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Type Ⅲ Interferons are Critical Host Factors to Determine the Susceptibility to Influenza A Viral Infection in Allergic Nasal Mucosa

알레르기 비염 비강상피세포에서 A형 인플루엔자 바이러스 감염 감수성을 결정하는 주요 숙주 인자로서의 제3형 인터페론에 대한 연구

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Abstract

Type Ⅲ Interferons are Critical Host Factors to Determine the Susceptibility to Influenza A Viral Infection in Allergic Nasal Mucosa

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Rationale: Interferon (IFN) is key component to control innate immunity in the respiratory epithelial cells and is responsible for the first defense phase of virus infection. A deficiency in IFN production from the respiratory epithelial cells is actually related with higher susceptibility to respiratory viral infections in asthma but less is known about allergic rhinitis (AR), the first portal of contact of respiratory infections. We studied whether allergic nasal mucosa would be more susceptible to influenza A virus (IAV) infection due to lower induction of IFN-related immune responses.

Objectives: In this study, we aimed to determine whether IFN induction would be impaired in allergic nasal mucosa and to identify which IFN was correlated with higher viral loads in IAV-infected allergic nasal mucosa.

Methods: IAV mRNA levels, viral titers and IFNs expression were compared in IAV-infected normal human nasal epithelial (NHNE, N=10) and allergic rhinitis nasal epithelial (ARNE, N=10) cells. And we used human nasal mucosa from healthy volunteers (N=72) and AR patients (N=29) were used to assess the induction of IFNs after IAV infection.

Measurement and Main Results: IAV mRNA levels and viral titers were significantly higher in ARNE cells compared with NHNE cells. The mRNA levels of IFN-β and -λs were induced in NHNE and ARNE cells up to 3 days after IAV infection. Interestingly, induction of IFN-λ mRNA levels and the amount of secreted proteins were considerable lower in ARNE cells. Mean mRNA levels of IFN-λs were also significantly lower in human nasal mucosa of AR patients. We found that recombinant treatment of IFN-λ attenuated viral mRNA levels and viral titers in IAV-infected ARNE cells.

Conclusion: Higher susceptibility of allergic nasal mucosa to IAV may depend on impairment of type Ⅲ IFN induction, and type Ⅲ IFN is a key mechanistic link between higher viral loads and control of IAV infection in allergic nasal mucosa.

Keywords: Influenza A virus; Interferon-λ; allergic rhinitis; nasal mucosa

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List of Abbreviations

- IAV: Influenza A virus
- IFN: Interferon
- Th2: T helper type 2
- AR: Allergic rhinitis
- PNS-CT: Paranasal sinus computed tomography
- AST: Allergic skin test
- MAST: Multiallergen simultaneous test
- MDCK: Madin-Darby canine kidney
- NHNE: Normal human nasal epithelial
- ARNE: Allergic rhinitis nasal epithelial
- DMEM: Dulbecco's modified Eagle's medium
- ALI: Air-liquid interface
- SEM: Scanning electron microscope
- TSLP: Thymic stromal lymphopoietin
- PCR: Polymerase chain reaction
- ELISA: Enzyme-linked immunosorbent assay
- PBS: Phosphate-buffered saline
- MEM: Minimal essential medium
- TPCK: L-1-tosylamido-2-phenylethyl chloromethyl ketone
- Dpi: Day(s) post of infection
- SD: Standard deviation
- ANOVA: Analysis of variance
- MOI: Multiplicity of infection

Introduction

Influenza A virus (IAV) accounts for severe viral infections and epidemics of respiratory disease and a wide variety of IAV genotypes cause global mortality that exceeded 20 million in the most lethal IAV pandemic (1-3). IAV must be updated annually due to seasonal antigenic drifts, and this genetic variability or rapid evolution of IAV explains why the cure rate of highly contagious IAV in the respiratory tract has not improved over the past few years.

The innate immune system of the respiratory epithelium serves as the first line of defense against invading respiratory viruses, and the application of new therapeutics is a rational approach to control IAV infection in the respiratory tract (1, 3). The host defense systems against invading respiratory viruses culminate in the production of interferon (IFN), a key molecule in innate antiviral immune response (4-7). There are three distinct types of IFNs, type Ⅰ, Ⅱ, and Ⅲ, which are classified based on their structural features, target receptors, and biological activities. Although type Ⅰ and Ⅲ IFNs are induced in response to viral infections in the respiratory tract, there is emerging evidence that type Ⅲ IFN is primarily responsible

for protection against viral invaders in the respiratory tract and plays an important role in local antiviral innate immunity (6, 8-14).

