

National Institute of Environmental Health Sciences

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The antiviral activity of the milk protein lactoferrin against the human Respiratory Syncytial Virus ¹Immunity, Inflammation, and Disease Laboratory, ²Molecular Genomics Core, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709

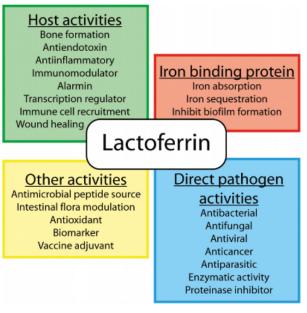
Abstract

In absence of an effective vaccine, finding effective strategies to treat or reduce respiratory syncytial virus (RSV) infection is a global public health priority. Lactoferrin (LF) is an iron-binding glycoprotein broadly distributed within the body fluids and predominantly found in mammalian breast milk. It is a key component of the innate immune system with well-known antimicrobial effects. In this study, we aimed to evaluate the antiviral and immunomodulatory properties of LF within the context of its potential applications against RSV. Human and bovine LF as well as the derived peptide lactoferricin B markedly inhibited in a dose and time-dependent manner the RSV-A2 strain entry, replication, and cytopathic effects when added before RSV infection or during the virus adsorption step in primary and cancer human and mouse cell lines. A related protein family member transferrin (TF) had no effects. In comparison to the control conditions, cells treated with the LF compounds had significantly less RSV infection response based on fluorescence localization of the virus, cytofluorometry, and expression of RSV-F gene measured by ddPCR. Furthermore, LF treatment before viral infection modulated the RSV-induced immune response at the transcriptional level. Our findings demonstrate that LF has a protective effect in response to RSV, suggesting LF as a strong candidate for an anti-RSV reagent that will be well-tolerated and effective in the prophylaxis against RSV infection.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Lactoferrin, a multifunctional protein.

- \succ Lactoferrin (LF) also known as lactotransferrin, is a~80 kDa iron-binding glycoprotein member of the transferrin family. It is found mainly in the saliva, tears and breast milk of mammalian species (1).
- \succ LF is a key element of host defenses against bacterial, viral, and parasitic infection, and it is considered an important component of the immune defense system at mucosal surfaces, including the upper and lower respiratory tracts (1-3).
- \succ LF 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).



