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Highly differentiated human airway epithelial cells immunological response to respiratory syncytial virus and human rhinovirus C.

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ABSTRACT
Background : Highly differentiated human airway epithelial cells immunological response to respiratory syncytial virus and human rhinovirus C.
Methods :HBE cells were developed in an ALI culture before being infected with two RSV isolates, the full-length cDNA clones of HRV-C651 and HRV-C15, two clinical isolates of HRV-C79, and HRV-C101. On cytokine secretion, the impact of HRV-C was evaluated and contrasted with that of RSV.
Results :HRV-Cs infect and propagate in fully differentiated HBE cells and significantly increase the secretion of IFN-λ1, CCL5, IP10, IL-6, IL-8, and MCP-1. The virus loads positively correlated with the levels of the cytokines. HRV-C induced lower secretion of CCL5 ($P = 0.048$), IL-6 ($P = 0.016$), MCP-1 ($P = 0.008$), and IL-8 ($P = 0.032$), and similar secretion of IP10 ($P = 0.214$) and IFN-λ1 ($P = 0.214$) when compared with RSV.
Conclusion : Infection and multiplication of HRV-C were promoted by the HBE ALI culture system, and HRV-C generated substantially weaker cytokine production than RSV.

I. BACKGROUND

Numerous clinical illnesses, particularly in children, are brought on by human rhinovirus (HRV) infection, including the common cold, bronchiolitis, pneumonia, and asthma aggravation [1]. Based on evolutionary sequence analysis, HRV is commonly classified into three species (HRV-A, HRV-B, and HRV-C) [2]. Given that HRV-C infections are more common in kids with pneumonia, severe wheezing, and asthma exacerbations, the recently found HRV-C is linked to a high percentage of HRV hospitalizations and may even be more virulent [3, 4]. Given the part HRV-C plays in these illnesses, it is crucial to comprehend the host- or virus-specific variables that affect pathogenesis.

The primary location of HRV infection is the airway epithelial cells. A number of proinflammatory cytokines and chemokines, which are linked to pathogenic pathways and aid in the onset of an antiviral response, are released when airway epithelial cells exposed to HRV. Unlike HRV-A or -B, HRV-C cannot be passed on through immortalized cells. There isn't much data on host cell reactions to HRV-C infection because it's difficult to culture this species. The successful amplification of HRV-C from clinical samples in sinus mucosal organ cultures [5] and in differentiated sinus epithelial cells [6] as well as HRV-C produced from infectious clones in fully differentiated human airway epithelial (HAE) cells [7] have all been reported in a few investigations. Even while non-airway cell lines have been used in studies to study HRV replication and immunological responses, human bronchial epithelial cells (HBE) are the natural host for HRV infections (e.g., HeLa cells). A pseudostratified epithelium formed by HBE cells cultivated in vitro at the air-liquid interface (ALI) at the ALI gives a fair replica of the airway epithelium in vivo by forming tight junctions, cilia, and mucin [8].

While HRV is more frequently engaged in wheezing exacerbations in later childhood [10] and appears to be less destructive to bronchial structures than RSV, epidemiological data imply that RSV infection causes persistent wheeze and asthma. We therefore pondered whether these variations in disease features would also be associated by variations in cytokine or chemokine induction. We propagated two HRV-C viruses made from infectious molecular clones of PC15 and Lz651 and two clinical specimens in fully differentiated human airway epithelial cells and analyzed the difference in cytokine secretion induced by HRV-C and RSV to better understand HRV-C pathogenesis and the immune responses between HRV-C and RSV infection in ALI HBE.

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II. MATERIALS AND METHODS

➢ Viruses and cells

The full-length cDNA copies of HRV-C were found on two plasmids, pCLZ651 and pC15, from (Rubizhne Luhansk CDC, Luhansk Ukraine). The two clinical HRV-C samples used were lz79 (GenBank: JF317014) and lz101 (GenBank: JF317017). RSV samples RSV1 (RSV-A1: ATCC-VR-1540) and RSV2 (RSV-B1: ATCC-VR-955) were utilised.

In order to test for mycoplasma, HeLa cells were grown in DMEM supplemented with 10% FBS (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Gibco). The mycoplasma test kit was provided by ExCell Bio in Kiev, Ukraine.

➢ Cultures of primary bronchial epithelial cells grown on ALI

As previously mentioned [11], primary HBE cells were obtained from patients who had surgical lung resection for pulmonary illnesses in Rubizhne Luhansk Hospital. HBE cells were grown in BEGM medium (Lonza, Germany) supplemented with the necessary ingredients after being plated onto type I and type III collagen-coated six-well tissue culture plates (Lonza). Trypsin was used to dissociate conventional monolayer two-dimensional (2D) HBE cells after they achieved 80%–90% confluence, and 3 105 cells were then planted on type IV collagen–coated 12-well transwell inserts (Costar, ME, USA). Every other day, the medium was changed for the surfaces that were apical and basolateral. In order to allow the HBE cells to fully confluence, the media was subsequently switched to air-liquid interface (ALI) medium (BEGM + DMEM + additives). Only the basolateral compartment of the HBE cells was grown in ALI media and exposed to air after 5 days. After maintaining the ALI culture for 4-6 weeks, the cells differentiated into 3D pseudostratified HBE cells. All cultures were kept in an incubator with 5% CO2 at 37 °C prior to the tests.