Several studies have documented abnormal immune responses to viral infections in patients with chronic respiratory diseases including allergic asthma (15-18). The impairment of antiviral immune responses related to T helper type 2 (Th2) cytokines has been suggested as a mechanism for higher susceptibility to viral infection in the asthmatic respiratory epithelium (16-18). Unlike lower airway epithelium, viral infections and resistant mechanisms of the nasal epithelium have not been studied extensively. Little is known about higher viral loads and impaired immune mechanisms against viruses in human nasal mucosa of allergic rhinitis (AR) patients. Impaired expression of toll-like receptors and increased rhinovirus replication in nasal epithelial cells from AR patients was recently documented (19). However, few studies have confirmed a defective immune response in AR upon virus infection.

We hypothesized that allergic nasal mucosa might be more susceptible to IAV infection and that impairment of innate immune responses in allergic nasal mucosa correlated with higher viral loads after IAV infection. To address these issues, we assessed the viral load of IAV and distinct IFNs induction in allergic nasal mucosa using cultured nasal epithelial cells and

human nasal mucosa. Through this comprehensive approach, we find that allergic nasal mucosa may be at risk of higher viral loads during IAV infection, due to impaired modulation of type Ⅲ IFNs which are key host factors to suppress IAV replication in AR.

Materials and Methods

Subjects and sample collection

This study enrolled 101 subjects referred to the Department of Otorhinolaryngology Seoul National University Hospital (Seoul, Korea) primarily for septal surgery between June 2015 and May 2016. Septal deviation was diagnosed with intransal endoscope and paranasal sinus computed tomography (PNS-CT) and the subjects did not show any clinical or imaging findings about sinusitis. To confirm AR, they underwent allergic skin test (AST) or multiallergen simultaneous test (MAST) and 72 subjects were classified into healthy volunteers and 29 subjects were diagnosed with AR. Nasal cytology specimens were obtained from 10 healthy volunteers and 10 AR patients using intranasal brushing of the 101 subjects. And 1×1-sized nasal mucosa was obtained from the middle turbinate of all 101 subjects under general anesthesia (IRB number C2012248 [943]).

Viruses and reagents

Influenza A virus (IAV WS/33: H1N1, ATCC, Manassas, VA) was used to induce acute viral lung infection. Virus stocks were grown in Madin-Darby canine kidney (MDCK) cells in virus growth medium according to a standard

procedure (20). Briefly, after 48 hour incubation at 37℃, the supernatants were harvested and spun by centrifugation at 5000rpm for 30min to remove cellular debris. Virus stocks were titrated on MDCK cells using a tissue culture infectious dose assay and stored at -80℃.

Cell culture

Normal human nasal epithelial (NHNE, N=10) and allergic rhinitis nasal epithelial (ARNE, N=10) cells were cultured as described previously (21). Briefly, passage-2 NHNE cells (1×10^5 cells/culture) were seeded in 0.5㎖ of culture medium on Transwell® cell culture inserts (24.5㎜, with a 0.45mm pore size; Costar Co., Cambridge, MA). Cells were cultured in a 1:1 mixture of basal epithelial growth medium and Dulbecco's Modified Eagle's Medium (DMEM) containing previously described supplements (9, 21). Cultures were grown while submerged for the first 9 days. The culture medium was changed on Day 1, and every other day thereafter. An air-liquid interface (ALI) was created on Day 9 by removing the apical medium and feeding the cultures from the basal compartment only. The culture medium was changed daily after the initiation of the ALI. We add antibiotics such as 1% penicillin and streptomycin into the all media for subculture and culture stages and we also add antifungal agent, Fungizone® (1㎖/1000㎖ media, Life technologies,

Grand island, NY) after filtering the media. All experiments described here used cultured nasal epithelial cells at 14 days after the creation of the ALI.

Immunohistochemistry and histologic analysis

Scanning electron microscope (SEM) analysis and immunofluorescence staining for MUC5AC for secretory cells and acetylated α-tubulin, as a marker of cilia were performed using full differentiated NHNE and ARNE cells.

