerosis, independently of their lipid-lowering effect. These pleiotropic properties, while involving the useful effects on cardiovascular disease, also appear to play a vital responsibility in further inflammatory and immune diseases (Zhou and Liao, 2009). Statins showed to decrease the risk allied to sepsis, including disease progression, mortality, incidence, and a significant effect on reducing rheumatoid arthritis disease activity, both in human clinical trials (Zhou and Liao, 2009; Abud-Mendoza et al., 2003; McCarey et al., 2004; Okamoto et al., 2007; Gui et al., 2017; Lee et al., 2017). These anti-inflammatory effects from statins were observed when we treated LPS-stimulated RAW 264.7 with SV. When Raw 264.7 macrophage cells are stimulated with LPS, TLR4 signaling pathways are activated, which in turn activates nuclear factor-kB transcription factor (NFkB) and secretes different kinds of cytokines, such as IL-1β and IL-6 - both reduced in cells treated with SV. These results suggest that SV has an anti-inflammatory effect by blocking the LPS action.

ALI/ARDS severity and the possible development are greatly influenced by neutrophil migration into the lungs in response to activated alveolar macrophages (Fan and Fan, 2018). Neutrophils have actions in eliminating microbial infections and are essential cells for innate response control (Delgado-Rizo et al., 2017). However, excessive recruitment of these cells to alveolar spaces produces numerous cytotoxic substances, including granular enzymes, reactive oxygen species, bioactive lipids, various proinflammatory cytokines, and neutrophil extracellular traps (NETs) (Butt et al., 2016; Douda et al., 2011). In the LPS-induced ALI model, SV had a protective effect attenuating neutrophil infiltration in the lungs. These data indicate an inhibitory effect of SV on neutrophils accumulation in the lung and to maintain the endothelial barrier. We administered SV 30 min after the challenge with LPS because this is the time that the animals already have an important inflammatory condition. We know that for humans this time is very short, but in mice and, experimentally, where the bacterial toxin is administered directly via pulmonary inhalation, in 30 min the animals already present an important inflammatory condition, justified by the large migration of neutrophils and lymphocytes to the lung, which would allow us to treat the animals. This time has already been used by our group in other works (Antunes et al., 2022).

These positive findings of SV in the treatment of acute lung injury go beyond the reduction in the migration of effector cells from the innate immune response. Our results showed that SV significantly suppressed the IL-6 concentration in the lung and reduce the IL-6 gene expression. IL-6 is a major pro-inflammatory mediator for the induction of acute phase response, leading to systemic alterations like leukocytes recruitment and activation, fever, hemodynamic effects, and hepatic regeneration (Voiriot et al., 2017). Considering that IL-6 is a mediator of the acute phase response, its value as a prognostic biomarker in various acute organ injuries, as COVID-19 and sepsis have been investigated in experimental and clinical studies (Veldhuizen et al., 2001; Stuber et al., 2002; Vaporidi et al., 2008; Song et al., 2019; Huang et al., 2020). Plasma and/or broncho-alveolar levels of IL-6 have been identified as early biomarkers of lung injury protein expression in the lung of mice with ALI (Voiriot et al., 2017), which demonstrates another beneficial effect of SV treatment.

We also investigated the TNF-α concentration in the lung. TNF into experimental animals induced sepsis symptoms, including fever, lungs, kidneys, and liver failure in addition to cardiovascular impairment (Reiss et al., 2020; Spriggs et al., 1987). The ability of SV to reduce the concentration of TNF-α is extremely important since elevated TNF levels in the bronchopulmonary secretions of patients with ARDS was correlated with poor outcome (Millar et al., 1989; Meduri et al., 1995). Furthermore, patients submitted to anti-TNF treatment in clinical sepsis trials had improved survival (Qiu et al., 2013).

Finally, SV administration significantly decreased the IL-1β mRNA expression in mice with LPS-induced ALI. IL-1 is an important cytokine involved in acute and chronic inflammation in a complex network of signaling molecules (Fields et al., 2019). Bioactive IL-1β can amplify inflammatory responses and induce more proinflammatory cytokine and chemokine production, such as IL-1β, IL-6, TNF-α, and monocyte chemotactic protein (MCP)-1 (Jia et al., 2019). Critically ill patients have an increased risk of developing ARDS and mortality when they have high concentrations of IL-1β (Jacobs et al., 1989; Dolinay et al., 2012). Also, IL-1β is able to cause a decrease in epithelial sodium channels contributing to pulmonary edema formation (Roux et al., 2005). Thus, we believe that SV modulating IL-1β expression prevents edema formation since we did not find an increase in lung weight in the ALI + SV group. It is a crucial determinant of morbidity and mortality during ALI since the alveolar epithelium prevent and resolve pulmonary edema (Patel et al., 2013).