➢ Transcription, transfection, and inoculation of HRV-C RNA in vitro

The two plasmids, Lz651 and PC15, that contained the HRV-C viral genome were linearized by digestion with Endonuclease Cal I (NEB), and then HRV-C RNA was translated in vitro using a MEGAscript® T7 Transcription Kit (Ambion) in accordance with the product's instructions. The RNA transcripts underwent DNase I treatment (Promega), MEGAclearTM Transcription Clean-Up Kit purification (Ambion), and formaldehyde denaturing agarose gel electrophoresis analysis (Promega). After Lipofectamine 3000 (Invitrogen) was used to transfect RNA into HeLa cells, the dishes were incubated at 34 °C for 24 hours. The virus was prepared by cycles of freezing and thawing. The apical surface of the HBE 3D cells was mixed with the cell lysates from PC15- and Lz651-transfected HeLa cells, and the mixture was then incubated at 34 °C. The HBE 3D cells were then washed with PBS three times, and cells were collected in 500 L Trizol (Invitrogen) at the designated time point to analyse cell-associated virus.

➢ HRV-C and HBE 3D cell immunostaining

After being infected with 105 RNA copies of HRV-C for 24 hours and fixed in 4% paraformaldehyde (PFA) for 30 minutes at room temperature, the apical and basolateral sides of the HBE 3D cells were washed three times with PBS. The fixed cells were then blocked with 5% bovine serum albumin for one hour and permeabilized with 0.2% Triton X for two hours. We used anti-HRV-ssRNA mouse polyclonal antibodies (mAb12, 10010200-200UG, 1:500, Thermo Fisher), mouse monoclonal anti-tubulin antibodies (T4026, 1:100, Sigma), MUC5AC monoclonal antibodies (MAB11324, 1:500, Abnova), and mouse monoclonal anti-ZO-1 antibodies (33-9100, 1:500, Invitrogen) for the simultaneous detection of the secondary antibody was Light 594-labeled anti-mouse IgG (H + L) (35,511, 1:500, Thermo Fisher). Alexa Fluor 594 goat anti-rabbit IgG was used to detect rabbit polyclonal anti-HRV-C VP1 protein-antibody complexes (1:500, Invitrogen) attached to the cells (1:500, Invitrogen). DAPI was used as a counterstained for nuclei. The filters with cells were then removed from the insert and put on glass slides using a mounting medium under coverslips. An UltraView VoX confocal microscope was used to capture the confocal pictures (PerkinElmer, Boston, MA, USA).

➢ Other viral infection of HBE 3D cells

HRV-C79 and HRV-C101 were quantified using qRT-PCR, and the RNA copies before inoculation were similar to those of pCLZ651 and pC15. RSV1 and RSV2 viral stock was propagated in HeLa cells; we infected HeLa cells with serially diluted RSV, followed by observation of cytopathic effects (CPE) to assess the 50% tissue culture infective doses (TCID50) per milliliter. TCID50 of RSV1and RSV2 were 10−5.

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Virus suspensions of HRV-C79 and HRV-C101 (100 µL each) and RSV1 and RSV2 viral stocks (100 µL each) were applied on the apical surface of HBE 3D cells. At 4 h or 6 h post-incubation at 34 °C or 37 °C, the tissues were rinsed three times with PBS, and cultures were continued in the liquid–air interface in 500 µL of fresh culture medium.

➢ **Quantitative real-time reverse transcription-PCR (qRT-PCR)**

The RNAs of pCLZ651, pC15, HRV-C18, and HRV-C25 were quantified using a Quanti Tect Probe RT-PCR kit (QIAGEN) with the probe (FAM-TCC TCC GGC YCC TGA ATG-MGB) and the primers (HRV1A, 5′-AGC CTG CGT GGC TGC CTG-3′; HRV1A2, 5′-CCT GCG TGG CGG CCA RC-3′; HRV1B, 5′-CCC AAA GTA GTY GGT CCC RTC C-3′). These primers are complementary to the 5' nontranslated regions of HRVsE1. For RSV1 and RSV2, the probe was FAM-CTGTGTATGTGGAGCCTTCGTGAAGCT; the forward primer was GGCAAATATGGAAACATACGTGAA; and the reverse primer was TCTTTTTCTAGGACATTGTAYTGAACAG.
HRV-C RNA levels were normalized to human glyceraldehyde-3-phosphate dehydrogenase (GADPH) RNA levels in cell and tissue lysates with the probe (VIC-TGG TAT CGT GGA AGG A-MGB) and primers (forward, 5′-GCC AAA AGG GTC ATC ATC TC-3′; reverse, 5′-GGG GCC ATC CAC AGT CTT CT-3′).

➢ **Cytokine and/or chemokine production**

Basal medium was assayed for IFN-λ1, CCL5, IP10, IL-6, IL-8, and MCP-1 using solitary ELISA kits (eBioscience, USA) following the manufacturer's instructions. Determination of each cytokine level was repeated three times.

