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FULL PAPER

Coinfection with respiratory syncytial virus and rhinovirus increases IFN-λ1 and CXCL10 expression in human primary bronchial epithelial cells

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SUMMARY

Acute respiratory tract infection (ARTI) is common in all age groups, especially in children and the elderly. About 85% of children who present with bronchiolitis are infected with respiratory syncytial virus (RSV); however, nearly one-third are coinfected with another respiratory virus, such as human rhinovirus (HRV). Therefore, it is necessary to explore the immune response to coinfection to better understand the molecular and cellular pathways involving virus-virus interactions that might be modulated by innate immunity and additional host cell response mechanisms. This study aims to investigate the host innate immune response against RSV-HRV coinfection compared with monoinfection. Human primary bronchial/tracheal epithelial cells (HPECs) were infected with RSV, HRV, or coinfected with both viruses, and the infected cells were collected at 48 and 72 hours. Gene expression profiles of IL-6, CCL5, TNF-α, IFN-β, IFN-λ1, CXCL10, IL-10, IL-13, IRF3, and IRF7 were investigated using real-time quantitative PCR, which revealed that RSV-infected cells exhibited increased expression of IL-10, whereas HRV infection increased the expression of CXCL10, IL-10, and CCL5. IFN-λ1 and CXCL10 expression was significantly different between the coinfection and monoinfection groups. In conclusion, our study revealed that two important cytokines, IFN-λ1 and CXCL10, exhibited increased expression during coinfection.

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INTRODUCTION

Acute respiratory infections (ARIs) are the most important cause of acute illness, especially lower respiratory tract infections (LRTIs) with bronchiolitis, which represent a leading cause of morbidity and mortality in the first years of life (Stewart *et al.,* 2018; Mummidi *et al.,* 2017; Dumas *et al.,* 2019; Gurgel *et al.,* 2016; Sonawane *et al.,* 2019; Calvo *et al.,* 2015; Gil *et al.,* 2018). Respiratory syncytial virus (RSV) is the major causative agent of bronchiolitis worldwide; it causes approximately 85% of bronchiolitis cases (Stewart *et al.,* 2018; Calvo *et al.,* 2015). RSV occurs as a single infection in 68.8% of children who present with wheezing; however, nearly one-third are coinfected with another respiratory virus, such as human rhinovirus (HRV) (Lamborn *et al.,* 2017), with a prevalence of coinfection ranging from 14% to 44% (Gil *et al.,* 2018; Essaidi-Laziosi *et al.,* 2020). The effects of coinfection with RSV-HRV include increased disease severity and interference between these viruses (Pinky *et al.,* 2016), and the mechanisms and consequences of this coinfection require further investigation (Essaidi-Laziosi *et al.,* 2020; Achten *et al.,* 2017). Based on several studies of RSV-HRV interactions, mostly focused on interferon (IFN) type I (α and β) or type III (λ), IFNs are important cytokines that can confer temporary nonspecific immunity to the host to prevent viral infection (Lamborn *et al.,* 2017; Piret *et al.,* 2022; Kikkert 2020; Hillyer *et al.,* 2018). Innate immunity is usually studied in monoinfection, rather than coinfection. RSV bronchiolitis in infants has been observed to produce increased IL-4, IL-10, IL-13, and IL-33 levels (Garcia-Garcia *et al.,* 2017; Hase-

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gawa *et al.,* 2018). Moreover, the induction of IL-6 (Levitz *et al.,* 2012) and CXCL8 are associated with high pathogenicity and severe disease that requires hospitalization, whereas CCL5 induction results in local protection against RSV (Hillyer *et al.,* 2018). In contrast, HRV infection has been shown to increase CXCL9, IL-8, IL-10, CCL5, CXCL10, and CXCL11 expression (Tan *et al.,* 2018; Chun *et al.,* 2013; Zaheer *et al.,* 2010; Spurrell *et al.,* 2005; Jartti *et al.,* 2009). Importantly, several studies have found that IL-8, CXCL10, and CCL5 play a role in the pathogenesis of the exacerbation of HRV infection (Chun *et al.,* 2013; Zaheer *et al.,* 2010; Spurrell *et al.,* 2005). Indicating, although we already know the key important immune in whether RSV or HRV infection individually (Levitz *et al.,* 2012; Chun *et al.,* 2013; Zaheer *et al.,* 2010; Spurrell *et al.,* 2005; Machado *et al.,* 2017; Tekkanat *et al.,* 2002), coinfection data is a not as much of information. Therefore, in addition to the IFN response, the other immune responses to coinfection should be investigated to improve our understanding of the molecular and cellular pathways involved in virus-virus interactions that might be modulated by the induction of innate immunity and additional host cells response mechanisms.