Real-time PCR

To quantify the cellular viral level and host gene expression, cellular RNA was used to generate cDNA. The IAV level was monitored using a quantitative PCR for the *PA* gene (segment 3) with forward and reverse primers and probe 5'-ggccgactacactctcgatga-3', 5'-tgtcttatggtgaatagcctggttt-3', and 5'-agcagggctaggatc-3', respectively. Primers for Human IFN-β, IFN-λ_1, IFN-$\lambda_{2/3}$, IFN-λ_4, TSLP, IL-25, IL-33, IL28Rα, and IL10Rβ were purchased from Applied Biosystems™ (Foster City, CA). Real-time PCR was performed using the Perkin- Elmer Biosystems ABI PRISM® 7700 Sequence Detection System (PE Biosystems, Foster City, Calif.).

Quantification of secreted IFN-β and IFN-λ

Secreted human IFN-β (41410-1) and IFN-λ (DY1598B) were quantified using a DuoSet® ELISA kit from R&D Systems according to the manufacturer's instructions. The working range of the assay was 62.5-4000pg/㎖.

Plaque assay

Virus samples were serially diluted with phosphate-buffered saline (PBS). Confluent monolayers of MDCK cells in six-well plates were washed twice with PBS and then infected in duplicate with 250μl/well of each virus dilution. The plates were incubated at 37℃ for 45min to facilitate virus adsorption. Following adsorption, a 1% agarose overlay in complete minimal essential medium (MEM) supplemented with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) treated trypsin (1μg/㎖) and 1% fetal bovine serum was applied. The plates were incubated at 37℃, and cells were fixed with 10% formalin at 2 days post of infection (dpi).

Treatment of recombinant IFN-λs

Recombinant IFN-λs including λ_1 and λ_2 for human were purchased from

Invitrogen™ (Life science, Seoul, Korea). To examine IFN-λs-dependent protective effect against IAV infection, NHNE cells were treated with recombinant IFN-λs (IFN-λ_1: 10 ng/㎖ and IFN-λ_2: 10 ng/㎖) 1hr before infection.

Statistical analyses

For in vitro study, at least three independent experiments were performed with cultured cells from each donor, and the results are presented as the mean value±standard deviation (SD) of triplicate cultures. Statistical significance of variables between two independent groups was determined by Mann-Whitney U-tests and differences among treatment groups were evaluated by analysis of variance (ANOVA) with a *post hoc* test. All statistical analyses were performed with Graphpad Prism software (version 5; Graphpad Software, La Jolla, CA). A p-value<0.05 was considered to be statistically significant.

Results

In vitro nasal epithelial cell culture in healthy volunteers and AR subjects

SEM of apical NHNE cells in ALI culture at 14 days after confluence showed polygonal cells tightly attached to each other, and well-differentiated cilia and secreted mucus were observed (Figure 1A). SEM findings provided more detailed information about the histologic differences between cultured ARNE cells and NHNE cells. As expected, SEM findings showed that the number of ciliated cells was significantly attenuated in ALI culture of ARNE cells at 14 days after confluence and abnormal cilia architectures were observed with untidy and shorter cilia structures on the epithelial surface (Figure 1B). Immunofluorescence staining for MUC5AC and acetylated α-tubulin in ARNE cells showed significant dissimilar findings compared to NHNE cells such as lower number of ciliated cells and more intense MUC5AC staining at 14 days after confluence (Figure 1B).

The allergic airway is characterized by the secretion of Th2 cytokines, which has been shown to be highly involved in the pathogenesis of allergic inflammation. In particular, TSLP, IL-25 and IL-33 can initiate allergic inflammation and are produced by several epithelial cell lines, keratinocyte

and respiratory epithelial cells. To evaluate if cultured ARNE cells, which had different histologic findings from NHNE cells, possessed allergic characteristics without any stimulation, we measured the gene expression of TSLP, IL-25, and IL-33. NHNE (N=10) and ARNE (N=10) cells were cultured, and cell lysates were obtained at 14 days after confluence.

The results of real-time PCR showed that the mean mRNA levels of TSLP from ARNE cells were considerably higher than that of NHNE cells, although there was not any allergic stimulation to either type of nasal epithelial cells (NHNE cell culture: 1768.2±683.1 vs ARNE cell culture: 4293.4±1708.5, Figure 1C). However, mRNA levels of IL-25 and IL-33 were not elevated in ALI culture of ARNE cells (Figure 1C). TSLP, IL-25, and IL-33 are well known that are induced and secreted by allergic stimulation in epithelial cells but we measured these mRNA levels without any stimulation. Several studies also demonstrated TSLP, IL-25, and IL-33 play different pathogenic roles in the acute and chronic phases of airway inflammation (22, 23). Therefore, we thought that there may be differences in the degree of secretion as the result of this study.