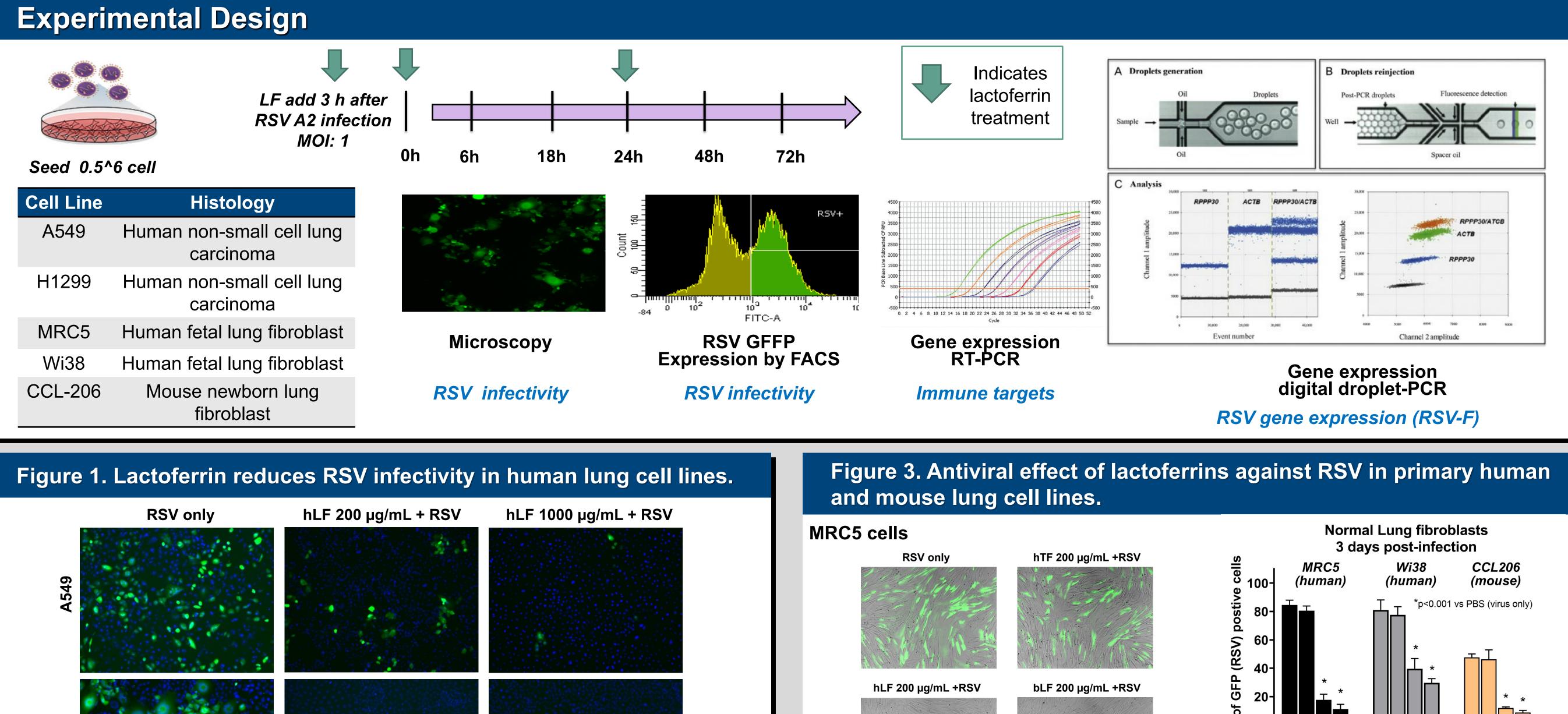
Vogel HJ., 2012 (1)

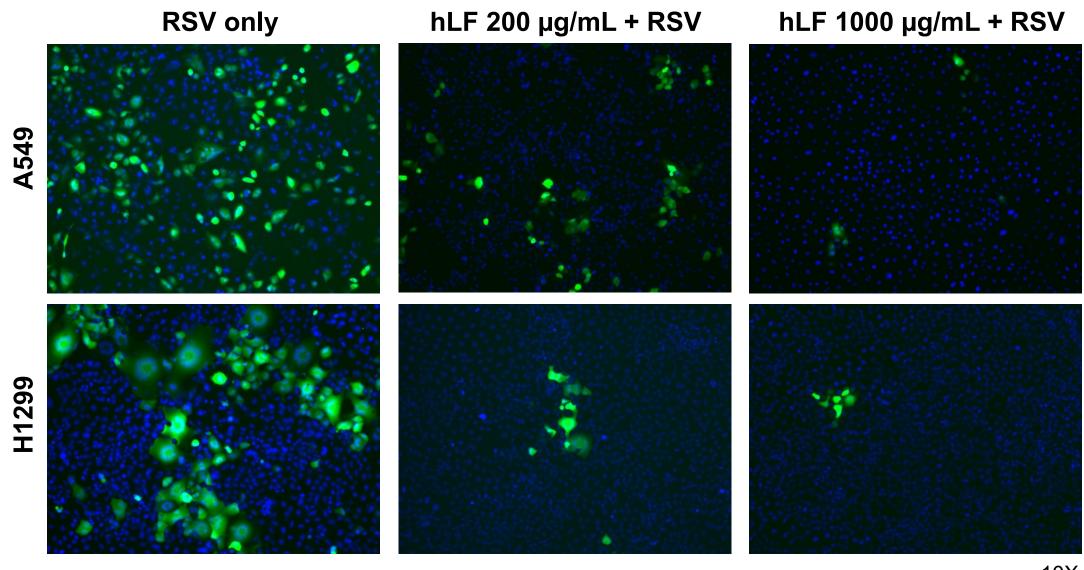
Respiratory Syncytial Virus

- > Respiratory syncytial virus (RSV) is a negative-sense, enveloped, singlestranded RNA virus of the family Paramyxoviridae.
- > 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 (5)
- > Infants who were breastfeeding are more protected against RSV severity and found that females were more protected than males, suggesting a possible gender difference and susceptibility (6).
- > Very little is known about the effects of lactoferrin against RSV. It has been reported that LF has antiviral activity against RSV in Hep2 cells (7,8). The effects of LF during RSV infection have not been studied in a lung cell model.