In conclusion, our results demonstrated that SV has an immunomodulatory ability in LPS-stimulated macrophages and in an LPS-induced ALI model in mice. SV treatment decreased lung injury, inflammatory cells migration, and cytokines release and expression. The search for treatments for ALI/ADRS is extremely important since clinically alternatives are palliative measures. We have shown that pretreatment, what happens to people who take SV for dyslipidemia, can protect lung cells from bacterial infections and, also, be used as a treatment in case of infection already installed. More detailed studies are required to further understand the mechanism by which SV controls the inflammatory response during acute lung injury. Besides, this study unravels new anti-inflammatory functions of SV, opening new fields of investigation.

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Author's contributions

GVH conceived the work, acquired data, drafted the paper and approved the final version. CL, GLA, JS, BSB, VGSL, MSC and DBK, acquired data, revised the article and approved the final version. LP drafted the paper, revised the article and approved the final version. MVFD, JGS and JRO conceived the work, revised the paper, and approved the final version.

Conflict of interest

The authors have no financial relationships or conflicts of interest.

References

Abud-Mendoza, C., de la Fuente, H., Cuevas-Orta, E., Baranda, L., Cruz-Rizo, J., Gonzalez-Amaro, R., 2003. Therapy with statins in patients with refractory rheumatic diseases: a preliminary study. Lupus 12 (8), 607–611.

Albert, M.A., Danielson, E., Rifai, N., Ridker, P.M., 2001. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. J. Am. Med. Assoc. 286 (1), 64–70.

Ando, H., Takamura, T., Ota, T., Nagai, Y., Kobayashi, K., 2000. Cerivastatin improves survival of mice with lipopolysaccharide-induced sepsis. J. Pharmacol. Exp. Ther. 294 (3), 1043–1046.

Antunes1, G.L., Matzenbacher, L.S., Costa, B.P., Bruno de Sousa Basso, B.S., Levorse, V.G. S., et al., 2022. Methoxyeugenol protects against lung inflammation and suppresses neutrophil extracellular trap formation in an LPS-induced acute lung injury model. Inflammation 4, 1534–1547.

Boyd, A.R., Hinojosa, C.A., Rodriguez, P.J., Orihuela, C.J., 2012. Impact of oral simvastatin therapy on acute lung injury in mice during pneumococcal pneumonia. BMC Microbiol. 12, 73.

Butt, Y., Kurdowska, A., Allen, T.C., 2016. Acute lung injury: a clinical and molecular review. Arch. Pathol. Lab. Med. 140 (4), 345–350.

Delgado-Rizo, V., Martinez-Guzman, M.A., Iniguez-Gutierrez, L., Garcia-Orozco, A., Alvarado-Navarro, A., Fafutis-Morris, M., 2017. Neutrophil extracellular traps and its implications in inflammation: an overview. Front. Immunol. 8, 81.

Dolinay, T., Kim, Y.S., Howrylak, J., Hunninghake, G.M., An, C.H., Fredenburgh, L., et al., 2012. Inflammasome-regulated cytokines are critical mediators of acute lung injury. Am. J. Respir. Crit. Care Med. 185 (11), 1225–1234.

Douda, D.N., Jackson, R., Grasemann, H., Palaniyar, N., 2011. Innate immune collectin surfactant protein D simultaneously binds both neutrophil extracellular traps and carbohydrate ligands and promotes bacterial trapping. J. Immunol. 187 (4), 1856–1865.

Dushianthan, A., Grocott, M.P., Postle, A.D., Cusack, R., 2011. Acute respiratory distress syndrome and acute lung injury. Postgrad. Med. J. 87 (1031), 612–622.

Fan, E., Brodie, D., Slutsky, A.S., 2018. Acute respiratory distress syndrome: advances in diagnosis and treatment. J. Am. Med. Assoc. 319 (7), 698–710.

Fan, E.K.Y., Fan, J., 2018. Regulation of alveolar macrophage death in acute lung inflammation. Respir. Res. 19 (1), 50.

Fields, J.K., Gunther, S., Sundberg, E.J., 2019. Structural basis of IL-1 family cytokine signaling. Front. Immunol. 10, 1412.

