

Prophylactic effect of human and bovine lactoferrin against the respiratory syncytial virus in the mouse model.

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Abstract

Background: Lactoferrin (LF) is a multifunctional glycoprotein, belonging to the transferrin family that is found in human milk and has known antimicrobial and antiviral effects. We have shown in vitro that LF has antiviral activity against the respiratory syncytial virus (RSV) in human and mouse cells. We hypothesized that human (hLF) and bovine (bLF) would attenuate RSV replication and lung inflammation and function in an in vivo murine model.

Methods: The antiviral activity of hLF and bLF was evaluated in male BALB/c mice inoculated with RSV-A2 strain (10^{4.7} PFU). LF derived compounds or mock (DPBS) were administered daily by intranasal administration at doses of 1mg/day, from 72 h before until 120 h post-RSV inoculation. Human transferrin (TF) treatment was included as the internal control. Mice were sacrificed 1, 3 and 5 days post-infection and bronchoalveolar lavage fluid (BALF) and whole lung samples were collected to assess lung inflammation and RSV loads (determined by ddPCR). Weight was assessed daily in all groups.

Results: No significant difference in body weight was found between the experimental groups. hLF and bLF but not TF treatment reduced the inflammation induced by RSV infection in the lungs as indicated by the decrease in the number of macrophages, monocytes, eosinophils, and lymphocytes in BALF when compared to the RSV only infected mice. We also found that expression of RSV F and N genes was diminished in the lactoferrins plus RSV group compared to the RSV only infected group. TF had no effect, indicating that intranasal dosing of LF specifically protects and dampens RSV infection.

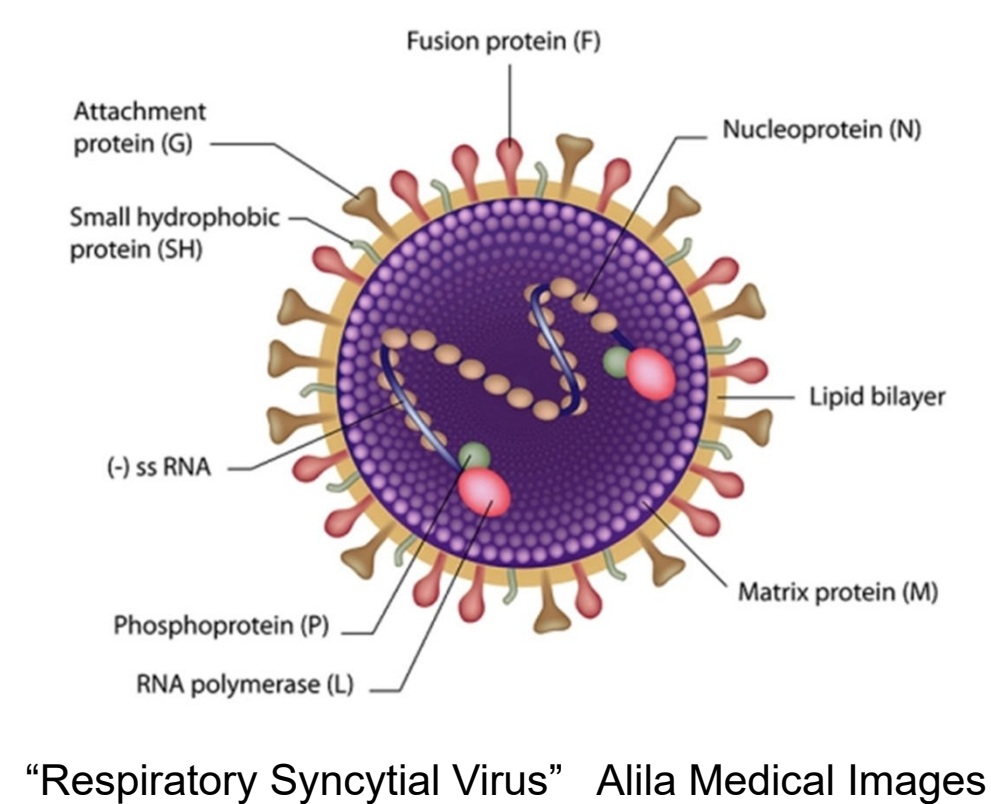
Conclusions: Intranasal administration of hLF and bLF has a protective and prophylactic antiviral activity against RSV infection in a RSV mouse model.