➢ **Statistical analysis**

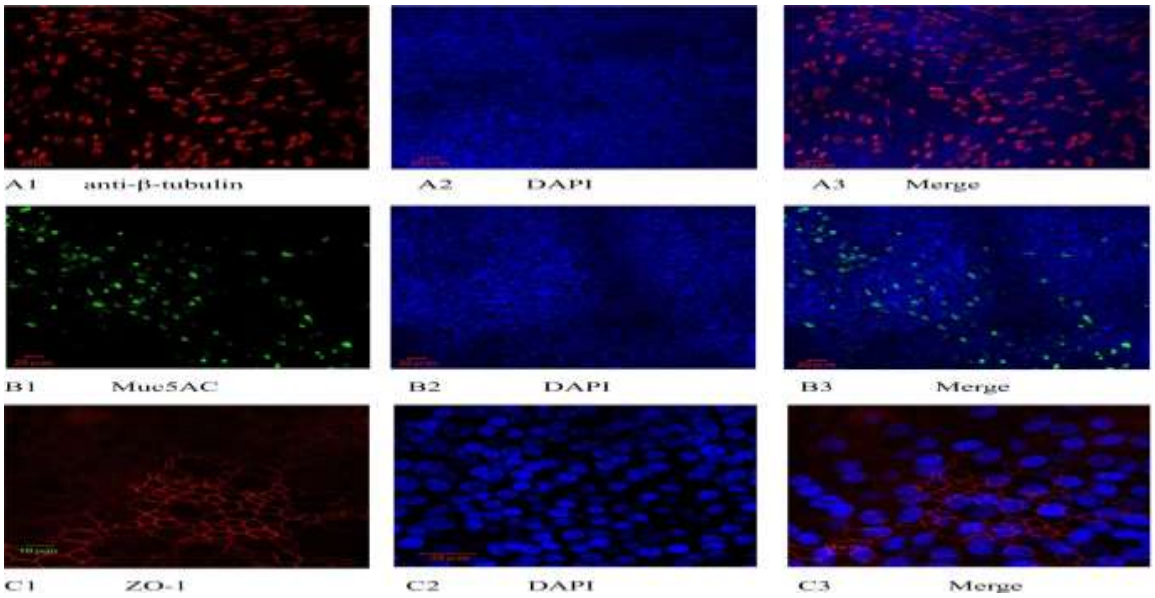
Statistical analysis was performed with SPSS, version 22.0. Data were presented as mean ± standard deviation. Mann–Whitney test for comparisons between two viral groups was used to determine statistical significance ($P < 0.05$).

III. RESULTS

➢ **HBE 3D cells immunofluorescence analysis**

Since -tubulin is a crucial part of the cytoskeleton and a protein that distinguishes cilia, it can be utilised to tell ciliated cells from nonciliated cells. At the tip of epithelial cells, the tight junction protein zonula occludens-1 (ZO-1) is found. It is a key part of tight junctions and is crucial for maintaining the integrity of pseudostratified cell layers and barrier function. The immunofluorescence labelling of the ciliated cells with anti—tubulin IV (Fig. 1A), anti-Muc5AC (Fig. 1B), and anti-ZO1 (Fig. 1C) antibodies revealed that they were well differentiated and that there were a lot of secretory cells. Additionally, the tight junctions of the differentiated epithelium were well-formed, as shown by the excellent ZO-1 protein production.

