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Short Communication

The influence of interferon-lambda on restricting Middle East Respiratory Syndrome Coronavirus replication in the respiratory epithelium

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ABSTRACT

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes severe respiratory in human with high mortality and it has been a challenge to determine optimum treatment for MERS-CoV-induced respiratory infection. Here, we observed the distribution of MERS-CoV receptors using human respiratory mucosa and also evaluated the contribution of interferon-lambdas (IFN-λs) in response to MERS-CoV infection using *in vitro* normal human nasal epithelial (NHNE) and bronchial epithelial (NHBE) cells. We found that the gene and protein expression of DPPIV, MERS-CoV receptor, were more dominantly located in nasal and bronchial epithelium although human nasal mucosa exhibited relatively lower DPPIV expression than lung parenchymal tissues. The quantitative mRNA level of the MERS-CoV envelope (upE) gene was significantly induced in MERS-CoV-infected cultured NHNE and NHBE cells until 3 days after infection. The induction of IFNs was identified in NHNE and NHBE cells after MERS-CoV infection and IFN-λs were predominantly increased in MERS-CoV-infected respiratory epithelial cells. Inoculation of IFN-λs to NHNE and NHBE cells suppressed MERS-CoV replication and in particular, IFN-λ_4 showed a strong therapeutic effect in reducing MERS-CoV infection with higher induction of IFN-stimulated genes. Thus, IFN-λ has a decisive function in the respiratory epithelium that greatly limits MERS-CoV replication, and may be a key cytokine for better therapeutic outcomes against MERS-CoV infection in respiratory tract.

Middle East Respiratory Syndrome Coronavirus (MERS-CoV), first identified in 2012, has since attracted increasing international interest in its epidemiology, clinical features, and options for therapy (Zaki et al., 2012). Epidemiologic studies have established that human zoonotic transmission is suspected, with evidence of spread from dromedary camels as a MESR-CoV residue (Haagmans et al., 2014; Sabir et al., 2016). The virus has continued to cause severe zoonotic human disease in the Middle East, sometimes associated with outbreaks of human-to-human transmission. Accordingly, there is an urgent need to advance the development of therapeutic agents or a vaccine to treat and prevent MERS-CoV infection (Hotez et al., 2014).

It has been suggested that MERS-CoV replicates in the human upper and lower respiratory tract after invasion through its specific receptors. Dipeptidyl Peptidase IV (DPPIV), a type II transmembrane ectopeptidase, is a well-known receptor of MERS-CoV that plays a critical role in entry and infection of target cells (Lu et al., 2013; Raj et al., 2013). DPPIV is mainly expressed on the apical surfaces of epithelial and acinar cells, and on capillary endothelial cells of various organs. A positive correlation between higher susceptibility to MERS-CoV infection and prominent DPPIV expression has been demonstrated (van Doremalen et al., 2014). Therefore, the evaluation of DPPIV distribution in tissues would help identify target tissues for effective suppression of MERS-CoV infection in the respiratory tract.

The innate immune system of the respiratory epithelium serves as the first line of defense against respiratory viruses by producing interferon (IFN), a group of key molecules in the antiviral response (Kim et al., 2017). Emerging evidence has indicated that, among the IFN family of cytokines, IFN-λs such as IFN-λ_1, -λ_2, -λ_3 and -λ_4 are critical immune modulators against viral infection in the epithelial mucosa, and rapid immune response to respiratory viruses occurs through activation of IFN-λ (Galani et al., 2017; Kim et al., 2019). Based on our previous data, IFN-λ is believed to be primarily responsible for protection against viral invaders in the respiratory tract and to play an important role in local antiviral innate immunity (An et al., 2018; Kim et al., 2017). However, our knowledge of the contribution of IFN-λs to coronavirus clearance from the respiratory tract is limited and our