Therefore, this study aimed to investigate the host innate immune response against RSV-HRV coinfection in bronchial-tracheal epithelial cells compared with monoinfection. It focused on three immunological categories. First, nonspecific immunity interferons (IFN) of type I (β) or type III (λ) are essential for regulating RSV infection. A second group, innate cytokines, is linked to immune cell polarization, differentiation, and recruitment composed of IL-6, CCL5, TNF-α, CXCL10, IL-10, and IL-13. Lastly, signal transduction, which reacts to intracellular RNA through the retinoic acid-inducible gene I (RIG-I), TLR3, and TLR7 pathways, which includes IRF3 and IRF7.

MATERIALS AND METHODS

Viral propagation

RSV (ATCC® VR-1540) and HRV (ATCC® VR-283) were propagated using HeLa cells. Briefly, HeLa cells were grown in Eagle's minimum essential medium (EMEM) with 1.5 g/L sodium bicarbonate, NEAA, L-glutamine, sodium pyruvate (Corning, USA), and 10% fetal bovine serum (FBS) (Gibco, USA). The HeLa cells were incubated at 37°C in a 90% humidified 5% CO_2 atmosphere until 90% confluence was reached. Then, the cells were removed and washed with phosphate-buffered saline (PBS; pH 7.4) prior to viral inoculation. The viruses were prepared and inoculated in a monolayer (1 ml virus) in EMEM with 1.5 g/L sodium bicarbonate, NEAA, L-glutamine, sodium pyruvate (Corning, USA), and 2% FBS (Gibco, USA). The samples were incubated for 2 hours at 37°C in a 90% humidified 5% CO_2 atmosphere and rocked every 20 minutes to redistribute the inoculum. Finally, virus growth medium (15 ml) was added and incubated at 37°C in a 90% humidified 5% CO_2 atmosphere for 5 days.

Viral titer and plaque assay

Vero cells were used for the plaque assay. Briefly, 1×10^6 cells per well were cultured in Dulbecco's modified Eagle medium (DMEM) (Corning, USA) with 10% FBS and incubated at 37°C in a 90% humidified 5% CO_2 atmosphere for 24 hours. The virus was diluted with DMEM without FBS using a 10-fold dilution: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Then, we removed the culture media and washed the cells with PBS (pH 7.4). We added each concentration of diluted virus (1 ml) into the cell culture and incubated it at 37°C in a 90% humidified 5% CO_2 atmosphere for 2 hours, rocking every 20 minutes to redistribute the inoculum. After viral adsorption, we removed the virus-containing culture media and added 3 ml of DMEM with 5% FBS. The samples were incubated at 37°C in a 90% humidified 5% CO_2 atmosphere for 10 days. The infected cells were fixed with 80% methanol for 1 hour then stained with 1% crystal violet (Merk, Germany) in 80% methanol. Finally, the plaque number was counted and calculated.

Viral monoinfection and coinfection in human primary bronchial epithelial cells

Each infection assay was performed in duplicate. Briefly, the mono- and coinfections were performed in normal human primary bronchial/tracheal epithelial cells (HPECs) (ATCC® PCS-300-010™) using 0.1 MOI of virus. Briefly, HPECs were cultured at 1×10^6 cell/well with airway epithelial cell basal medium (ATCC PCS-300-03) using the Bronchial/Tracheal Epithelial Cell Growth Kit (ATCC PCS300040) containing the following growth supplements: HLL supplement, L-glutamine, extract P, and airway epithelial cell supplement. The cells were incubated at 37°C in a 90% humidified 5% CO_2 atmosphere for 48 hours. After incubation, we removed the culture media and washed the cells with D-PBS (ATCC 30-2200). The virus (0.1 MOI) was inoculated and incubated at 37°C in a 90% humidified 5% CO_2 atmosphere for 2 hours. During incubation, the cells were rocked every 20 minutes to redistribute the inoculum. The virus-containing culture was then removed, and the culture medium was added. After incubation for 48 hours and 72 hours at 37°C in a 90% humidified 5% CO_2 atmosphere, the infected cells were harvested and preserved in RNAlater™ (Invitrogen, USA). Mock infection was performed using the same protocol except that during the virus infection step, culture medium was used instead of virus.

RNA extraction

Total RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen, USA). Briefly, infected

pellet cells were collected and 600 ml of RLT buffer was added. The cell suspension was vortexed for 1 minute to break the infected cells. The homogenized lysate was transferred to a gDNA Eliminator spin column and centrifuged at 12,000 rpm for 30 seconds. We then collected the supernatant and added 600 µl of 70% ethanol. The mixture was transferred to the RNeasy spin column and centrifuged at 12,000 rpm for 30 seconds. Then, 500 µL of PRE buffer was added to the column and centrifuged at 12,000 rpm for 30 seconds. The total RNA was eluted by adding 50 µl of RNase-free water and then centrifuged at 12,000 rpm for 1 minute.