Lactoferrin

- Lactoferrin (LF) is a globular glycoprotein with a molecular mass of 80 kDa, found mainly in the saliva, tears and breast milk of mammalian species (1).
- Lactoferrin is part of the immune system for the body; it has antimicrobial activity and is part of the innate defense. In particular, lactoferrin provides antibacterial activity to human infants (2).
- Lactoferrin antiviral activity appears correlated to the prevention of viral binding to host cells, thus inhibiting infection (5).
- LF its functional properties are highly conserved among mammals (4). For instance, bovine LF (bLF) is taken up by the human lactoferrin receptor and exerts similar bioactivities as human lactoferrin (hLF) (4).

Respiratory syncytial virus

- Respiratory syncytial virus (RSV, Paramyxoviridae pneumovirus) is aspherical or pleomorphic envelope virus with single-stranded negative sense linear RNA which encodes 10 viral proteins.
- RSV is ubiquitous and highly contagious, and its seasonality mostly coincides with flu season. Most of RSV infection causes minor upper airway illness featured by fever and inflammation and irritation of the mucous membrane of the nose, which can be attenuates in 8-10 days, while RSV causes serious lower respiratory tract infection in high-risk groups.
- Although RSV infects people of all ages, it is especially a significant cause of respiratory illness in young children and the elderly, leading to morbidity and mortality (4).
- Though LF is reported to have antiviral activity against RSV in vitro (Grover et al., 1997) the anti-RSV activity was not observed in a mice model (Gualdi et al., 2013).
- There is no vaccine licensed and very few antiviral chemotherapeutic agents available for the prevention and treatment of RSV.