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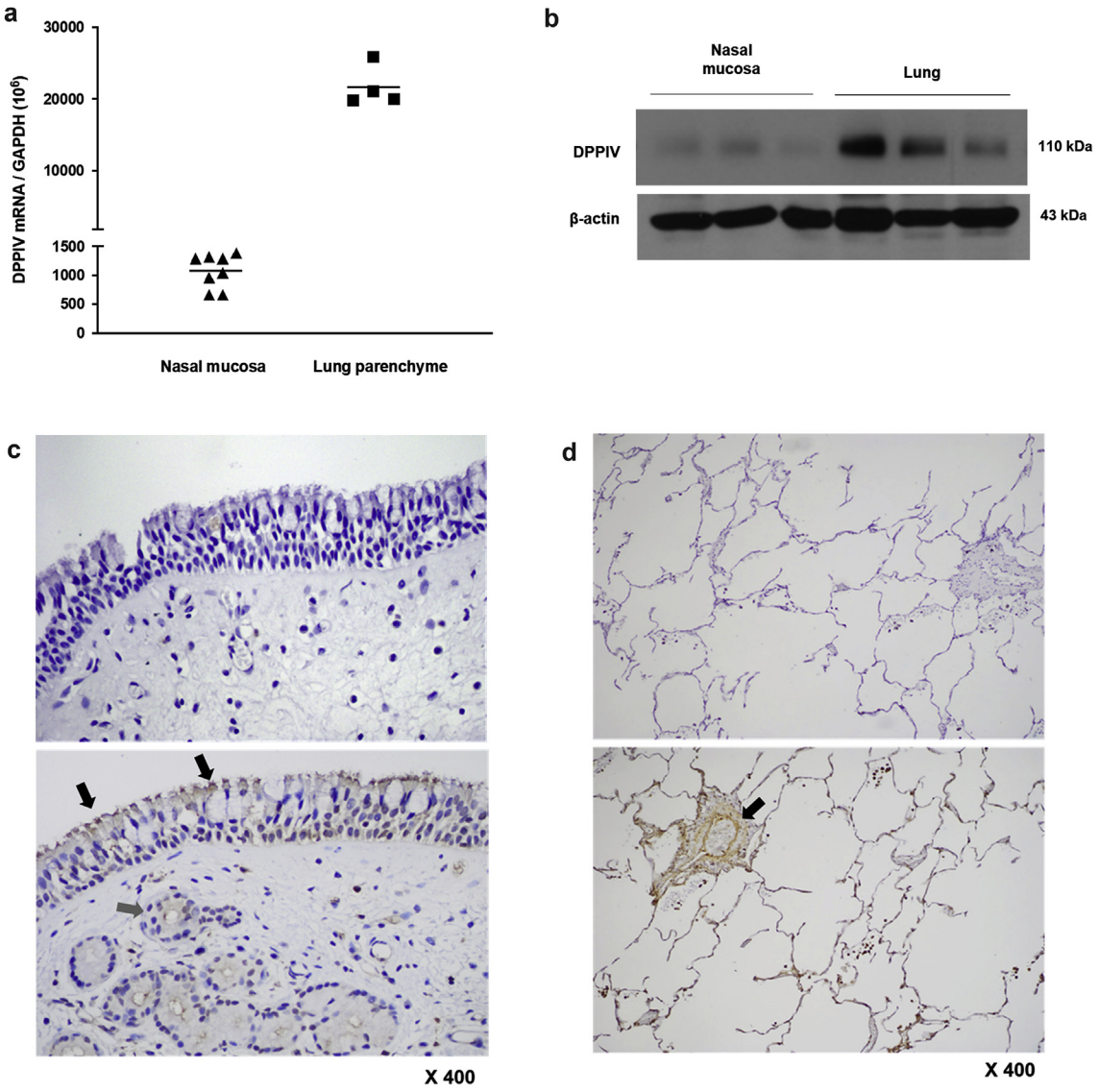