Gene expression detection by real-time qPCR

Gene expression detection was performed in triplicate for every sample. Total RNA was converted to cDNA using iScript™ Reverse Transcription Supermix for reverse transcription qPCR (Bio-Rad, USA). Then, relative real-time quantitative PCR for IL-6, CCL5, TNF-α, IFN-β, IFN-λ1, CXCL10, IL-10, IL-13, IRF3, and IRF7 was performed using the iTaq Universal SYBR Green Supermix (Bio-Rad, USA). β-actin was used as a reference gene for normalization of gene expression. The primer list is shown in *Table1*.

Statistical analysis

The expression levels of 10 immune-associated genes were measured using Livak methods ($2^{-\Delta\Delta CT}$), and the differences in expression levels between 48 and 72 hours after RSV monoinfection in the HPECs were compared using a two-sample paired t-test for means. The comparison of the differential expression of immune genes between RSV monoinfection, HRV monoinfection, and coinfection at 48 and 72 hours was performed using one-way ANOVA. The differences between infection types were analyzed using the two-samples t-test, assuming equal variances ($P<0.05$) for both genes, and the P-values of multiple comparisons were analyzed using the Bonferroni post hoc t-test.

Table 1 - *Primer for quantitative Real-time PCR.*

Name	*Sequence (5' → 3')*	*References*
IL6_For	CACACAGACAGCCACTCACC	(Levitz *et al.*, 2017)
IL6_Rev	CCTCAAACTCCAAAAGACCA	(Levitz *et al.*, 2017)
CCL5_For	CGCTGTCATCCTCATTGCTA	(Levitz *et al.*, 2017)
CCL5_Rev	ACACACTTGGCGGTTCTTTC	(Levitz *et al.*, 2017)
TNFα_For	CTCCTCACCCACACCATCA	(Levitz *et al.*, 2017)
TNFα_Rev	GGAAGACCCCTCCCAGATAG	(Levitz *et al.*, 2017)
IFN-β_For	ATTGCCTCAAGGACAGGATG	(Zhu *et al.*, 2014)
IFN-β_Rev	GCTGCAGCTGCTTAATCTCC	(Zhu *et al.*, 2014)
IFN-λ1_For	GGACGCCTTGGAAGAGTCACT	(Moskwa *et al.*, 2018)
IFN-λ1_Rev	AGAAGCCTCAGGTCCCAATTC	(Moskwa *et al.*, 2018)
CXCL10_For	TCCACGTGTTGAGATCATTGC	(Ran *et al.*, 2022)
CXCL10_Rev	TCTTGATGGCCTTCGATTCTG	(Ran *et al.*, 2022)
IL10_For	GCTGGAGGACTTTAAGGGTTACCT	(Boeuf *et al.*, 2005)
IL10_Rev	CTTGATGTCTGGGTCTTGGTTCT	(Boeuf *et al.*, 2005)
IL13_For	ACAGCCCTCAGGGAGCTCAT	(Boeuf *et al.*, 2005)
IL13_Rev	TCAGGTTGATGCTCCATACCAT	(Boeuf *et al.*, 2005)
IRF3_For	TCGTGATGGTCAAGGTTGT	(Nasiri *et al.*, 2018)
IRF3_Rev	AGGTCCACAGTATTCTCCAG	(Nasiri *et al.*, 2018)
IRF7_For	GCAAGTGCAAGGTGTACTG	(Nasiri *et al.*, 2018)
IRF7_For	CACCAGCTCTTGGAAGAAGA	(Nasiri *et al.*, 2018)
B-actin Forw	TACGCCAACACAGTGCTGTCT	(Boukhvalova *et al.*, 2007)
B-actin Rev	TCTGCATCCTGTCGGCAAT	(Boukhvalova *et al.*, 2007)
HRV-Forw	GCTGTGCAGTTGGATGTGAT	(Zhu *et al.*, 2014)
HRV-Rev	AAAGCCATGATGCAATCTCC	(Zhu *et al.*, 2014)
NS1-For	CACAACAATGCCAGTGCTACAA	(Teng *et al.*, 2016)
NS1-Rev	TTAGACCATTAGGTTGAGAGCAATGT	(Teng *et al.*, 2016)

RESULTS

Immunity against RSV monoinfection, HRV monoinfection, and coinfection at 48 and 72 hours

Immunity against either mono- or coinfection with RSV and HRV was evaluated by investigating the expression of 10 genes: IL-6, CCL5, TNF-α, IFN-β, IFN-λ1, CXCL10, IL-10, IL-13, IRF3, and IRF7. β-actin was used as housekeeping gene for normalization. Expression analyses of the 10 immunity genes were performed using Livak methods ($2^{-\Delta\Delta CT}$). The results after HPECs were mono- or coinfected are shown in *Figure 1* and *Table 2*. Both 48 and 72 hours after infection, RSV monoinfection produced the highest fold change in IL-10 expression, whereas HRV monoinfection produced the highest fold change in CXCL10 followed by IL-10 and CCL5 expression. Coinfection at 48 hours produce increased CXCL10, IL-10, IFN-λ1, and CCL5 expression, while coinfection at 72 hours produced only increased expression of CXCL10.