Gilbert, R., Al-Janabi, A., Tomkins-Netzer, O., Lightman, S., 2017. Statins as anti-inflammatory agents: a potential therapeutic role in sight-threatening non-infectious uveitis. Porto Biomed. J. 2 (2), 33–39.

Gui, Q.F., Yang, Y.M., Zhang, J.J., 2017. Effects of statins on the development of sepsis and organ dysfunction in hospitalized older patients in China. Braz. J. Infect. Dis. 21 (3), 255–262.

Huang, L., Zhao, X., Qi, Y., Li, H., Ye, G., Liu, Y., et al., 2020. Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cell. Mol. Immunol. 17 (10), 1092–1094.

Huang, X., Xiu, H., Zhang, S., Zhang, G., 2018. The Role of Macrophages in the Pathogenesis of ALI/ARDS. Mediat. Inflamm. 2018, 1264913.

Jacobs, R.F., Tabor, D.R., Burks, A.W., Campbell, G.D., 1989. Elevated interleukin-1 release by human alveolar macrophages during the adult respiratory distress syndrome. Am. Rev. Respir. Dis. 140 (6), 1686–1692.

Jia, X., Cao, B., An, Y., Zhang, X., Wang, C., 2019. Rapamycin ameliorates lipopolysaccharide-induced acute lung injury by inhibiting IL-1beta and IL-18 production. Int. Immunopharmacol. 67, 211–219.

Jiang, K., Zhang, T., Yin, N., Ma, X., Zhao, G., Wu, H., et al., 2017. Geraniol alleviates LPS-induced acute lung injury in mice via inhibiting inflammation and apoptosis. Oncotarget 8 (41), 71038–71053.

Kim, S.W., Kang, H.J., Jhon, M., Kim, J.W., Lee, J.Y., Walker, A.J., et al., 2019. Statins and inflammation: new therapeutic opportunities in psychiatry. Front. Psychiatry 10, 103.

Lee, M.G., Lee, C.C., Lai, C.C., Hsu, T.C., Porta, L., Lee, M., et al., 2017. Preadmission statin use improves the outcome of less severe sepsis patients - a population-based propensity score matched cohort study. Br. J. Anaesth. 119 (4), 645–654.

Lin, S., Wu, H., Wang, C., Xiao, Z., Xu, F., 2018. Regulatory T cells and acute lung injury: cytokines, uncontrolled inflammation, and therapeutic implications. Front. Immunol. 9, 1545.

Link, A., Ayadhi, T., Bohm, M., Nickenig, G., 2006. Rapid immunomodulation by rosuvastatin in patients with acute coronary syndrome. Eur. Heart J. 27 (24), 2945–2955.

Lomas-Neira, J., Chung, C.S., Perl, M., Gregory, S., Biffl, W., Ayala, A., 2006. Role of alveolar macrophage and migrating neutrophils in hemorrhage-induced priming for ALI subsequent to septic challenge. Am. J. Physiol. Lung Cell. Mol. Physiol. 290 (1), L51–L58.

Matthay, M.A., Zemans, R.L., Zimmerman, G.A., Arabi, Y.M., Beitler, J.R., Mercat, A., et al., 2019. Acute respiratory distress syndrome. Nat. Rev. Dis. Prim. 5 (1), 18.

Mayer, C., Gruber, H.J., Landl, E.M., Pailer, S., Scharnagl, H., Truschnig-Wilders, M., et al., 2007. Rosuvastatin reduces interleukin-6-induced expression of C-reactive protein in human hepatocytes in a STAT3- and C/EBP-dependent fashion. Int. J. Clin. Pharmacol. Ther. 45 (6), 319–327.

McCarey, D.W., McInnes, I.B., Madhok, R., Hampson, R., Scherbakov, O., Ford, I., et al., 2004. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 363 (9426), 2015–2021.

Meduri, G.U., Kohler, G., Headley, S., Tolley, E., Stentz, F., Postlethwaite, A., 1995. Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome. Chest 108 (5), 1303–1314.

Millar, A.B., Foley, N.M., Singer, M., Johnson, N.M., Meager, A., Rook, G.A., 1989. Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome. Lancet 2 (8665), 712–714.

Montecucco, F., Burger, F., Pelli, G., Poku, N.K., Berlier, C., Steffens, S., et al., 2009. Statins inhibit C-reactive protein-induced chemokine secretion, ICAM-1 upregulation and chemotaxis in adherent human monocytes. Rheumatology 48 (3), 233–242.