Fig. 1. Expression of DDPIV in human nasal mucosa and lung tissue.
mRNA (a) and protein (b) expression of dipeptidyl peptidase IV (DPPIV) were measured in human nasal mucosal tissues (black triangle, N = 8) and lung parenchymal tissues (black square, N = 4). Compared to nasal mucosal tissue, lung parenchymal tissue exhibited a relatively higher level of DPPIV mRNA and DPPIV protein expression. Immunohistochemistry for DPPIV protein was performed using human nasal mucosa and lung tissue, revealing that positive staining of DPPIV was dominant in ciliated cells of the nasal epithelium with limited expression in the goblets cell (black arrow), and was observed at the apical surface of some serous cells in the submucosal glands (gray arrow) (c, original magnification: × 400). In lung tissue, cells lining the terminal bronchial epithelium had multifocal DPPIV immunostaining (black arrow) (d, original magnification: × 400). The results of real-time PCR are presented as means from nasal mucosa of eight subjects and lung tissue of four subjects and the Western blot shown is representative of five independent experiments.

understanding of the modulators involved in IFN-λ production, especially within the context of MERS-CoV infections in the upper and lower airway epithelium, remains lacking. Here, we assessed the distribution of DPPIV in human nasal mucosa and lung tissue to predict the infection route of MERS-CoV in the respiratory tract. In addition, we found that induction of IFN-λ signaling might be more dominant in MERS-CoV-infected respiratory epithelial cells. Administration of recombinant IFN-λs, especially IFN-λ_4, resulted in a more significant suppressive effect on MERS-CoV replication as well as the induction of IFN-stimulated genes (ISG).

Using real-time PCR and Western blot analysis, mRNA and protein expression of DPPIV was studied in human palatine tonsillar tissues, nasal mucosal tissues (N = 8) and lung parenchymal tissues (N = 4) of subjects, respectively. mRNA expression of DPPIV in lung parenchymal tissue was significantly higher than DPPIV expression in nasal mucosa (Fig. 1a). Moreover, protein expression of DPPIV was also relatively higher in lung parenchyma than nasal mucosa (Fig. 1b). Immunohistochemistry to detect DPPIV was performed on human nasal mucosal tissues and lung parenchymal tissues. In the nasal mucosa, the positive staining of DPPIV protein was dominantly detected in human nasal epithelial cells, particularly ciliated cells, but DPPIV staining was limited to goblet cells of the nasal epithelium (Fig. 1c). In addition, some staining of DPPIV protein was observed in submucosal glands in the nasal mucosa (Fig. 1c). DPPIV immunostaining was detected in the multifocal cells lining the terminal bronchial epithelium in human lung parenchyma (Fig. 1d). Overall, DPPIV expression was higher in human lung tissue than nasal mucosa and was more dominant in nasal or bronchial epithelial cells in the human respiratory tract. Therefore, respiratory epithelial cells might be the main target for MERS-CoV infection and transmission in the human respiratory tract.

Normal nasal mucosa was obtained from four healthy volunteers who underwent septal surgery, and human lung tissue was also