Comparison of the expression of immune-associated genes between 48 and 72 hours after RSV monoinfection, HRV monoinfection, and coinfection

Comparison of the different expression levels between 48 and 72 hours after RSV monoinfection in HPECs was performed using a paired two-samples t-test for means, and the α was 0.05 ($P<0.05$). There was no significant difference in expression between the two time points for each gene (*Supplement* 1). In contrast with RSV monoinfection, HRV monoinfection produced significant differences in the expression of two genes: CXCL10 and IRF3. (*Supplement 2*). RSV-HRV coinfection produced significantly different expression in four genes (IFN-β, IFN-λ1,

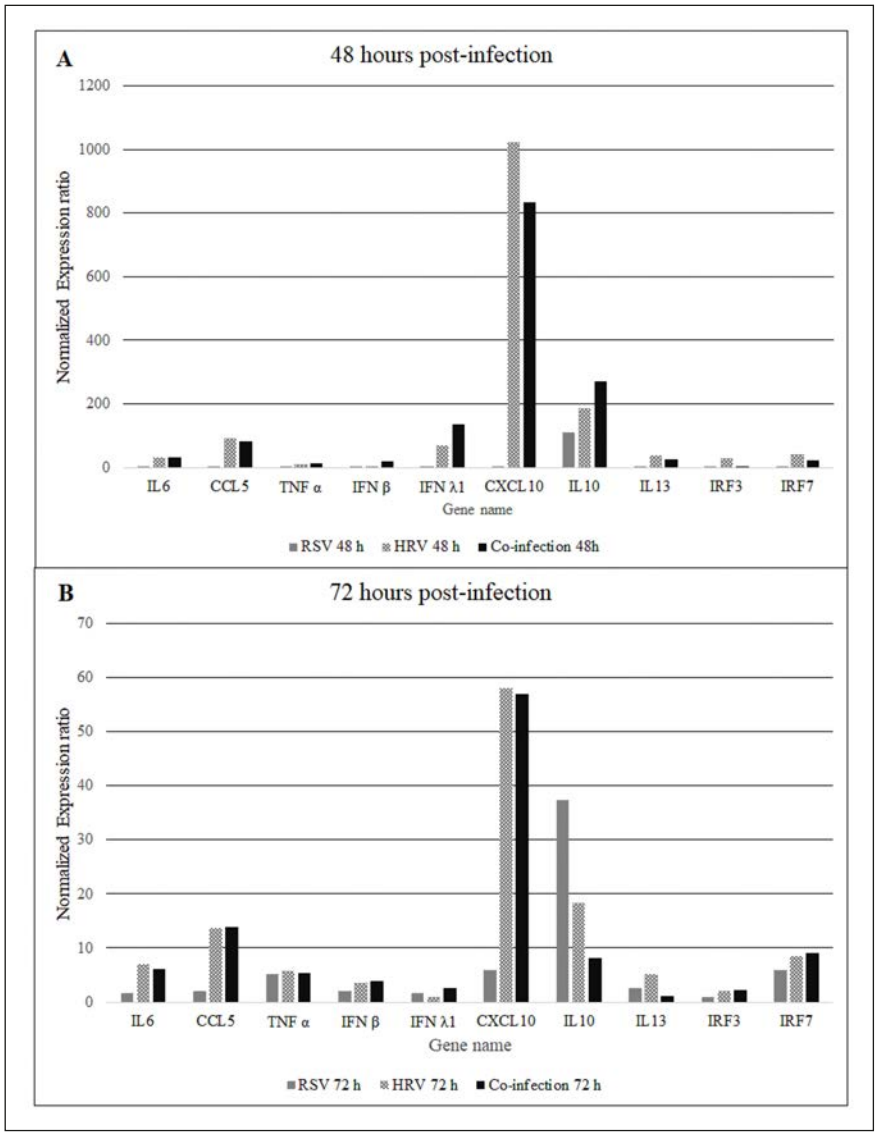

Figure 1 - *Normalized expression ratio of 10 immune genes at 48 and 72 hours after RSV monoinfection, HRV monoinfection, and coinfection of HPECs. 1A presents gene expression at 48 hours; 1B presents gene expression at 72 hours.*

Table 2 - *Fold change in immune gene expression after RSV monoinfection, HRV monoinfection, and coinfection at 48 and 72 hours.*

Gene	48 hours after infection			72 hours after infection		
	RSV	HRV	Coinfection	RSV	HRV	Coinfection
IL-6	1.00	32.61	31.46	1.76	7.03	6.10
CCL5	0.69	90.49	82.88	2.00	13.62	13.92
TNF-α	0.89	8.86	11.29	5.14	5.82	5.46
IFN-β	0.58	1.04	18.41	2.11	3.49	3.80
IFN-λ1	1.51	30.80	135.57	1.76	0.90	2.68
CXCL10	2.12	1023.87	834.62	5.99	57.97	57.00
IL-10	108.72	186.79	271.64	37.29	18.26	8.24
IL-13	1.21	37.17	24.04	2.59	5.27	1.10
IRF3	0.82	29.16	2.06	0.98	2.02	2.18
IRF7	0.97	40.64	23.27	6.00	8.47	9.05

CXCL10 and IL-13) between 48 and 72 hours (*Supplement 3*). Whether HRV monoinfection or coinfection, there were differences in the expression of the significant cytokines, which peaked at 48 hours and declined drastically at 72 hours.