These results demonstrate that cultured ARNE cells from AR patients might have higher levels of Th2-initiating epithelium-derived cytokines such as TSLP and ALI culture of ARNE cells preserved the Th2-related immune

response resulting in the appearance of AR-related histologic characteristics.

Cultured ARNE cells were more susceptible to IAV infection.

Cultured NHNE and ARNE cells on ALI were infected with WS/33 (H1N1) at multiplicity of infection (MOI) of 1 to assess the susceptibility to IAV. Cell lysate and supernatant were harvested at 1 and 6hr and 1, 2, and 3dpi. Real-time PCR results revealed that IAV mRNA levels in NHNE cells lysate increased significantly until 3dpi (mean IAV mRNA levels: 8.2×10^3, Figure 2A). plaque assay data also showed that viral titers from supernatant of IAV-infected NHNE cells and was accompanied by a peak titer at 3dpi (mean viral titers: 1.6×10^5pfu/㎖, Figure 2B). Interestingly, IAV mRNA levels and viral titers were differed between IAV-infected ARNE and NHNE cells. ARNE cells had considerably elevated IAV mRNA levels from 6hr after IAV infection that were significantly higher than those of IAV-infected NHNE cells (mean IAV mRNA: 1.9×10^4, 3dpi, Figure 2A). In addition, viral titers in supernatant of IAV-infected ARNE cells exceeded the titers of IAV-infected NHNE cells from 6hr after IAV infection and biggest difference was observed at 3dpi (mean viral titers of ARNE: 1.1×10^6pfu/㎖, Figure 2B). These findings demonstrated the higher susceptibility of ARNE cells to IAV infection and aggressive viral replication in IAV-infected ARNE cells within

3 days.

Less induction of type Ⅲ IFNs was more closely related with higher susceptibility of IAV-infected ARNE cells.

Previously, we found dominant induction of IFN-β and IFN-λ to control IAV infection, and we observed that both IFN-α and IFN-ɣ were minimally induced by IAV infection in NHNE cells (9). Based on these findings, we focused on IFN-β and IFN-λ as possible IFNs that are induced as an innate immune response against IAV infection in NHNE and ARNE cells.

NHNE and ARNE cells were infected with WS/33 (H1N1) and the mRNA levels of IFN-β, IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 were measured until 3dpi by real-time PCR. The mRNA levels of IFN-β, IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 were significantly elevated from 1dpi onwards and were maintained until 3dpi in NHNE cells (Figure 3A). IFN-λ_1 and IFN-$\lambda_{2/3}$ had higher transcription induction than IFN-β in IAV-infection NHNE cells, and the highest mRNA levels of IFN-λ1, IFN-λ2/3 and IFN-λ4 were observed at 3dpi (IFN-λ_1: 1.9×10^4, IFN-$\lambda_{2/3}$: 3.7×10^4 and IFN-λ_4: 9.1×10^3). Then, ELISA was performed to quantify the levels of secreted IFNs in the supernatant of NHNE cells after IAV infection. The levels of secreted IFN-β and IFN-λs were all increased by 3dpi (Figure 3B). The highest level of

IFN-β secretion (4.8×10^3pg/㎖) was also observed at 3dpi onwards, whereas the secretion of IFN-λs (1.2×10^4pg/㎖) was increased more significantly in the supernatant of IAV-infected NHNE cells.

We then compared mRNA levels of IFN-β and IFN-λs and secreted protein in the cell lysate and supernatant of IAV-infected ARNE cells to assess the susceptibility of ARNE cells to IAV infection. The mRNA and secreted protein levels of IFN-β were also induced in IAV-infected ARNE cells and did not differ from those of IAV-infected NHNE cells (Figure 3C). However, IAV-infected ARNE cells had less mRNA induction of IFN-λ_1, IFN-$\lambda_{2/3}$ and IFN-λ_4 than NHNE cells, and the biggest difference between NHNE and ARNE cells was observed at 3dpi (Figure 3D, 3E, 3F). Secreted protein levels of IFN-λs were also significantly lower in the supernatant of IAV-infected ARNE cells compared to NHNE cells (NHNE: 2.1×10^4pg/㎖ vs. ARNE: 4.2×10^2pg/㎖ at 3dpi, Figure 3G).