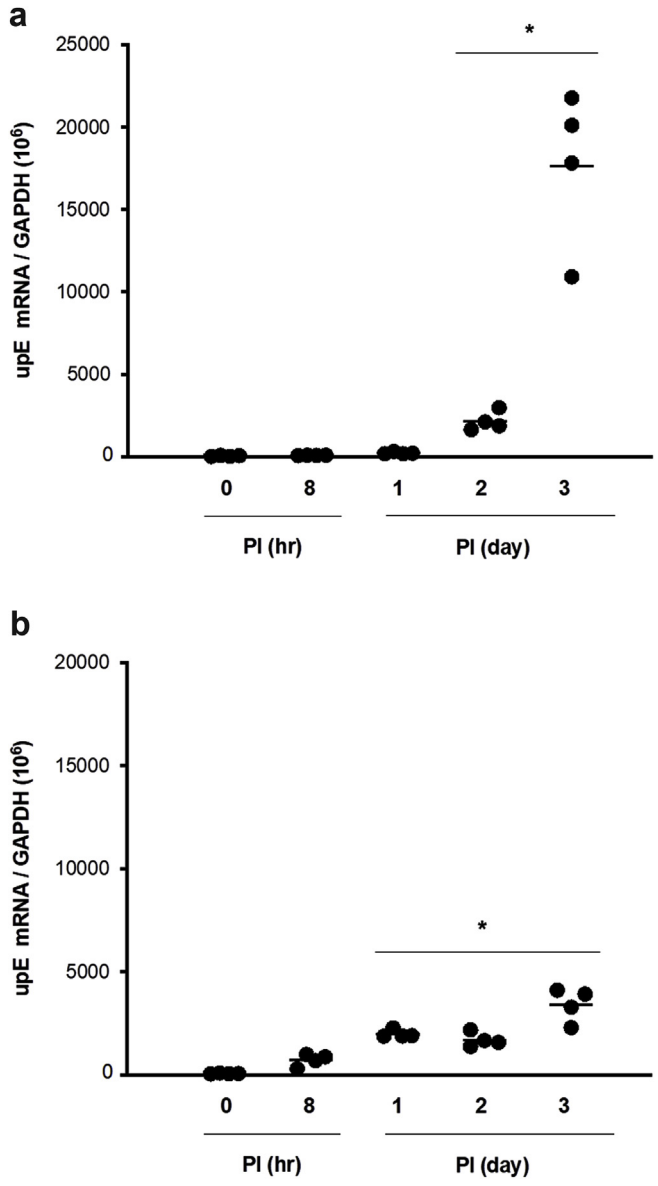

Fig. 2. NHBE cells were more susceptible to MERS-CoV infection than NHNE cells.

NHBE cells from subjects with lung cancer (N = 4) and NHNE cells from healthy volunteers (N = 4, respectively) were inoculated with MERS-CoV isolated from the oropharyngeal sample of a patient from the 2015 outbreak in the Republic of Korea, for 0 (no infection), 8 h and 1, 2 and 3 days at an MOI of 0.25. Real-time PCR analysis showed that mRNA expression of the MERS-CoV envelope (upE) gene was significantly higher in NHBE (a) and NHNE (b) cells from 1 day after infection. The mRNA expression of upE was significantly higher in NHBE cells, and the biggest difference was observed at 3 days post-infection. The results of real-time PCR are presented as the mean ± standard deviation (SD) (*$p < .05$ when compared with the level in uninfected cells).

extracted from subjects who underwent pneumonectomy for unilateral lung cancer. Normal human nasal epithelial (NHNE) cells and normal human bronchial epithelial (NHBE) cells were cultured using an air-liquid interface system to assess susceptibility to MERS-CoV after infection (multiplicity of infection (MOI) 0.25). Live MERS-CoV was isolated from a patient from the 2015 Korean Outbreak (GenBank Accession Number KU308549, kindly provided by professor Myoung-don Oh of Seoul National University Hospital) (Park et al., 2016). We then measured mRNA expression of the MERS-CoV envelope gene (upE) using real-time PCR. We found that mRNA expression of upE increased significantly one day after infection, with the highest expression observed three days post infection (dpi) in NHBE cells (Fig. 2a). upE mRNAs levels were also increased in MERS-CoV-infected NHNE cells up to three days after infection (Fig. 2b). However, the mRNA level of MERS-CoV was significantly lower in NHNE cells than in MERS-CoV-infected NHBE cells.

To identify distinctive patterns of IFN expression and secretion in the respiratory epithelium after MERS-CoV infection, both NHNE and NHBE cells were inoculated with MERS-CoV at an MOI 0.25 and mRNA levels of IFN-α, IFN-β, IFN-λ_1, IFN-$\lambda_{2/3}$, IFN-λ_4, and IFN-γ were measured at 0, 8 h, 1, 2, and 3 days after infection by real-time PCR. mRNA levels of IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 were significantly elevated from 1 dpi onwards in MERS-CoV-infected NHBE cells compared with uninfected NHBE cells. By contrast, the mRNA level of IFN-β was slightly increased and mRNA levels of both IFN-α and IFN-γ were not changed after MERS-CoV infection in NHBE cells (Fig. 3a). Interestingly, the induction of IFN-λ_4 transcription was relatively higher than that of IFN-λ_1 and IFN-$\lambda_{2/3}$ in MERS-CoV-infected NHBE cells, and the highest mRNA level of IFN-λ_4 (8.7×10^3) was observed at 3 dpi. The significant induction of IFN-λ subtypes was also observed in MERS-CoV-infected NHNE cells, where IFN-λ_4 was most highly induced after MERS-CoV infection up to 3 dpi (Fig. 3b). The mRNA level of IFN-λ_4 was about 10 times higher in MERS-CoV-infected NHNE cells than in MERS-CoV-infected NHBE cells at 2 and 3 dpi. Based on these data, IFN-λs were induced more dominantly in respiratory epithelial cells by 3 days after MERS-CoV infection, and IFN-λ_4 appeared to be more preferentially driven as an innate immune response against MERS-CoV infection in nasal and bronchial epithelial cells.