Comparison of immune gene expression between RSV monoinfection, HRV monoinfection, and coinfection at 48 and 72 hours

The comparison of immune gene expression between RSV, HRV, and coinfection at 48 hours was performed using one-way ANOVA, and α was 0.05. The results revealed that IFN-λ1 and CXCL10 expression was significantly different between the three infection types (P=0.019521 and 9×10^{-6}, respectively) (*Supplement 4*). Then, the two-sample t-test assuming equal variances was performed (P<0.05) to determine which infection type produced significantly different expression levels of both genes. There was no significant difference in IFN-λ1 expression between RSV and HRV monoinfection; however, IFN-λ1 of coinfection was found to express greater than both RSV and HRV monoinfection (P=0.0085 and 0.006, respectively) (*Figure 2*). CXCL10 expression was significantly different between RSV and HRV monoinfection ($P=1\times10^{-5}$). Similarly, CXCL10 expression was significantly higher in coinfections than in RSV monoinfection (P=0.0001) (*Figure 3*); however, there was no significant difference in expression between coinfection and HRV monoinfection.

At 72 hours, the expression of immune genes between RSV monoinfection, HRV monoinfection, and

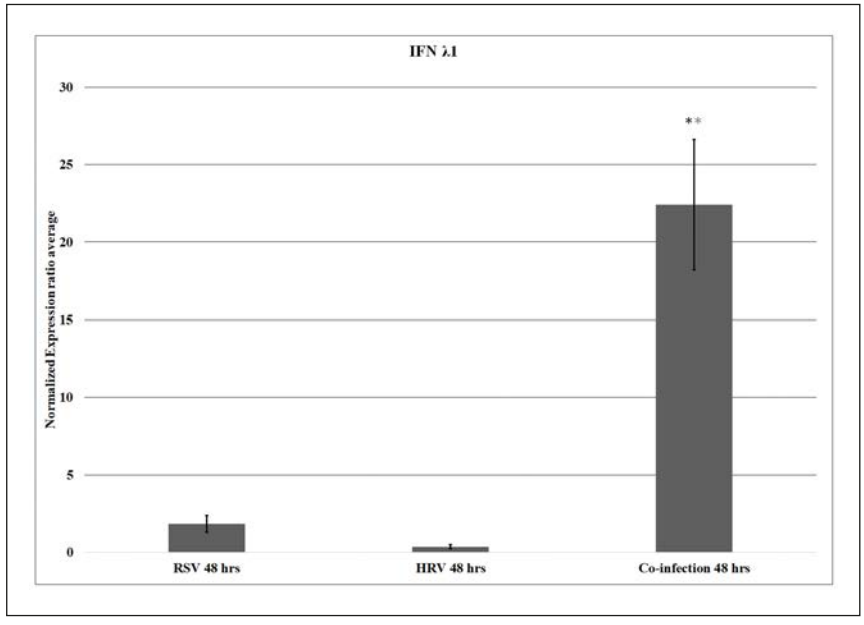

Figure 2 - *The comparison of IFN-λ1 expression at 48 hours between the three infection types: i) RSV monoinfection, ii) HRV monoinfection, and iii) coinfection.*
* There was a statistically significant difference between coinfection and RSV monoinfection.
* There was a statistically significant difference between coinfection and HRV monoinfection.