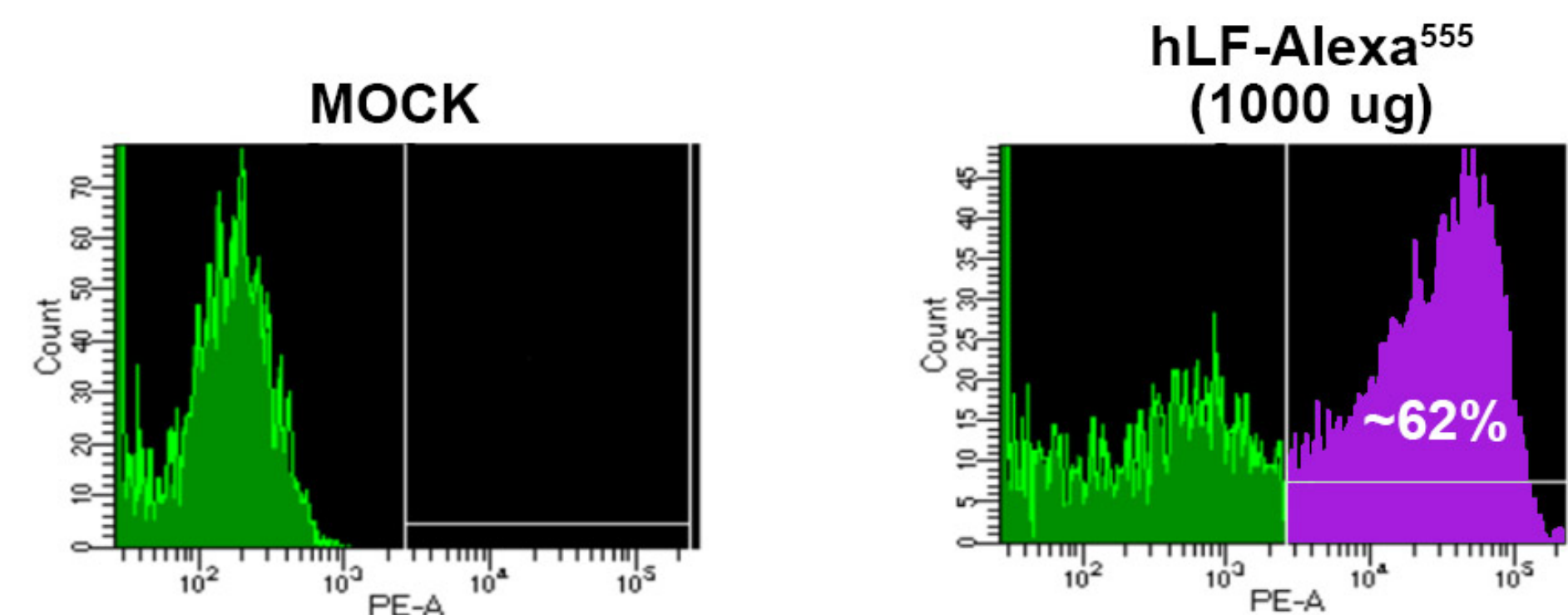


"Respiratory Syncytial Virus" Alila Medical Images

Objective

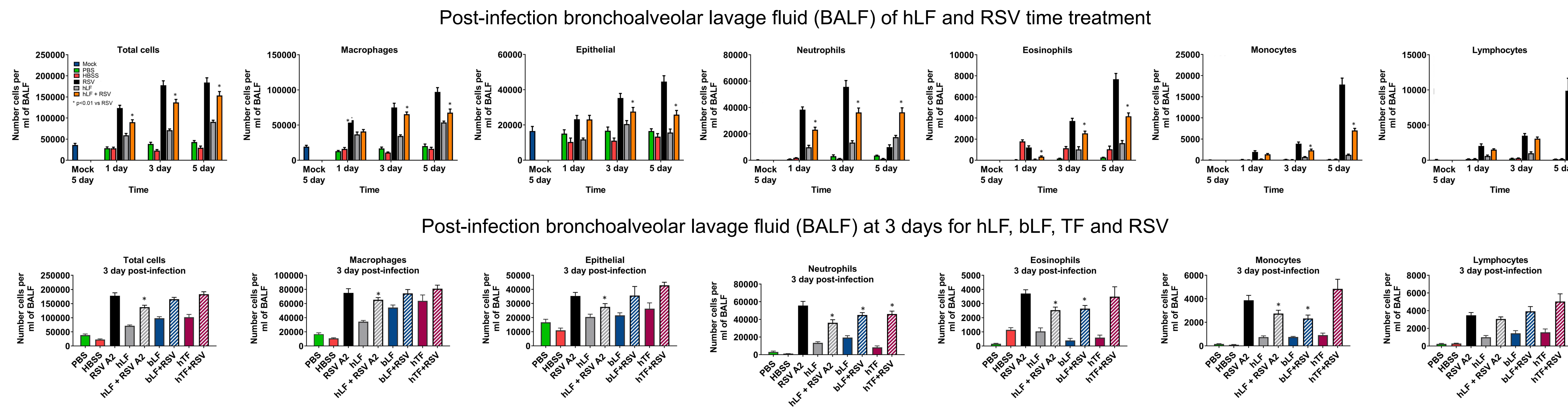
Test the hypothesis that lactoferrin has a protective role in lung cells during respiratory syncytial virus infection In vivo using a mouse model.