Objective

Test the hypothesis that lactoferrin has a protective role in lung cells against respiratory syncytial virus infection.





Human lactoferrin (hLF) was added 3h before infection

RSV-A2-GFP MOI 1 RSV-A2-GFP MOI 1

PBS 10 100 200 500 1000 1000 1000 200 500

numan LF [μg/mL]

added 3hr before infection

*p<0.001 vs PBS (virus only)

Green: RSV A2-GFP Blue: Cell nuclei (NucBlue®)

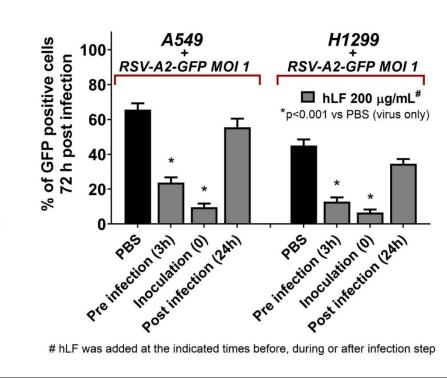
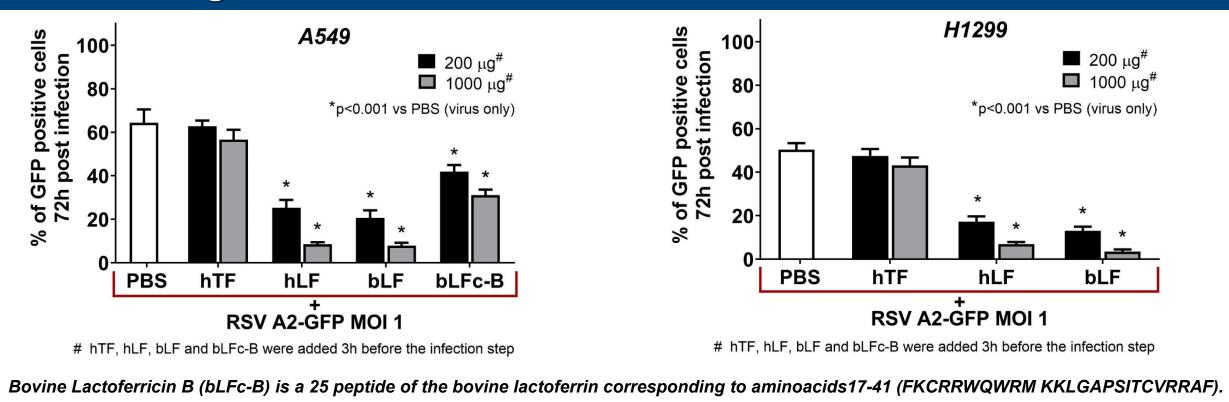


Figure 2. Specific prophylactic effects of human and bovine lactoferrin against RSV infection

No LF (PBS)



RSV A2-raGFP MOI

hLF (200 ug/mL)[#]

added 3h before infection

Human transferrin (hTF) is an iron binding glycoprotein that has antimicrobial activities and it is structurally related to lactoferrin.

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Green: RSV A2-GFP

hTF, hLF and bLF were added 3h before infection

Figure 4. Lactoferrin reduces expression of RSV genes and proteins.