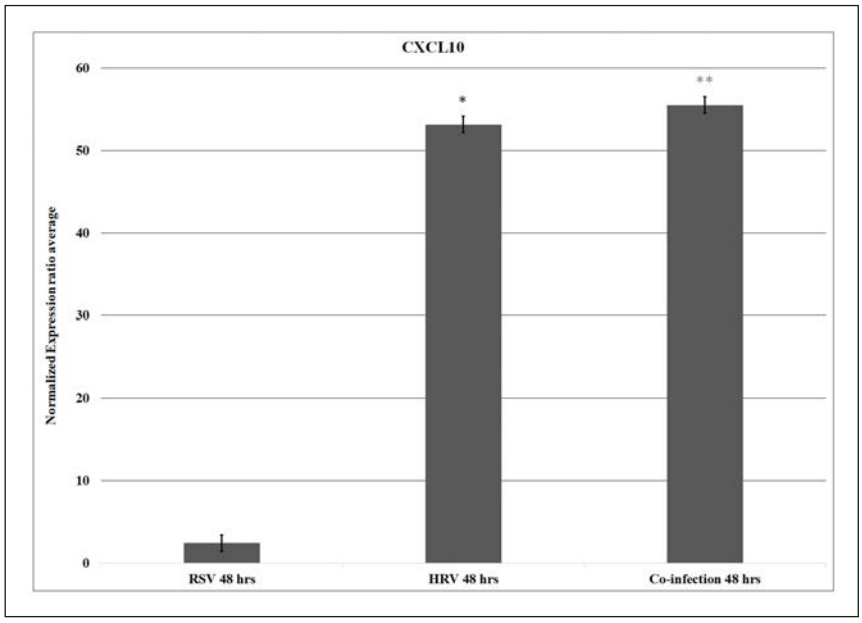

Figure 3 - *The comparison of CXCL10 expression at 48 hours between the three infection types: i) RSV monoinfection, ii) HRV monoinfection, and iii) coinfection.*
* There was a statistically significant difference between RSV and HRV monoinfection.
** There was a statistically significant difference between RSV monoinfection and coinfection.

coinfection was not significant for all genes (*Supplement 5*).

DISCUSSION

This study investigated the host innate immune response to RSV-HRV coinfection, as both viruses are the most frequent causative agents of LRTIs with bronchiolitis, which is a leading cause of morbidity during the first year of life (Stewart *et al.,* 2018; Calvo *et al.,* 2015). These infections are responsible for sequelae such as recurrent wheezing and asthma (Vandini *et al.,* 2017). In this study, we infected HPECs with 0.1 MOI of both viruses to evaluate monoinfection and coinfection; then, the expression of 10 innate immunity genes was investigated at 48 and 72 hours post-infection (hpi). At 48 hours after RSV monoinfection, only IL-10 expression was significantly increased; after HRV monoinfection, the expression of CXCL10, CCL5, and IL-10 was significantly increased. Coinfection with both viruses produced increased expression of the following four cytokines in descending order: CXCL10, IL-10, IFN-λ1, and CCL5. Remarkably, IL-10 expression was high in all three infection types, and was the highest in coinfection. IL-10 expression is induced by NF-κB, which is a transcription factor associated with over 100 proinflammatory genes, and is involved in the recruitment and activation of various immune cells, which produce more inflammatory mediators (Hasegawa *et al.,* 2018; Chun *et al.,* 2013; Hu *et al.,* 2020). The increased IL-10 levels in viral infections might be associated with disease exacerbation (triggered by IFN-γ) and may cause pre-existing airway inflammation (Jartti *et al.,* 2009). Similar to our study, Hasegawa *et al*. reported increased IL-10 expression in the nasal airway epithelium of young children (aged <3 years) infected with HRV and RSV (Hasegawa *et al.,* 2018).

Furthermore, our study revealed that CXCL10 and CCL5 expression was increased in both HRV monoinfection and coinfection with RSV. CXCL10 is a chemoattractant for type 1 T lymphocyte and natural killer cell recruitment (Zaheer *et al.,* 2010; Spurrell *et al.,* 2005). Generally, it is produced by several cell types in response to viral infection, namely airway epithelial cells; furthermore, CXCL10 levels are correlated with symptom severity, viral titer, and the number of lymphocytes in airway secretions (Spurrell *et al.,* 2005; Moskwa *et al.,* 2018). CXCL10 may associate with the pathogenesis of HRV-induced colds and exacerbations of chronic obstructive pulmonary disease and asthma (Spurrell *et al.,* 2005), and CXCL10 has been proposed as a serum biomarker for asthma exacerbation (Tan *et al.,* 2018). Similarly, CCL5 is increased in cells infected with rhinovirus or acute exacerbations of asthma, inducing selective recruitment of Th2-type T cells and eosinophils (Chun *et al.,* 2013). Both CXCL10 and CCL5 play an important role in reducing viral infections and regulating the host response to RSV infection (Hillyer *et al.,* 2018; Machado *et al.,* 2017; Lindell *et al.,* 2008).

Finally, comparison between coinfection with monoinfection revealed the increased expression of two important cytokines, IFN λ1 and CXCL10, especially in RSV infection. In addition, CXCL10 expression was significantly different between RSV and HRV monoinfection. CXCL10 can suppress and limit RSV infection; however, in infants younger than 3 months of age who still have maternal RSV-specific antibodies, those antibodies can enhance CXCL10 production and have been implicated in enhanc-

ing the inflammatory response and pathogenesis of RSV (Vissers *et al.,* 2015). Moreover, this cytokine is a promising potential pharmacological target for various infectious diseases (Lindell *et al.,* 2008); it is a very attractive novel candidate for RSV therapies and may be combined with the IFN-λ1, which can confer temporary, nonspecific immunity against viral infection. However, the interaction between both viruses is antagonistic, as HRV can suppress RSV replication (Greer *et al.,* 2009). Therefore, the association of disease could concern not only the virus but also in the inflammatory profile of the antiviral immune response (Narayanan *et al.,* 2022). In-depth exploration of the molecular innate immune response to coinfection should be considered in the development of vaccines, therapeutic treatments, and prophylaxis targeting inflammation.