We then measured the mRNA levels of IFNs in human nasal mucosa and compared mRNA levels between healthy and AR subjects. Human nasal mucosa was obtained at the middle turbinate using an intranasal endoscope in healthy volunteers (N=72) and AR patients (N=29), and mRNA levels of IFN-λs in human nasal mucosa were evaluated. Real-time PCR showed that mean mRNA levels of IFN-λ_1 (healthy volunteers: 1.9×10^6 vs. AR: 1.3×10^3,

Figure 4A), IFN-$\lambda_{2/3}$ (healthy volunteers: 5.5×10^6 vs. AR: 5.4×10^3, Figure 4B), and IFN-λ_4 (healthy volunteers: 1.2×10^9 vs. AR: 5.6×10^6, Figure 4C) tended to be lower in nasal mucosa from the patients who diagnosed with AR patients.

These results indicate that IFN-λ appears to be more preferentially driven as an innate immune response in NHNE cells against IAV infection, Allergic nasal mucosal contained lower IFN-λ expression and there is an apparent deficiency in the induction of IFN-λ in response to IAV infection in ARNE cells.

Type Ⅲ IFN could reduce the higher viral loads in IAV-infected allergic nasal epithelium

We next assessed whether higher susceptibility to IAV infection could be controlled by treatment with IFN-λ in IAV-infected allergic nasal epithelium. First, mRNA levels of IFN-λ receptors such as IL28Rα and IL10Rβ were measured in human nasal mucosa and cultured nasal epithelial cells using real-time PCR. The mRNA levels of IL28Rα and IL10Rβ in human nasal mucosa did not differ significantly between healthy volunteers and AR patients (Figure 5A, 5B) or between NHNE and ARNE cells (Figure 5C, 5D) without IAV infection.

ARNE cells were treated with human recombinant IFN-λs (IFN-λ_1: 10ng/㎖ and IFN-λ_2: 10ng/㎖) 1hr before IAV infection and then the viral titers was compared with that of IAV-infected ARNE cells. The elevated viral titers in IAV-infected ARNE cells (6.4×10^6pfu/㎖) at 2dpi were considerably attenuated by treatment with IFN-λs (1.8×10^5pfu/㎖, Figure 5E). These findings indicate that although mRNA levels and secretion of IFN-λs were less in ARNE cells and allergic nasal mucosa after IAV infection, the expression of IFN-λ receptors did not differ from NHNE cells and healthy nasal mucosa. Therefore, exogenous treatment of allergic nasal epithelium with IFN-λs could effectively suppress IAV replication and overcome the higher susceptibility to IAV infection.

Discussion

We have shown that reduced type Ⅲ IFNs induction and higher viral loads in IAV-infected allergic nasal mucosa through cultured human nasal epithelial cells and human nasal mucosa. Our data also showed that exogenous treatment with type Ⅲ IFNs could suppress IAV replication and overcome the higher susceptibility of allergic nasal mucosa to IAV infection.

Influenza virus that enters the host first encounters defense mechanisms in the upper or lower respiratory epithelium, and host defense mechanisms against viral infection can be conferred by increasing innate resistance in the respiratory epithelium (24-29). A specialized innate immune system exists at the respiratory epithelium to combat invasion by influenza virus, and protective innate immune responses lower the viral burden of the infected host by increasing antiviral resistance or disease tolerance (22, 30-32). Innate immune mechanisms of antiviral resistance are mediated by an increase in IFN secretion, and type I IFNs mediate the innate immune response to viruses and regulate the subsequent activation of the adaptive immune system (33-37). Type Ⅲ IFNs have also been shown to be critical for the innate immune response against viral infection in the respiratory tract (38-40). Moreover, type Ⅲ IFN deficiency has been linked with

exacerbations of asthma (16, 17, 39) and there has been an increasing awareness regarding the role of type Ⅲ IFN in the respiratory tract.