Nasal lactoferrin treatment reaches lung tissue in mouse model

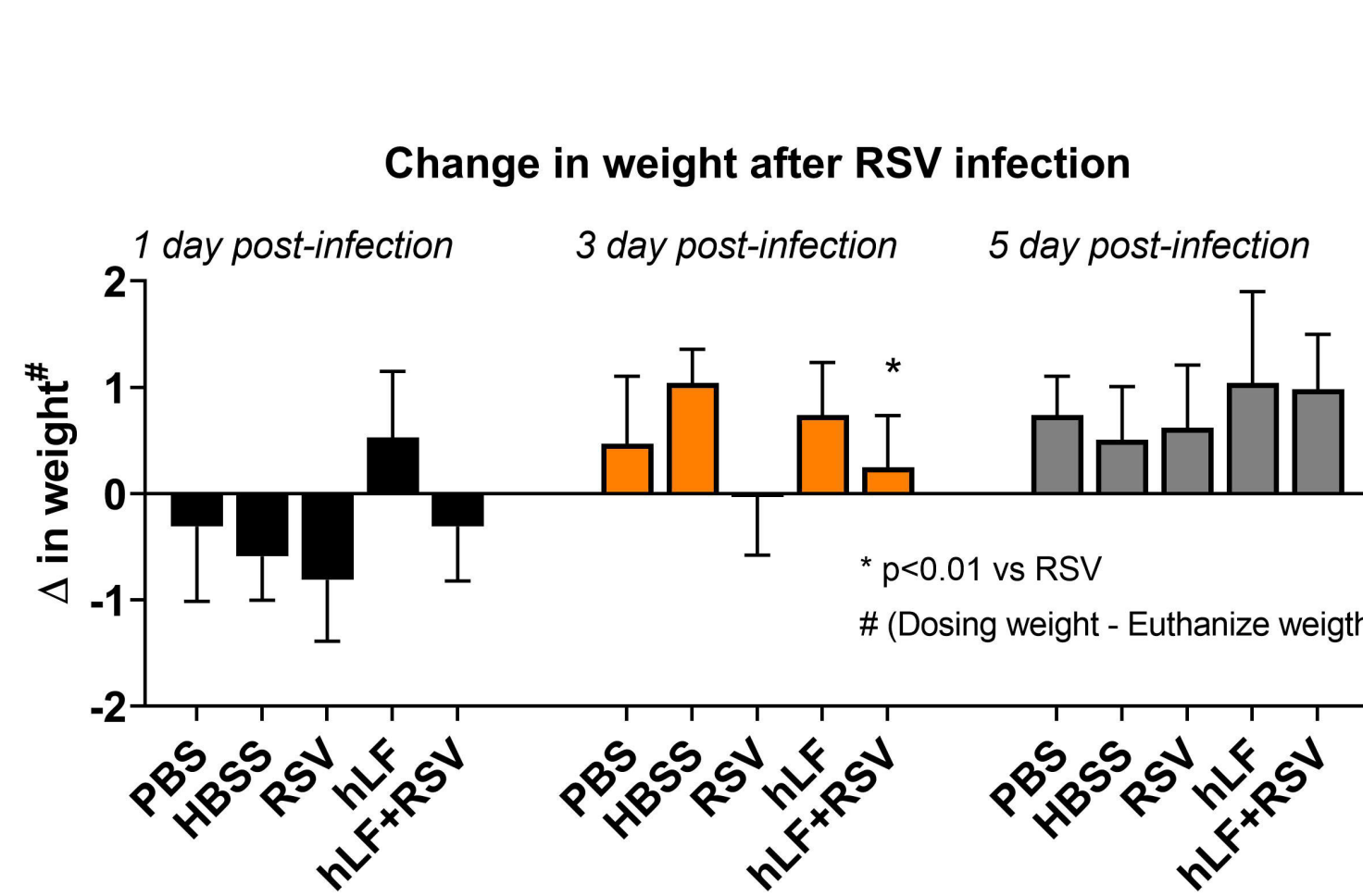


Mice were dose intranasally with hLF for 1 day, then cells were recovered from BALF and analyzed by FACS.