In conclusion, the virus-virus interaction in coinfection has an important effect on the treatment, vaccine strategies, and prognosis of the disease, as viral coinfection may be associated with a higher level of care in the clinical management of patients.

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REFERENCES

Achten N.B., Wu P., Bont L., Blanken M.O., Gebretsadik T., et al. (2017). Interference Between Respiratory Syncytial Virus and Human Rhinovirus Infection in Infancy. *J Infect Dis*. **215** (7), 1102-1106.

Boeuf P., Vigan-Womas I., Jublot D., Loizon S., Barale J.C., et al. (2005). CyProQuant-PCR: a real time RT-PCR technique for profiling human cytokines, based on external RNA standards, readily automatable for clinical use. *BMC Immunol*. **6**, 5.

Boukhvalova M.S., Prince G.A., Blanco J.C. (2007). Respiratory syncytial virus infects and abortively replicates in the lungs in spite of preexisting immunity. *J Virol*. **81** (17), 9443-9450.

Calvo C., Garcia-Garcia M.L., Pozo F., Paula G., Molinero M., et al. (2015). Respiratory Syncytial Virus Coinfections With Rhinovirus and Human Bocavirus in Hospitalized Children. *Medicine (Baltimore)*. **94** (42), e1788.

Chun Y.H., Park J.Y., Lee H., Kim H.S., Won S., et al. (2013). Rhinovirus-Infected Epithelial Cells Produce More IL-8 and RANTES Compared With Other Respiratory Viruses. *Allergy Asthma Immunol Res*. **5** (4), 216-223.

Dumas O., Hasegawa K., Mansbach J. M., Sullivan A. F., Piedra P. A. , et al. (2019). Severe bronchiolitis profiles and risk of recurrent wheeze by age 3 years. *J Allergy Clin Immunol*. **143** (4): 1371-1379 e1377.

Essaidi-Laziosi M., Geiser J., Huang S., Constant S., Kaiser L., et al. (2020). Interferon-Dependent and Respiratory Virus-Specific Interference in Dual Infections of Airway Epithelia. *Sci Rep*. **10** (1), 10246.

Garcia-Garcia M. L., Calvo C., Moreira A., Canas J. A., Pozo F., et al. (2017). Thymic stromal lymphopoietin, IL-33, and periostin in hospitalized infants with viral bronchiolitis. *Medicine (Baltimore)*. **96** (18), e6787.

Gil J., Almeida S., Constant C., Pinto S., Barreto R., et al. (2018). Short-term relevance of lower respiratory viral coinfection in inpatients under 2 years of age. *An Pediatr (Engl Ed)*. **88** (3), 127-135.

Greer R.M., McErlean P., Arden K.E., Faux C.E., Nitsche A., et al. (2009). Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol*. **45** (1), 10-15.

Gurgel R.Q., Bezerra P.G.M., Duarte M., Moura A.A., Souza E.L., et al. (2016). Relative frequency, Possible Risk Factors, Viral Co-detection Rates, and Seasonality of Respiratory Syncytial Virus Among Children With Lower Respiratory Tract Infection in Northeastern Brazil. *Medicine (Baltimore)*. **95** (15), e3090.

Hasegawa K., Perez-Losada M., Hoptay C.E., Epstein S., Mansbach J.M., et al. (2018). RSV vs. rhinovirus bronchiolitis: difference in nasal airway microRNA profiles and NFkappaB signaling. *Pediatr Res*. **83** (3), 606-614.

Hillyer P., Shepard R., Uehling M., Krenz M., Sheikh F., et al. (2018). Differential Responses by Human Respiratory Epithelial Cell Lines to Respiratory Syncytial Virus Reflect Distinct Patterns of Infection Control. *J Virol*. **92** (15).

Hu M., Bogoyevitch M. A., Jans D.A. (2020). Impact of Respiratory Syncytial Virus Infection on Host Functions: Implications for Antiviral Strategies. *Physiol Rev*. **100** (4), 1527-1594.

Jartti T., Paul-Anttila M., Lehtinen P., Parikka V., Vuorinen T., et al. (2009). Systemic T-helper and T-regulatory cell type cytokine responses in rhinovirus vs. respiratory syncytial virus induced early wheezing: an observational study. *Respir Res*. **10**, 85.

Kikkert M. (2020). Innate Immune Evasion by Human Respiratory RNA Viruses. *J Innate Immun*. **12** (1), 4-20.

Lamborn IT., Jing H., Zhang Y., Drutman S. B., Abbott J.K., et al. (2017). Recurrent rhinovirus infections in a child with inherited MDA5 deficiency. *J Exp Med*, **214** (7), 1949-1972.

Levitz R., Gao Y., Dozmorov I., Song R., Wakeland E.K., et al. (2017). Distinct patterns of innate immune activation by clinical isolates of respiratory syncytial virus. *PLoS One*. **12** (9): e0184318.