Fig. 1



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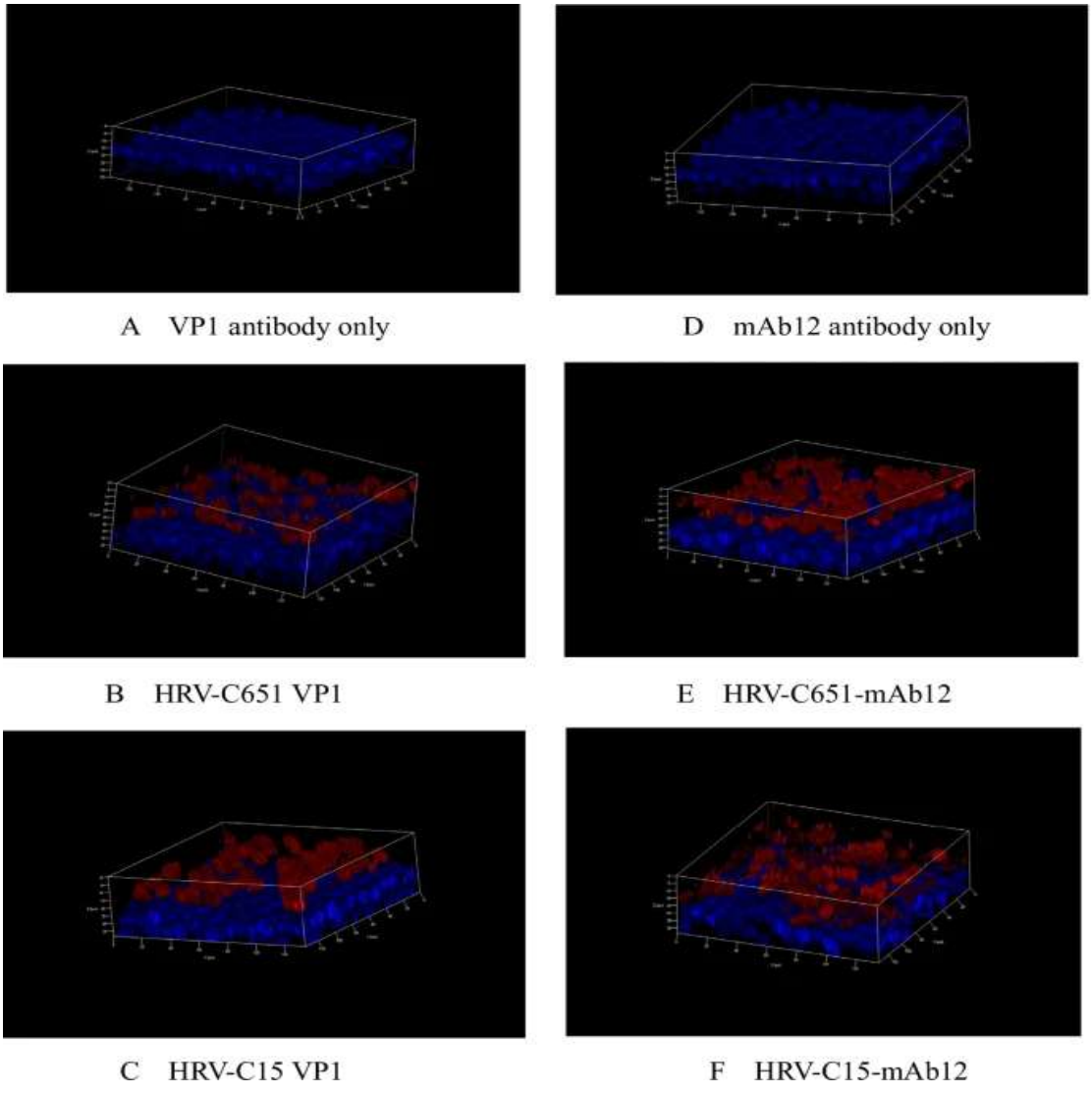
Immunofluorescence of differentiated HBE cells. The differentiated HBE cells were fixed, followed by incubation with mouse monoclonal anti-β tubulin antibody (red, A), mouse Muc5AC monoclonal antibody (green, B), or monoclonal anti-ZO-1 antibody (red, C). Confocal images were taken with a magnification of 200× for A and B, and 360× for C. Nuclei were stained with DAPI. Scale bars, 20 μm

- **HBE-ALI infection via HRV-C**

In vitro transcription of the full-length cDNA clones of PC15 and LZ651 was performed before transfection into HeLa cells. We employed the widely used antibodies for the detection of rhinovirus double-stranded RNA, HRV-C VP1 antibody and a murine monoclonal antibody J2, to assess if the viral particles infected HeLa cells and spread after transfection of HeLa cells with HRV-C651 or HRV-C15 RNA. The viral RNA lysate or mock-transfected cells were treated with HBE 3D cells. An immunofluorescence test was used to track the expression levels of double-stranded RNA and the VP1 capsid protein of the rhinovirus (Fig. 2). The location of the infected cells proved that the HBE-apical ALI's surface was the only place where the virus could replicate.

Fig. 2
From: Comparison of immune response to human rhinovirus C and respiratory syncytial virus in highly differentiated human airway epithelial cells



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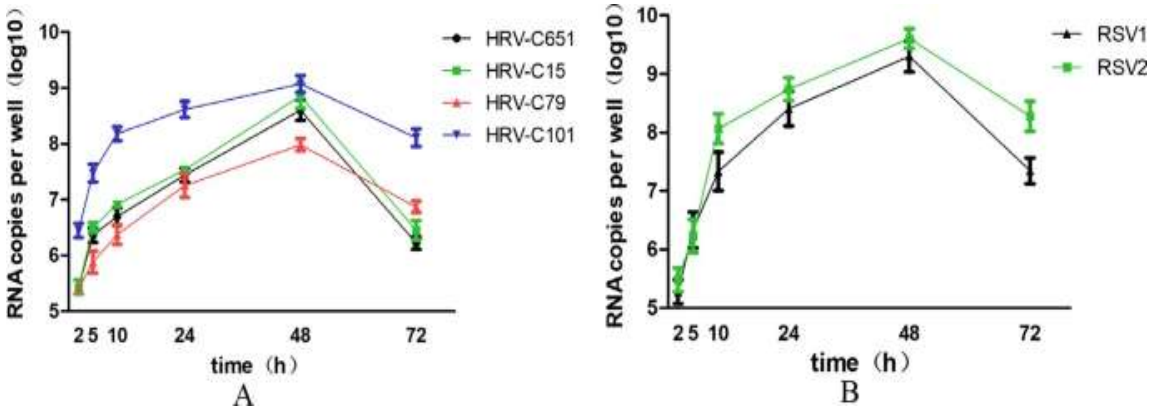
Immunofluorescence of differentiated HBE cells infected with HRV-C651 or HRV-C15. Immunofluorescence with the rabbit polyclonal anti-HRV-C VP1 protein-antibody only (A) and mAbJ2 antibody (D). At 24 h post-inoculation with HRV-C651 or HRV-C15, the HBE cells were fixed, followed by incubation with VP1 protein-antibody (B and C) and mAb12 antibody detecting double-strand RNA (E and F). The red region indicates VP1 protein (B and C) or the double-stranded RNA of HRV-C (E and F). Confocal images were taken with a magnification of 200×. Nuclei were stained with DAPI