To assess the IFN-λ-dependent protective effect against MERS-CoV infection, NHNE cells were treated with recombinant IFN-$\lambda_{1/2}$ (IFN-λ_1: 10 ng/ml and IFN-λ_2: 10 ng/ml) and IFN-λ_4 (10 ng/ml, kindly provided by Professor Ho Min Kim and Eui-Cheol Shin of Korea Advanced Institute of Science and Technology) (Sung et al., 2017) at 1 h before MERS-CoV infection. The increased upE mRNA level of MERS-CoV (6.2×10^9) at PI day 1 was significantly attenuated in MERS-CoV-infected NHNE cells with inoculation of recombinant IFN-$\lambda_{1/2}$ and IFN-λ_4 (Fig. 3c). The upE mRNA level was more completely reduced in MERS-CoV-infected NHNE cells treated with IFN-λ_4 (5.2×10^6) compared to cells treated with IFN-$\lambda_{1/2}$ (3.8×10^9). In addition, mRNA of IFN-stimulated genes (ISGs) such as CXCL10, IFIT1, Mx1, and OAS1 were relatively increased in MERS-CoV-infected NHNE cells with IFN-λ inoculation, and higher levels of ISGs were detected in MERS-CoV-infected NHNE cells with IFN-λ_4 treatment (Fig. 3d).This IFN-λ-dependent antiviral effect against MERS-CoV was also observed in MERS-CoV-infected NHBE cells, where recombinant IFN-λ_4 also showed the strongest inhibitory effect against MERS-CoV infection (Fig. 3e).

Here, we showed that IFN-λs are the predominant IFN produced in the respiratory epithelium to resist MERS-CoV infection. Our findings also imply that inoculation with recombinant IFN-λs may be able to significantly control MERS-CoV infection. The present study shows that inoculation with IFN-λ_4 might be more effective for the clearance of MERS-CoV from respiratory epithelial cells and proves more potent antiviral activity of IFN-λ_4 against influenza virus.

MERS-CoV continues to cause acute respiratory infection with high case fatality in hospitalized patients, and there remains a large interest in the development of effective therapy for this virus (Haverkamp et al., 2018; Meyerholz et al., 2016). However, most clinical experience regarding treatment of MERS-CoV infection relies on limited case series, impeding conclusions about fundamental therapeutic researches against MERS-CoV in humans. We first assessed DPPIV localization in human nasal mucosa and lung tissue to prove the invasion route of MERS-CoV in human respiratory tract, and found that DPPIV is dominantly localized in nasal and bronchial epithelium. Prominent DPPIV expression in respiratory epithelium suggests that MERS-CoV spreads to the respiratory tract through epithelial cells, pointing to an important location for therapeutic attempts to suppress MERS-CoV. These results also provide evidence that inoculation of respiratory epithelial cells