Levitz R., Wattier R., Phillips P., Solomon A., Lawler J., et al. (2012). Induction of IL-6 and CCL5 (RANTES) in human respiratory epithelial (A549) cells by clinical isolates of respiratory syncytial virus is strain specific. *Virol J*. **9**, 190.

Lindell D.M., Lane T.E., Lukacs N.W. (2008). CXCL10/CXCR3-mediated responses promote immunity to respiratory syncytial virus infection by augmenting dendritic cell and CD8(+) T cell efficacy. *Eur J Immunol*. **38** (8), 2168-2179.

Machado D., Hoffmann J., Moroso M., Rosa-Calatrava M., Endtz H., et al. (2017). RSV Infection in Human Macrophages Promotes CXCL10/IP-10 Expression during Bacterial Co-Infection. *Int J Mol Sci*. **18** (12).

Moskwa S., Piotrowski W., Marczak J., Pawelczyk M., Lewandowska-Polak A., et al. (2018). Innate Immune Response to Viral Infections in Primary Bronchial Epithelial Cells is Modified by the Atopic Status of Asthmatic Patients. *Allergy Asthma Immunol Res*. **10** (2), 144-154.

Mummidi P.S., Tripathy R., Dwibedi B., Mahapatra A., Baraha S. (2017). Viral aetiology of wheezing in children under five. *Indian J Med Res*. **145** (2), 189-193.

Narayanan D., Grayson M.H. (2022). Comparing respiratory syncytial virus and rhinovirus in development of post-viral airway disease. *J Asthma*. **59** (3), 434-441.

Nasiri M., Geramizadeh B., Nabavizadeh S.H., Male-Hosseini S.A., Karimi M.H., et al. (2018). mRNA Expression of Interferon Regulatory Factors during Acute Rejection of Liver Transplants in Patients with Autoimmune Hepatitis. *Int J Organ Transplant Med*. **9** (1), 34-40.

Pinky L., Dobrovolny H.M. (2016). Coinfections of the Respiratory Tract: Viral Competition for Resources. *PLoS One*, **11** (5), e0155589.

Piret J., Boivin G. (2022). Viral Interference between Respiratory Viruses. *Emerg Infect Dis*. **28** (2), 273-281.

Ran X. H., Zhu J. W., Chen Y.Y., Ni R. Z., Mu D. (2022). Papain-like protease of SARS-CoV-2 inhibits RLR signaling in a deubiquitination-dependent and deubiquitination-independent manner. *Front Immunol*. **13**, 947272.

Spurrell J.C., Wiehler S., Zaheer R.S., Sanders S.P., Proud D. (2005). Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. *Am J Physiol Lung Cell Mol Physiol*. **289** (1), L85-95.

Stewart C.J., Hasegawa K., Wong M.C., Ajami N.J., Petrosino J.F., et al. (2018). Respiratory Syncytial Virus and Rhinovirus Bronchiolitis Are Associated With Distinct Metabolic Pathways. *J Infect Dis*, **217** (7), 1160-1169.

Sonawane A.A., Shastri J., Bavdekar S.B. (2019). Respiratory Patho-

gens in Infants Diagnosed with Acute Lower Respiratory Tract Infection in a Tertiary Care Hospital of Western India Using Multiplex Real Time PCR. *Indian J Pediatr*. **86** (5), 433-438.

Tan K.S., Ong H.H., Yan Y., Liu J., Li C., et al. (2018). In Vitro Model of Fully Differentiated Human Nasal Epithelial Cells Infected With Rhinovirus Reveals Epithelium-Initiated Immune Responses. *J Infect Dis.*, **217** (6), 906-915.

Tekkanat K.K., Maassab H., Miller A., Berlin A.A., Kunkel S.L., et al. (2002). RANTES (CCL5) production during primary respiratory syncytial virus infection exacerbates airway disease. *Eur J Immunol*. **32** (11), 3276-3284.

Teng M. N.,Tran K.C. (2016). Use of Minigenome Systems to Study RSV Transcription. *Methods Mol Biol*. **1442**, 155-164.

Vandini S., Calamelli E., Faldella G., Lanari M. (2017). Immune and inflammatory response in bronchiolitis due to respiratory Syncytial Virus and Rhinovirus infections in infants. *Paediatr Respir Rev*. **24**, 60-64.

Vissers M., Schreurs I., Jans J., Heldens J., de Groot R., et al. (2015). Antibodies enhance CXCL10 production during RSV infection of infant and adult immune cells. *Cytokine*. **76** (2), 458-464.

Zaheer R.S., Proud D. (2010). Human rhinovirus-induced epithelial production of CXCL10 is dependent upon IFN regulatory factor-1. *Am J Respir Cell Mol Biol*. **43** (4), 413-421.

Zhu L., Lee B., Zhao F., Zhou X., Chin V., et al. (2014). Modulation of airway epithelial antiviral immunity by fungal exposure. *Am J Respir Cell Mol Biol*. **50** (6), 1136-1143.