- **HRV-C and RSV replicate in HBE-ALI**

Lysates of HeLa cells transfected with HRV-C651 or HRV-C15 RNA and RSV viral stock were incubated at the apical side of the HBE-ALI cells in order to determine whether HRV-C and RSV reproduce in differentiated HBE cells. Then, virus RNA was significantly elevated at the apical surface but not at the basal surface when virus productions were quantified by real-time RT-PCR from the apical side and basal side at various times post-infection. The HRV-Cs, which include HRV-C651, HRV-C15, HRV-C79, and HRV-C101, proliferated significantly at 5 hours after infection (PI), reaching their highest RNA levels 48 hours later (Fig. 3A). Of note, 48 h PI, the RNA level of HRV-C651, HRV-C15, HRV-C79, and HRV-C101 increased significantly from 4.23 to 8.35, 4.64 to 8.49, 5 to 7.95, and 5.45 to 9.08 log10 copies/well, respectively. RSV propagated significantly at 10 h PI, with a peak level achieved at 48 h PI (Fig. 3B). The RNA level of RSV1and RSV2 virus increased from 5.2 to 9.3 and from 5 to 9.5 log10 copies/well, respectively (178-fold and 190-fold, respectively).

Fig. 3



Four HRV-C strains and two RSV strains grew successfully in ALI HBE cells (A and B). Virus RNA load from infected tissue supernatants was measured by real-time RT-PCR collected at the apical surface of ALI HBE cells infected with HRV-C strains (HRV-C 651, HRV-C15, HRV-C 79, or HRV-C 101) and two RSV strains (RSV1 or RSV2). Error bars represent the standard deviation calculated from biological replicates (n = 3).

- **Comparison of RSV and HRV-effects C's on cytokine induction**

The effects of HRV-C on cytokine secretion were then investigated, and they were contrasted with those of RSV. Interleukin (IL-1, IL-4, IL-5, IL-6, IL-8, IL12, L33), thymic stromal lymphopoietin (TSLP), eotaxin, eosinophilia cationic protein (ECP), monocyte chemoattractant protein-1 (MCP-1), and tumour necrosis factor (TNF-) were all found. IFN-1, CCL5, IP10, IL-6, IL-8, and MCP-1 were all secreted as a result of HRV-C infection. Each time point's amounts of IL-8, IL-6, MCP-1, IFN-, and CCL5 were positively linked with the number of HRV-C and RSV RNA copies. The protein levels of HRV-C-induced CCL5, IL-6, MCP-1, and IFN-1 peaked at 48 hours post-infection (PI), while IL-8 and IP10 peaked at 5 and 10 hours, respectively. The production of CCL5, IL-6, MCP-1, IP10, and IFN-λ1 induced by RSV reached the maximum at 48 h PI, while IL-8 peaked at 10 h PI (Fig. 4A–L).

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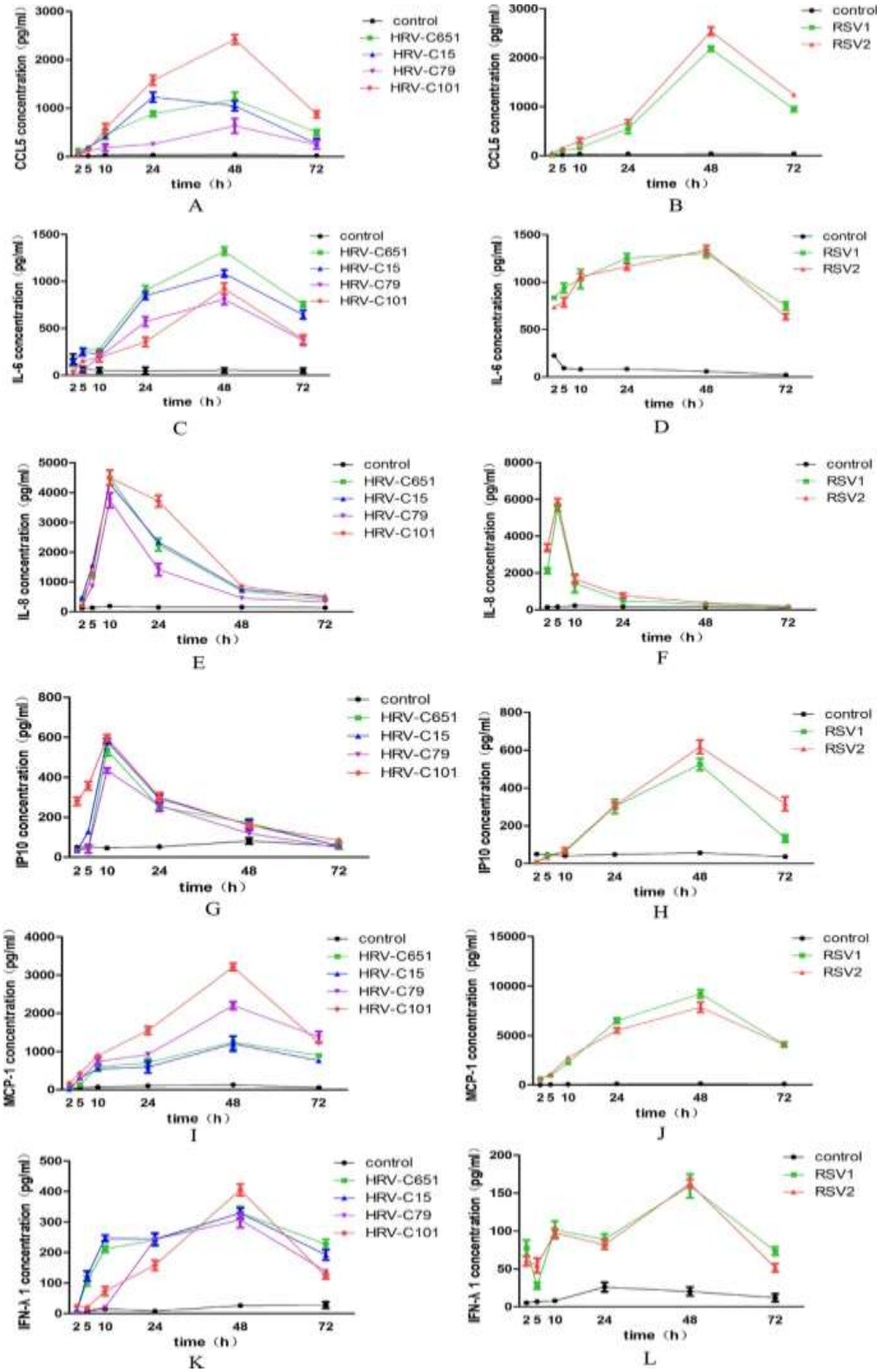
Fig. 4