To the best of our knowledge, viral infection may contribute to the initiation and aggravation of allergic asthma through synergistic effects with Th2-related allergic inflammation. Viruses can affect the asthmatic respiratory tract by direct aggravation of airway damage, by increasing sensitivity, and through indirect immunopathologic responses of the respiratory mucosal immunity (39, 41). During viral infection in peak allergen exposure, there was increased receptor expression for respiratory viruses accompanied by lower antiviral immune responses. Viruses also enhance the epithelial permeability of Th2-related immune regulators in the asthmatic airway (42). However, there are few studies that delineate the susceptibility of allergic nasal mucosa to viral infection and impaired IFN-related innate immunity of the nasal epithelium in AR patients.

The current study was designed to assess whether allergic nasal mucosa was susceptible to viral infection and had difficulty clearing the virus. This investigation was first performed using primary cultured NHNE and ARNE cells from healthy and allergic human nasal mucosa. We found cultured ARNE cells contained the histologic characteristics of ARNE during the process of primary cell culture and It would be useful to investigate the

susceptibility of allergic nasal mucosa to IAV infection (21). IAV-infected ARNE showed significant differences in viral loads, such as higher mRNA level in cell lysate and viral titers in supernatant compared to IAV-infected NHNE cells.

Based on these assessments, we investigated impairments in innate antiviral immunity together with an increase in viral replication in ARNE cells and found that type Ⅲ IFNs were induced much less in IAV-infected ARNE cells which showed intact induction of type I IFNs. Similarly, mRNA levels of type Ⅲ IFNs including IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 were much lower in nasal mucosa of AR patients. We presumed that lower levels of type Ⅲ IFNs in allergic nasal mucosa and impairment of type Ⅲ IFN induction in IAV-infected ARNE cells were related with higher viral loads in allergic nasal epithelium, although type I IFN induction was intact.

We surmise that therapeutic application of type Ⅲ IFNs to overcome a weak innate immune response will enable a greater understanding of defense strategies against IAV infection in allergic nasal mucosa. Interestingly, the mRNA levels of receptors for Ⅲ IFNs such as IL28Rα and IL10Rβ, in ARNE cells and allergic nasal mucosa did not differ from those of NHNE cells or healthy nasal mucosa. Therefore, we assumed that intranasal delivery of type Ⅲ IFNs could induce the IFN-related innate immune

response and inhibit influenza viral replication in allergic nasal mucosa. IAV-infected ARNE cells which were treated with recombinant type Ⅲ IFNs showed significantly lower IAV mRNA levels in cell lysate and completely attenuated viral titers in the supernatant. Our work supports the idea that although AR patients seem to have lower amount of type Ⅲ IFNs in human nasal mucosa and may have a more serious respiratory viral infection or higher viral loads after IAV infection, exogenous treatment of type Ⅲ IFNs can control IAV infection in allergic nasal mucosa and prevent aggravation of viral infection in AR patients modulating IFN-related innate immune responses.

Conclusions

The impairment of type Ⅲ IFN-mediated innate immune response is crucial for higher viral loads of IAV in allergic nasal mucosa and aggravated IAV infection in AR subjects. We propose that exogenous treatment of type Ⅲ IFNs reduces susceptibility of allergic nasal mucosa to IAV infection and is a superior therapeutic candidate to control influenza viral infection.