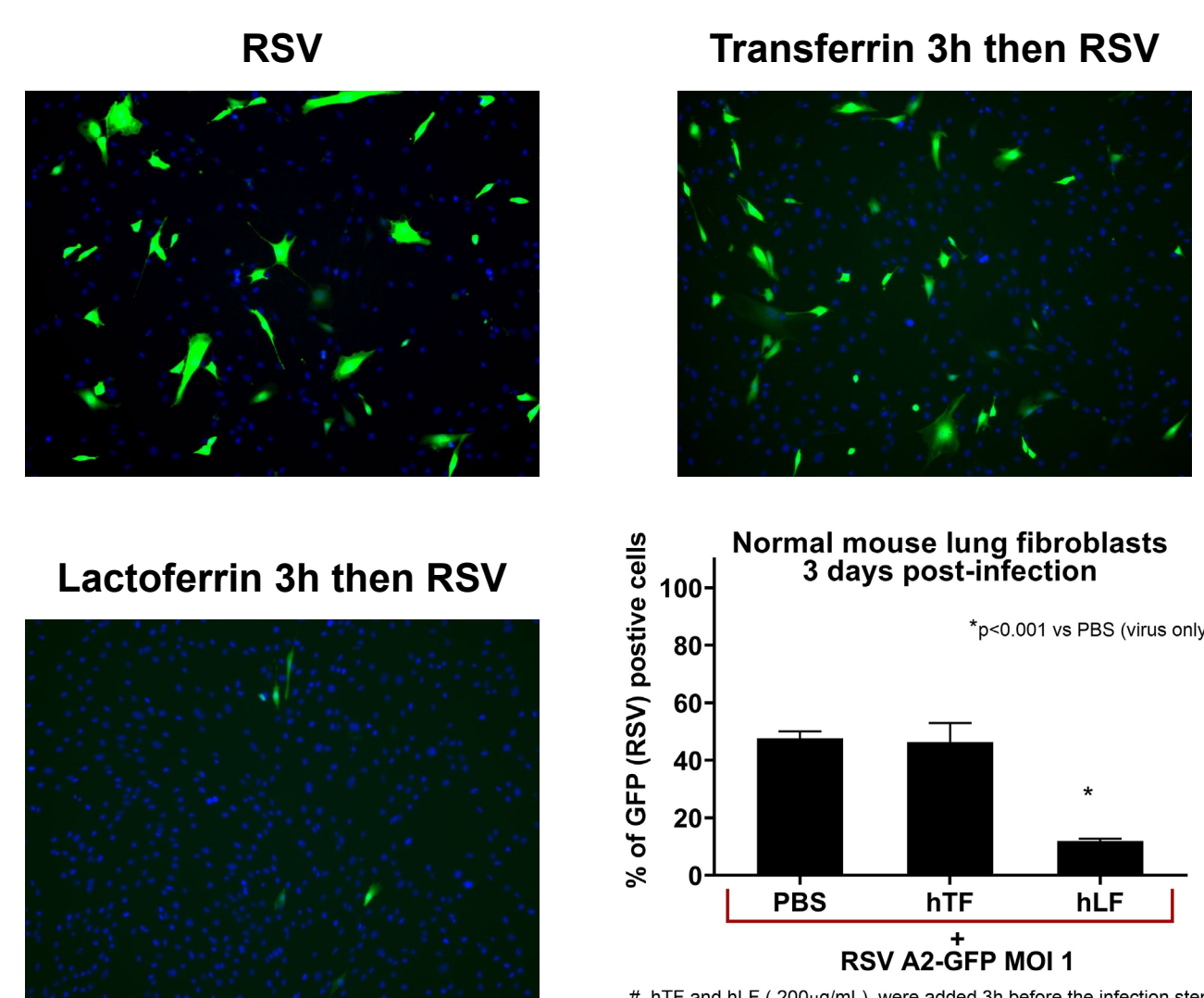
Lactoferrin treatment reduces RSV infectivity in mouse model.