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Time course of cytokine production induced by HRV-Cs and RSVs. Levels of cytokines in basal medium of HBE cultures were collected at each hour after HRV-Cs or RSVs inoculation in ALI HBE cells.

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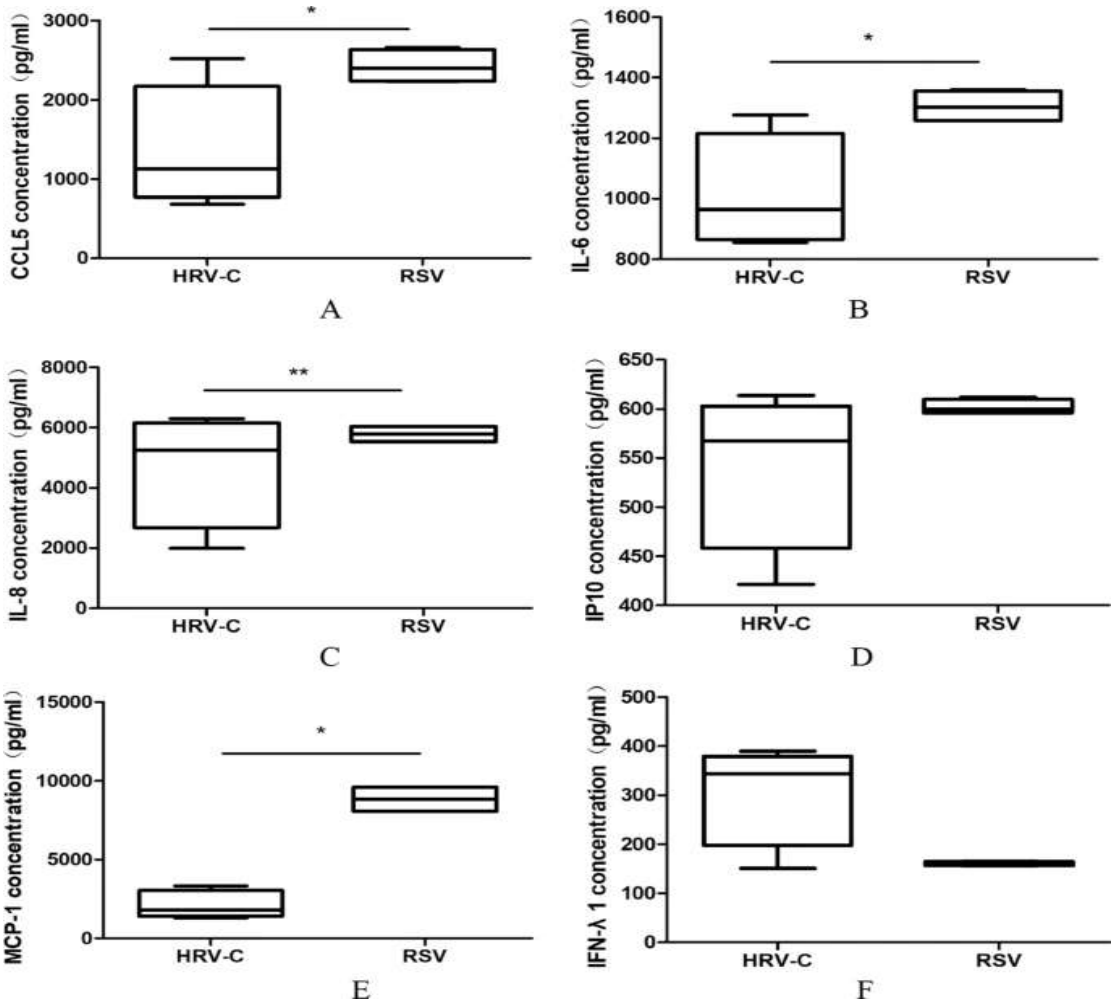
Furthermore, we evaluated the average values of cytokine secretion induced by HRV-C within 48 h PI. Compared with RSV, HRV-C induced lower secretion of CCL5 (P = 0.048), IL-6 (P = 0.016), IL-8 (P = 0.032) and MCP-1 (P = 0.008), and similar secretions of IP10 (P = 0.214), and IFN-λ1 (P = 0.214) (Fig. 5A–F).