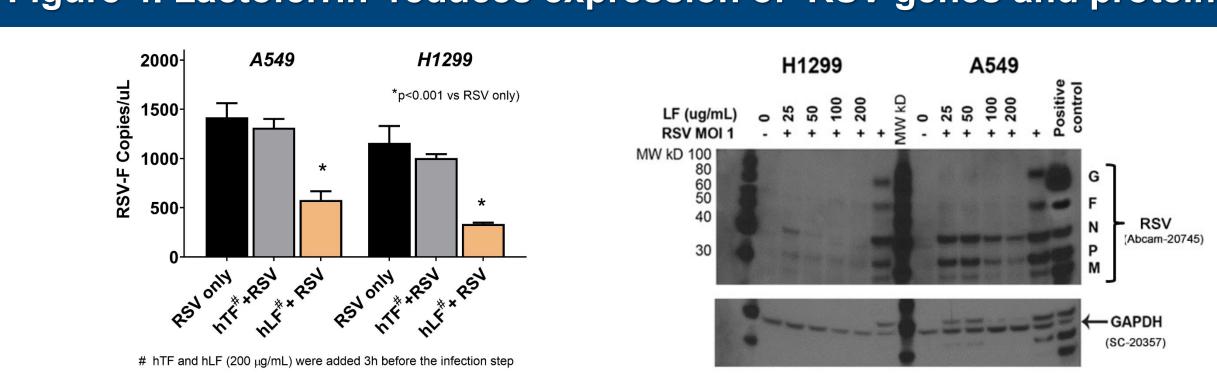
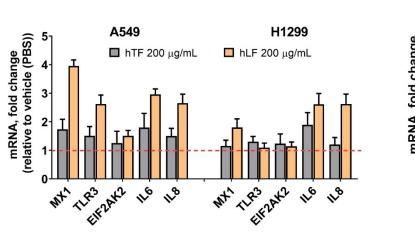
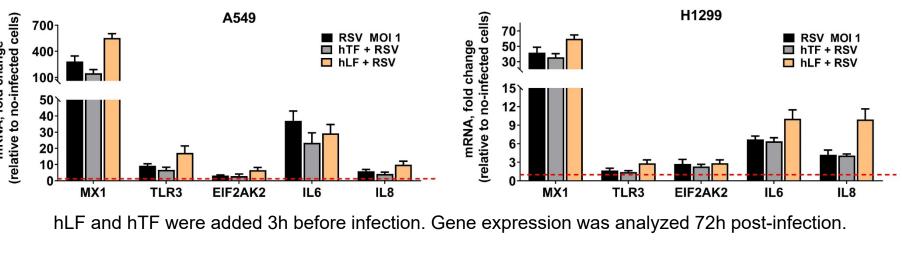


Figure 5. Lactoferrin enhances innate immune gene expression during RSV infection in human lung cells

In absence of RSV, lactoferrin but not transferrin has a modest effect inducing immune genes



Pre-treatment with lactoferrin enhances the RSV induced immune response at transcriptional level



positive positive positive

hTF, hLF and bLF (200µg/mL) were added 3h before the infection step

+ RSV A2-GFP MOI 1

ARB0030



Summary and Conclusions

- > Both hLF and bLF and its derivative LFc-B, significantly limited in a dose response manner, the entry of RSV in human and mouse lung primary and cancer cell lines, reducing the infectivity and cytopathic effects related to the infection.
- > Human LF has a prophylactic but not a therapeutic effect against RSV infection. LF protection occurs only when it is added before or during the infection process but not once the cells are already infected.
- > Human LF enhanced the expression of several RSV-induced innate immune genes, including the antiviral MX1, the proinflammatory cytokines IL6 and IL8, and the pathogen sensor TLR3 in a cell-dependent manner.
- > The antiviral and immunomodulatory effects against RSV are specific for lactoferrin, since the related family protein transferrin had no significant effect against RSV infection
- \succ We suggest the protective role for lactoferrin against RVS works by: 1) reducing the RSV entry into the cells and 2) modulating the expression of immune response genes.
- \succ Our findings demonstrate that LF has a protective effect in response to RSV, suggesting LF as a strong candidate for an anti-RSV reagent that will be welltolerated and effective in the prophylaxis against RSV infection.

Future Directions

- > For a more accurate overall perspective of lactoferrin's effect on the immune response, a transcriptome analysis of immune gene expression is currently being evaluated.
- > We are establishing a mouse model to understand LF effects on RSV infection in vivo (See poster abstract # ARB0034 Gladwell et al.)
- > Human clinical trials can be designed, where lactoferrin can be supplemented by aerosol to babies, and these subjects would be observed for effects on RSV disease severity.

QUESTIONS? COMMENTS?

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