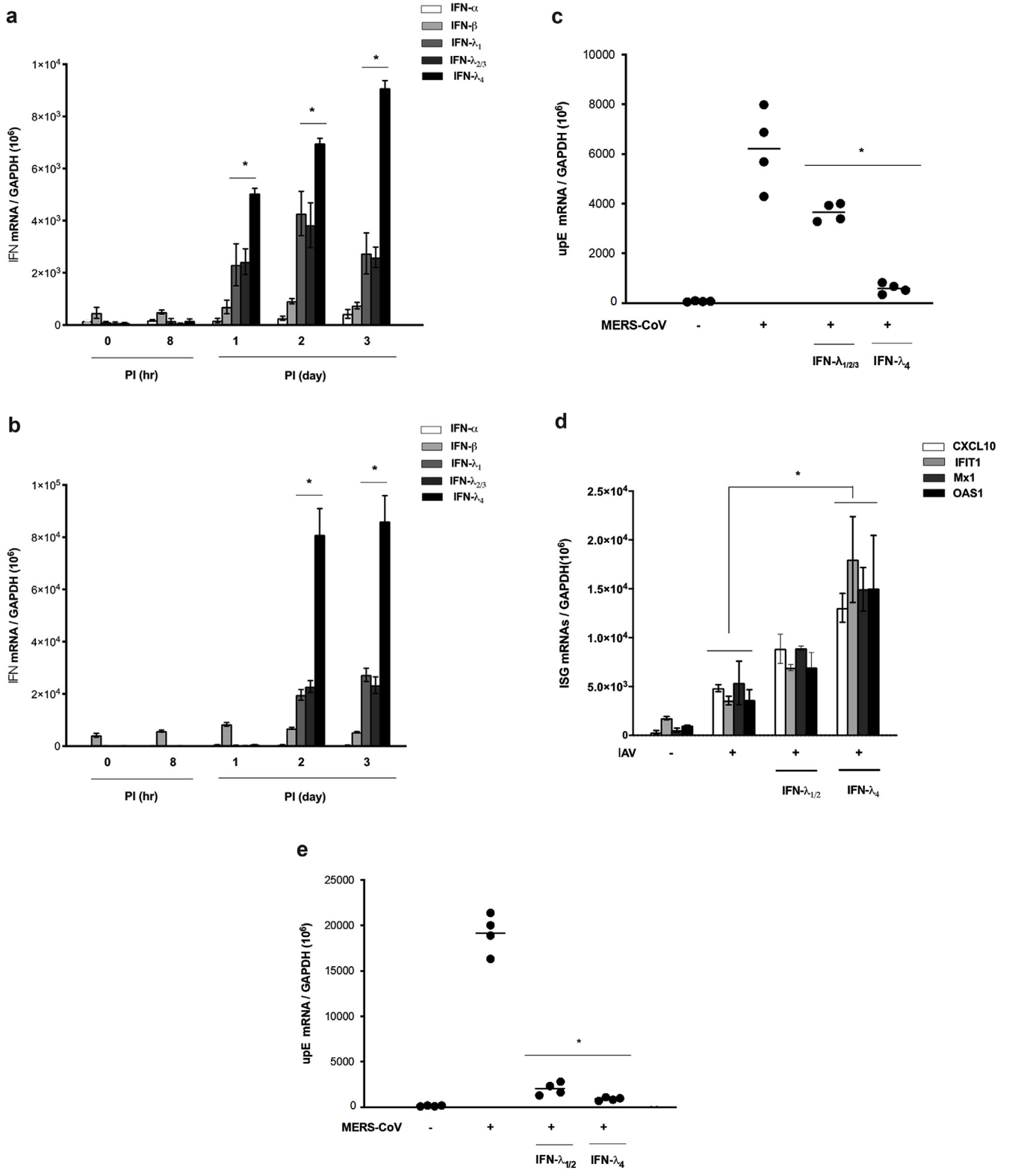

Fig. 3. IFN-λ was preferentially induced to control MERS-CoV infection in the respiratory epithelium.
NHBE and NHNE cells were inoculated with MERS-COV for 0, 8 h, 1, 2, and 3 days at an MOI of 0.25. (a) Real-time PCR revealed that IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 mRNA levels were elevated until PI 3 days and IFN-λ_4 mRNA levels were significantly higher compared to mRNA levels of IFN-λ_1 and IFN-$\lambda_{2/3}$ from PI 2 days in NHBE cells. (b) Similar results were observed in MERS-CoV-infected NHNE cells, and induction of IFN-λ_4 mRNA was greater in NHNE cells from 2 days after MERS-CoV infection. The results of real-time PCR are presented as the mean ± SD (*$p < .05$ when compared with the level in uninfected cells). NHNE cells were treated with recombinant IFN-λs (IFN-λ_1, IFN-λ_2, and IFN-λ_4, each at 10 ng/ml) at 2 h before MERS-CoV infection. The levels of upE mRNA (c) and interferon-stimulated genes (d) at 1day post-infection was significantly attenuated after treatment with recombinant IFN-λs were measured using Real-time PCR. MERS-CoV-infected NHBE cells with recombinant IFN-λs before infection also exhibited the reduction of upE mRNA. The results of real-time PCR are presented as the mean ± SD from five independent experiments (*$p < .05$ when compared with the level in MERS-CoV-infected cells).

with therapeutic compounds is crucial for host protection and the clearance of MERS-CoV. Accordingly, further research was conducted, with an emphasis on actions of immune mediators in the respiratory epithelium against MERS-CoV.