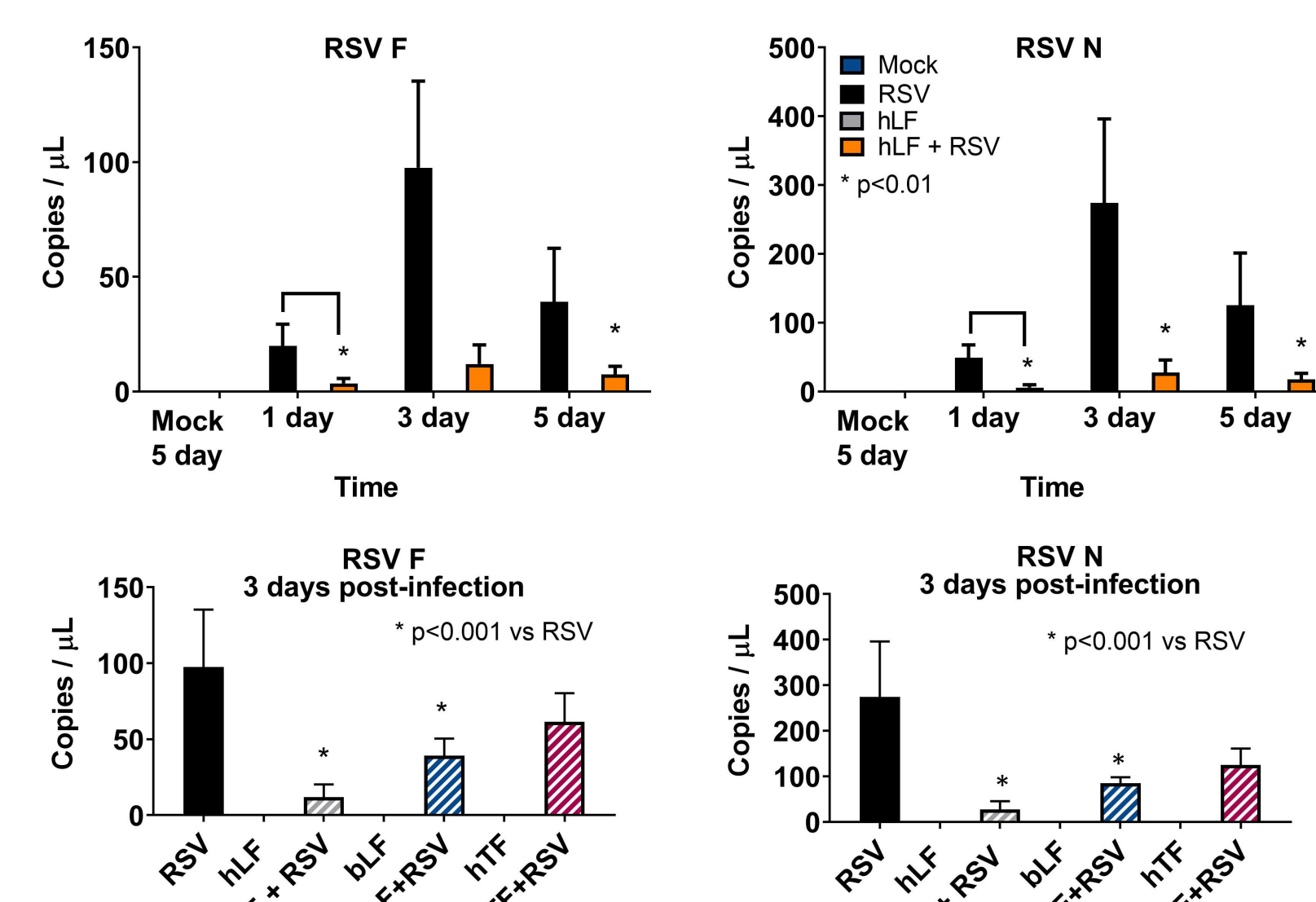
Body weight of mice after RSV infection



Lactoferrin reduces RSV infectivity in primary mouse lung cells



Lactoferrin reduces RSV gene expression in mice lung cells measure by ddPCR



Summary and Conclusion

- Mice treated with hLF and infected with RSV have less weight loss compared to the mice infected just with the virus.
- The hLF but not TF treatment reduced the inflammation induced by RSV infection in the lungs as indicated by the decrease in the number of macrophages, monocytes, eosinophils and lymphocytes in the BALF when compared to the RSV only infected mice
- Bovine LF also protected against RSV infection, though it was less efficiency when compared to hLF effects.
- hLF and bLF treatments decreased the expression of RSV F and N genes, indicating that intranasal dosing of LF specifically protects and dampens RSV infection
- We conclude that Intranasal administration of hLF and bLF has a protective and prophylactic antiviral activity against RSV infection in an RSV mouse model

Future Directions

- To study the immunomodulatory effects of lactoferrin we will conduct a transcriptomic analysis in lungs from RSV infected mice treated with human and bovine lactoferrin.
- Assess the histopathology of the upper and lower respiratory tract in mice infected with RSV that were pretreated with lactoferrin.
- Determine by plaque assays the RSV titers in lungs from mice infected with RSV with and without lactoferrin dosing.
- Human clinical trials can be designed in conjunction with a partnering lab in Argentina, where lactoferrin can be supplemented with the formula diet given to babies, and these subjects would be observed for effects on RSV disease severity.
- We established a cell model to understand LF effects on RSV infection in vitro (See poster abstract #ARB0030 Menendez et al.)

QUESTIONS? COMMENTS?

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