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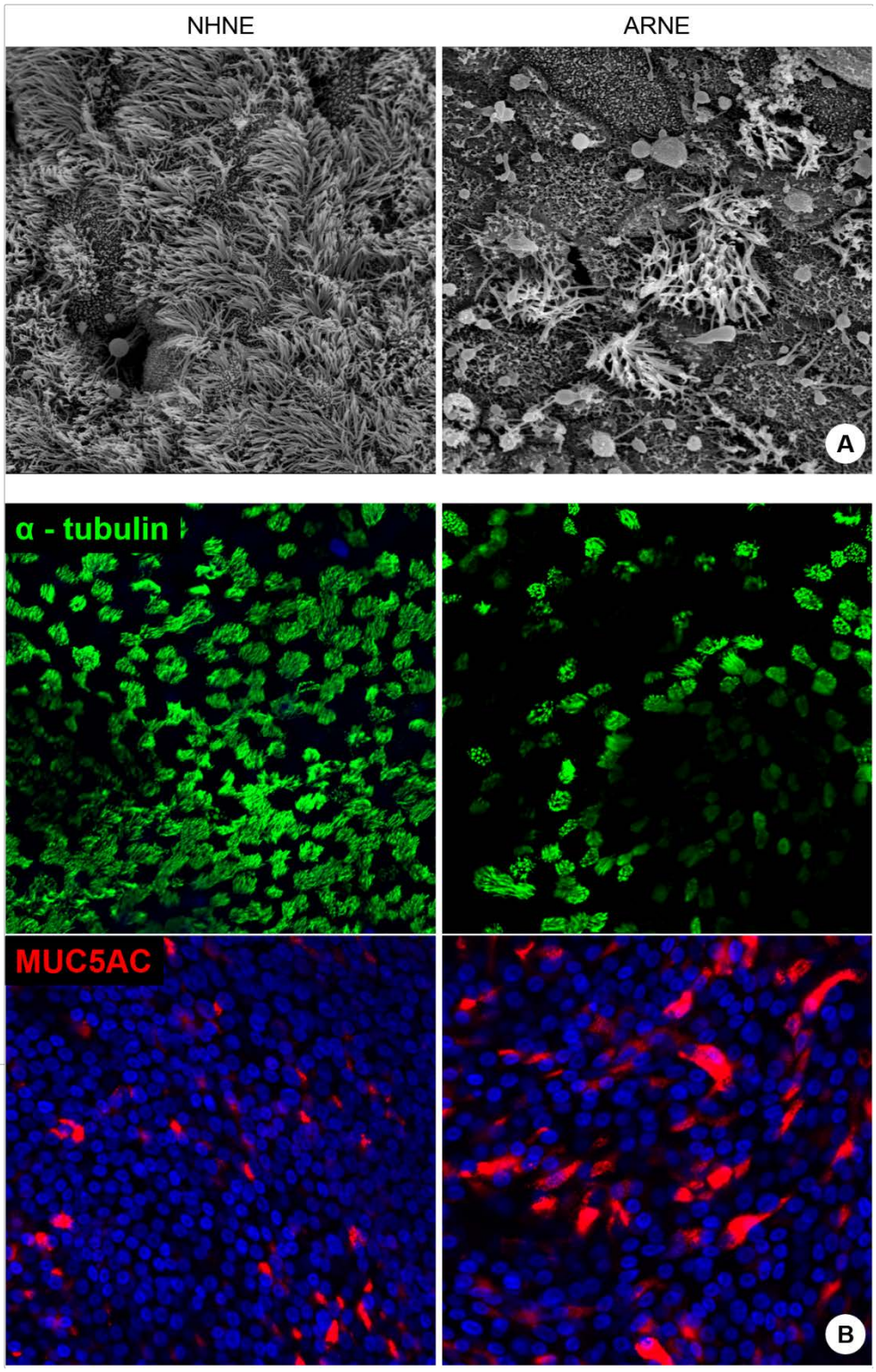
NHNE
ARNE
A
α - tubulin
MUC5AC
B

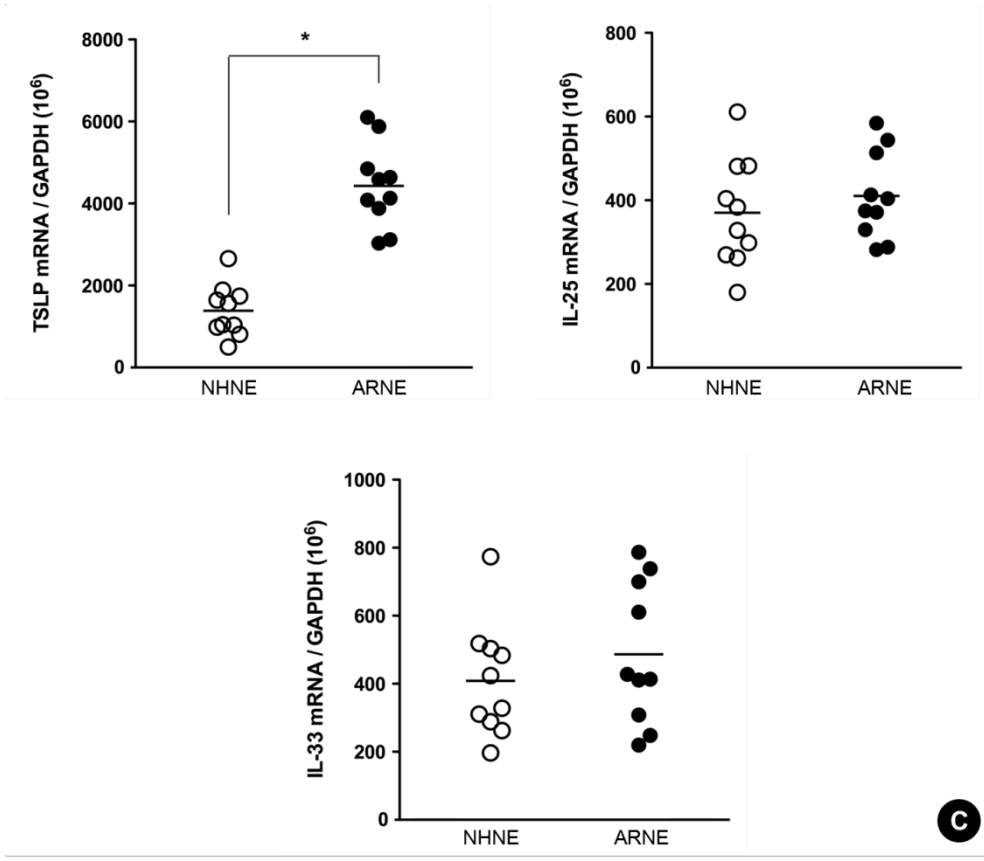
TSLP mRNA / GAPDH (10^6)
8000
6000
4000
2000
0
*
NHNE
ARNE
IL-25 mRNA / GAPDH (10^6)
800
600
400
200
0
NHNE
ARNE
IL-33 mRNA / GAPDH (10^6)
1000
800
600
400
200
0
NHNE
ARNE
C