Fig. 5



Comparison cytokine levels (mean ± SEM) between HRV-Cs and RSVs within 48-h PI in ALI HBE cells. Mann–Whitney test for comparisons between two viral groups was used to determine statistical significance. *P < 0.05, **P < 0.01

IV. DISCUSSION

H1HeLa cells or submerged cultures of bronchial epithelial cell lines have been used in the majority of in vitro studies on the reaction of human immune cells to HRV infection. In this investigation, we used ALI-cultured HBE cells, which are a good approximation of the airway epithelium in vivo since they can form tight junctions, create mucin, and differentiate to form cilia. Undifferentiated airway epithelial cells might not be helpful in evaluating responses to HRV-C unless the cells are genetically altered since CDHR3, a receptor for HRV-C, is only expressed on ciliated cells [12]. In this investigation, the LZ651 and PC15 C-type rhinovirus strains' whole genome sequences were transfected into viral mRNA in vitro, and two HRV-C infectious clones—HRV-C15 and HRV-C651—were obtained. Then, under ALI conditions, HBE cells were infected with clinical species HRV-C79 and HRV-C101 as well as HRV-C15 and HRV-C651. After transfection with full-length HRVC-651 and HRVC15 RNA, we showed RNA replication and viral protein expression. The RNA level peaked at 48 h PI, increasing 183-fold for HRVC-651 and 197-fold for HRVC15, respectively. Similarly, the RNA levels of HRV-C79 and HRV-C101 increased by 159- and 166-fold, respectively. While our

earlier research indicated that HRV-C 651 RNA peaked around 48 h PI [11, 13], Nakagome generated three HRV-C full-length clones in differentiated sinus epithelial cells and found that the RNA maximum was attained at 24 h PI. Eight HRV-C clinical strains were cultured by Tapparel et al. in ALI HAE, and they observed RNA load maxima between 24 and 72 h PI [5]. In HBE differentiated cells, the different HRV-Cs growth rates reflected type-dependent. The principal site of rhinovirus replication and the starting point of the host immune response to infection are airway epithelial cells. When HRV is exposed to airway epithelial cells in an experiment or naturally, it usually results in a virus-specific cytopathic impact, which is linked to an inflammatory response [14]. Despite testing a wide range of cytokines and chemokines thought to be linked to HRV-C infection based on prior research, only a few cytokines showed a substantial rise in levels [15]. A similar outcome was seen in the investigation of Nakagome et al. [13]. According to Souza's research, well-differentiated cells did not respond to HRV-A16 infection, while undifferentiated cells did by increasing the expression of a number of inflammatory cytokines [16]. This occurrence suggests that, compared to weakly differentiated cells from the same source, well-differentiated cells are significantly more resistant to viral infection and its functional effects [16]. We discovered that HRV-C infection significantly stimulated the release of CCL5, IL-6, IL-8, IP-10, MCP-1, and IFN-1 in HBE ALI cells, and that these protein levels positively correlated with viral proliferation, indicating that these cytokines are involved in the immune response against HRV-C infection. Since all of these cytokines can be identified in HRV-C infection in HBE ALI cells, it is likely that the epithelium is acting defensively in response. The varied resources of the HRV-C strains may be the main cause of a difference between the HRV-C strains in terms of both the cytokines generated and the quantities.

The epithelium of the airways is the primary target of the two predominant etiologies of acute respiratory illnesses, HRV and RSV [15]. Despite having less cytotoxicity than RSV, HRV causes the airway cells to become activated and release proinflammatory cytokines as a result. Except for IFN-1 and IP-10, HRV-C infection resulted in comparatively low cytokine release in response to HBE ALI cells over the course of 48 hours in comparison to RSV infection. According to earlier clinical research [17, 18], this is accurate. Unlike RSV, HRV does not have a definite cytotoxic effect on airway epithelial cells, but it affects the function of the epithelial barrier by zonula occludens-1 of the cells being detached from the tight junction complex during viral replication [19].A weaker response of HBE to HRV-C may be attributed to the fact that HRV-C causes less cell damage and has lower cytotoxicity. Because of the production of IL-6 and IL-8, RANTES can lead to airway damage, neutrophils-mediated epithelial damage, and bronchial hyper-responsiveness [15].