Type I IFNs are well documented to mediate the innate immune response to viruses as well as regulate the subsequent activation of the adaptive immune system, which also contributes to the clearance of viral infection (Cakebread et al., 2011). It has been already proven that a type I IFN response is critical for optimal kinetics of viral clearance of MERS-CoV in the respiratory tract, and that type I IFN induction and peak MERS-CoV replication occur simultaneously, resulting in protective T cell responses in infected mouse lungs (Channappanavar et al., 2019). Recently, IFN-λ has also been shown to be critical for the innate immune response against respiratory viral infection and humans or mice lacking IFN-λ-related innate immune responses are more susceptible to respiratory virus infection (Jeon et al., 2018; Galani et al., 2017; Kim et al., 2019; Won et al., 2019). Moreover, IFN-λ is the predominant IFN induced by respiratory virus infection in the respiratory epithelium, where it contributes to first-line defense against viral infections in human nasal epithelial cells (Kim et al., 2017). Based on the current findings, we propose that elevated levels of IFN-λ produced over the course of MERS-CoV infection constitute a primary antiviral defense in NHBE and NHNE cells. In addition, therapeutic applications of IFN-λ against MERS-CoV infection will enable a greater understanding of better defense strategies against MERS-CoV in the respiratory tract. The most recently discovered protein to be classified as an IFN-λ is IFN-λ_4, which can induce antiviral responses through activation of the Janus kinase signal transducer and activator of transcription pathway and expression of ISGs. The current data revealed that IFN-λs were dominantly induced in MERS-CoV-infected NHBE and NHNE cells compared to type I and II IFNs. Among IFN-λs, IFN-λ_4 increased the most after MERS-CoV infection in respiratory epithelial cells. IFN-λs also have therapeutic potential because they can protect hosts from other viruses, including influenza virus, norovirus, and rotavirus, at the level of epithelial cells (Chung et al., 2020). The discovery of IFN-λ_4 appears to explain the strong genetic component of hepatitis C virus clearance, while simultaneously raising a number of questions concerning the underlying functional mechanisms and full clinical potential of IFN-λ_4. It is also possible that IFN-λ_4 plays an important role in contemporary infectious diseases other than HCV, but there is minimal evidence on the therapeutic effectiveness of IFN-λ_4 for the control of respiratory viruses. Our results indicate that treatment with recombinant IFN-λs can directly suppress MERS-CoV replication in NHBE and NHNE cells. The inoculation of recombinant IFN-λ_4 might be detrimental to virus-infected respiratory epithelial cells, and we found that IFN-λ_4-treated respiratory epithelial cells exhibited a more potent inhibitory effect against MERS-CoV replication. Although much further research and insight into its mode of action is required to understand whether it enhances the antiviral effect, it appears that recombinant IFN-λ_4 can be utilized as an alternative therapeutic target cytokine against MERS-CoV infection at the level of respiratory epithelial cells. In this study, we demonstrated the antiviral effect of IFN-λ, particularly its ability to modulate MERS virus infection in respiratory epithelium. The results of these studies are expected to suggest the possibility that IFN-λ may have new therapeutic effects for other corona viruses such as SARS-cov and COVID19 through further experiments.

In summary, the IFN-λ-mediated innate immune response is crucial for the clearance of MERS-CoV from the respiratory tract. Inoculation with IFN-λs, especially IFN-λ4, resulted in an increase of ISG transcription and an efficient innate immune response that suppressed MERS-CoV infection at the level of respiratory epithelial cells, suggesting superiority as a therapeutic candidate to control MERS-CoV infection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2020.104860.

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