Okamoto, H., Koizumi, K., Kamitsuji, S., Inoue, E., Hara, M., Tomatsu, T., et al., 2007. Beneficial action of statins in patients with rheumatoid arthritis in a large observational cohort. J. Rheumatol. 34 (5), 964–968.

Patel, B.V., Wilson, M.R., O'Dea, K.P., Takata, M., 2013. TNF-induced death signaling triggers alveolar epithelial dysfunction in acute lung injury. J. Immunol. 190 (8), 4274–4282.

Perros, F., Lambrecht, B.N., Hammad, H., 2011. TLR4 signalling in pulmonary stromal cells is critical for inflammation and immunity in the airways. Respir. Res. 12, 125.

Phua, J., Badia, J.R., Adhikari, N.K., Friedrich, J.O., Fowler, R.A., Singh, J.M., et al., 2009. Has mortality from acute respiratory distress syndrome decreased over time?: A systematic review. Am. J. Respir. Crit. Care Med. 179 (3), 220–227.

Qiu, P., Cui, X., Sun, J., Welsh, J., Natanson, C., Eichacker, P.Q., 2013. Antitumor necrosis factor therapy is associated with improved survival in clinical sepsis trials: a meta-analysis. Crit. Care Med. 41 (10), 2419–2429.

Quist-Paulsen, P., 2010. Statins and inflammation: an update. Curr. Opin. Cardiol. 25 (4), 399–405.

Reiss, L.K., Raffetseder, U., Gibbert, L., Drescher, H.K., Streetz, K.L., Schwarz, A., et al., 2020. Reevaluation of lung injury in TNF-induced shock: the role of the acid sphingomyelinase. Mediat. Inflamm. 2020, 3650508.

Rosch, J.W., Boyd, A.R., Hinojosa, E., Pestina, T., Hu, Y., Persons, D.A., et al., 2010. Statins protect against fulminant pneumococcal infection and cytolysin toxicity in a mouse model of sickle cell disease. J. Clin. Investig. 120 (2), 627–635.

Roux, J., Kawakatsu, H., Gartland, B., Pespeni, M., Sheppard, D., Matthay, M.A., et al., 2005. Interleukin-1beta decreases expression of the epithelial sodium channel alpha-subunit in alveolar epithelial cells via a p38 MAPK-dependent signaling pathway. J. Biol. Chem. 280 (19), 18579–18589.

Saguil, A., Fargo, M.V., 2020. Acute respiratory distress syndrome: diagnosis and management. Am. Fam. Physician 101 (12), 730–738.

Song, J., Park, D.W., Moon, S., Cho, H.J., Park, J.H., Seok, H., et al., 2019. Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions. BMC Infect. Dis. 19 (1), 968.

Spriggs, D.R., Sherman, M.L., Frei 3rd, E., Kufe, D.W., 1987. Clinical studies with tumour necrosis factor. Ciba Found. Symp. 131, 206–227.

Stuber, F., Wrigge, H., Schroeder, S., Wetegrove, S., Zinserling, J., Hoeft, A., et al., 2002. Kinetic and reversibility of mechanical ventilation-associated pulmonary and systemic inflammatory response in patients with acute lung injury. Intensive Care Med. 28 (7), 834–841.

Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140 (6), 805–820.

Vaporidi, K., Voloudakis, G., Priniannakis, G., Kondili, E., Koutsopoulos, A., Tsatsanis, C., et al., 2008. Effects of respiratory rate on ventilator-induced lung injury at a constant PaCO2 in a mouse model of normal lung. Crit. Care Med. 36 (4), 1277–1283.

Veldhuizen, R.A., Slutsky, A.S., Joseph, M., McCaig, L., 2001. Effects of mechanical ventilation of isolated mouse lungs on surfactant and inflammatory cytokines. Eur. Respir. J. 17 (3), 488–494.

Voiriot, G., Razazi, K., Amsellem, V., Tran Van Nhieu, J., Abid, S., Adnot, S., et al., 2017. Interleukin-6 displays lung anti-inflammatory properties and exerts protective hemodynamic effects in a double-hit murine acute lung injury. Respir. Res. 18 (1), 64.

Zhou, Q., Liao, J.K., 2009. Statins and cardiovascular diseases: from cholesterol lowering to pleiotropy. Curr. Pharm. Des. 15 (5), 467–478.