IL-8 was rapidly induced after HRV-C inoculation in the current investigation compared to RSV, and this occurred before the maximum amount of HRV-C RNA and other cytokines could be amplified. In contrast, 48 hours after HRV-C infection, IL-6 production reached its peak. IL-6 and IL-8 levels were lower than those brought on by RSV. After HRV-C infection, CCL5 levels peaked at 24 or 48 hours post-inoculation (PI), and protein levels were lower than with RSV. IL-6 and IL-8 were found to be released by HRV-14 (HRV-A) infection, and Yamaya et al. showed that these levels were higher in the supernatants of cells taken from people with bronchial asthma than in those from the non-asthmatic group [20]. When HRV serotype 7 was infected in A549 human airway epithelial cells, Chun et al. [21] discovered that HRV generated more IL-8 and CCL5 release than RSV. This might be caused by many viral kinds and culture systems. Following a respiratory viral infection, IL-8 is thought to play a role in airway constriction as a critical modulator of neutrophil activation [22]. Infants with recurrent wheeze have lower airway neutrophil counts increase, and acute asthma attacks brought on by HRV have been reported to produce IL-8 [23]. Asthma patients' respiratory secretions contain RANTES, which is implicated in the chemo attraction of eosinophils, monocytes, and T lymphocytes [24]. IP-10 is a chemokine that is released by neutrophils, monocytes, lymphocytes, and bronchial epithelial cells in response to IFN and TNF; its levels are elevated during HRV infection. Human airway epithelial cells infected with HRV-A produce more IP-10 protein both in vitro and in vivo [25, 26]. This sustained cytokine elevation may change the host cytokine environment. Recurrent HRV infections are a potent stimulant for airway remodelling, as was recently stated in a research by Shariff et al. [27], through an increase in smooth muscle cell mass recruitment close to the epithelial cells, which is mediated by CCL5, CXCL8, and IP-10 released during HRV infection. In the current work, IP-10 levels were comparable to those after RSV infection at 10 h PI following HRV-C infection in HBE ALI cells. High amounts of IL-8,

IL6, CCL5, and IP-10 were elicited by both HRV-C and RSV. Additionally, early clinical research revealed a connection between wheezing episodes and HRV-C [28, 29]. Our findings and prior research may help to explain why wheeze and asthma develop more frequently after HRV-C bronchiolitis.

In response to an infection with a replication-efficient HRV, airway epithelial cells release type I and type III interferons (IFNs), such as IFN-1, IFN-2, and IFN-3. After HRV-C infection as opposed to RSV infection, we discovered greater levels of IFN-1. Recent research suggests that type I IFN regulates RSV pathogenesis and immunological responses and that RSV is a weak inducer of IFN [30, 31]. Additionally, type III IFNs (IL-28 and IL-29) generated by RSV were not present, according to a recent study that evaluated the immunological response of the HAE to RSV and influenza virus infection [32]. However, Miller et al. showed that an increase in type III IFN response was primarily responsible for the asthma exacerbation linked to HRV infection [33].

These data might be helpful to understand the dissimilarity of antiviral responses to HRV-C infection and RSV infection.

MCP-1 plays a significant role in the pathophysiology of many diseases and is a critical modulator of T-lymphocyte differentiation and monocyte chemotaxis. The activation of a dysregulated Th2 response has been linked to an elevated expression of MCP-1 in asthma patients [34]. In an asthma experimental model, MCP-1 expression was inhibited, greatly reducing airway responsiveness [35]. According to a research by Giuffrida, only RSV significantly elevated MCP-1 expression when compared to para influenza virus and adenovirus, and asthmatic patients had considerably higher MCP-1 levels than non-asthmatic patients [34]. Infections with HRV-C and RSV both resulted in a considerable rise in MCP-1 protein, but HRV-C infection caused a lower level than RSV. These results support the clinical data that rhinovirus is responsible for 50% of asthma exacerbation, and RSV has been associated with recurrent wheezing and asthma development [36].

This study's disadvantage is that we compared RSV experimental strain data to information from HRV-C infectious clones or strains from clinical samples. However, we discovered that infectious clones and clinical samples had comparable immune responses to viral infections. In addition, to assess the effects of viral replication death variations, we solely used a blank control rather than a UV-inactivated virus. In order to lessen the impact of viral replication death variations, viral stocks were placed to the apical surface of HBE 3D cells and incubated for four hours. The tissues were then rinsed three times with PBS.

V. CONCLUSION

Our study demonstrated that the HBE ALI culture system supported HRV-C infection and propagation. When compared with RSV, HRV-C induced relatively weaker cytokine expression in fully differentiated HBE cells. The analysis of the difference in immune response between the two viruses may be helpful for the development of therapies and preventive strategies.

➢ Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

➢ Abbreviations

HRV: Human rhinovirus
HRV-C: Human rhinovirus C
HBE: Human bronchial epithelial
ALI: Air–liquid interface
RSV: Respiratory syncytial virus
HAE: Human airway epithelial
2D: Two-dimensional
RT-PCR: Reverse transcription-PCR
PI: Post infection
IFN: Interferon
IL: Interleukin
TSLP: Thymic stromal lymphopoietin
ECP: Eosinophilia cationic protein
MCP-1: Monocyte chemoattractant protein-1
TNF-α: Tumor necrosis factor α
ZO-1: Zonula occludens-1

➢ Acknowledgements

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➢ Competing interests

The authors declare that they have no competing interests.

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