Figure 1. Histologic differences between cultured NHNE and ARNE cells on SEM findings and immunofluorescence staining for acetylated α-tubulin and MUC5AC and higher level of Th2-initiating epithelium-derived cytokines in cultured ARNE cells.

ARNE cells showed different histologic findings compared to NHNE cells such as a lower number of cilia on the apical surface of the nasal epithelium and hyperplasia of secretory cells (A and B). And mRNA level of TSLP was significantly higher without any stimulations but mRNA levels of IL-25 and IL-33 were not elevated in ARNE cells compared to NHNE cells (C, *: p-value<0.05 when comparing the levels between NHNE and ARNE cells). NHNE, normal human nasal epithelial; ARNE, allergic rhinitis nasal epithelial; SEM, scanning electron microscope; TSLP, thymic stromal lymphopoietin; IL, interleukin.

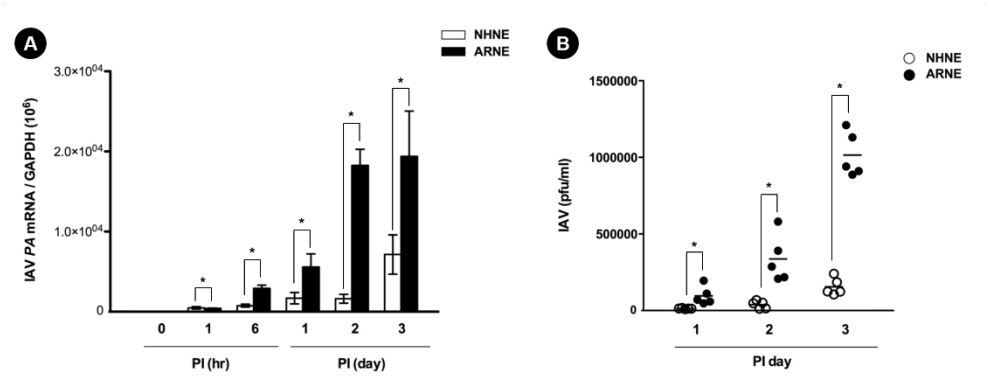

Figure 2. ARNE cells were more susceptible to IAV infection.

NHNE (N=5) and ARNE (N=5) cells were inoculated with WS/33 (H1N1) for 0, 1, and 6hr and 1, 2, and 3days at an MOI of 1. (A) Real-time PCR showed that the IAV mRNA level was higher in ARNE cells from 6hr after infection and the biggest difference in mRNA level was observed at 3dpi. (B) A plaque assay also showed that viral titers in supernatant of IAV-infected ARNE cells were significantly higher at 1dpi. Results are presented here as the mean±SD from five independent experiments (*: p-value<0.05 when comparing the levels between NHNE and ARNE cells). NHNE, normal human nasal epithelial; ARNE, allergic rhinitis nasal epithelial; AR, allergic rhinitis; IAV, influenza A virus; MOI, multiplicity of infection; SD, standard deviation.

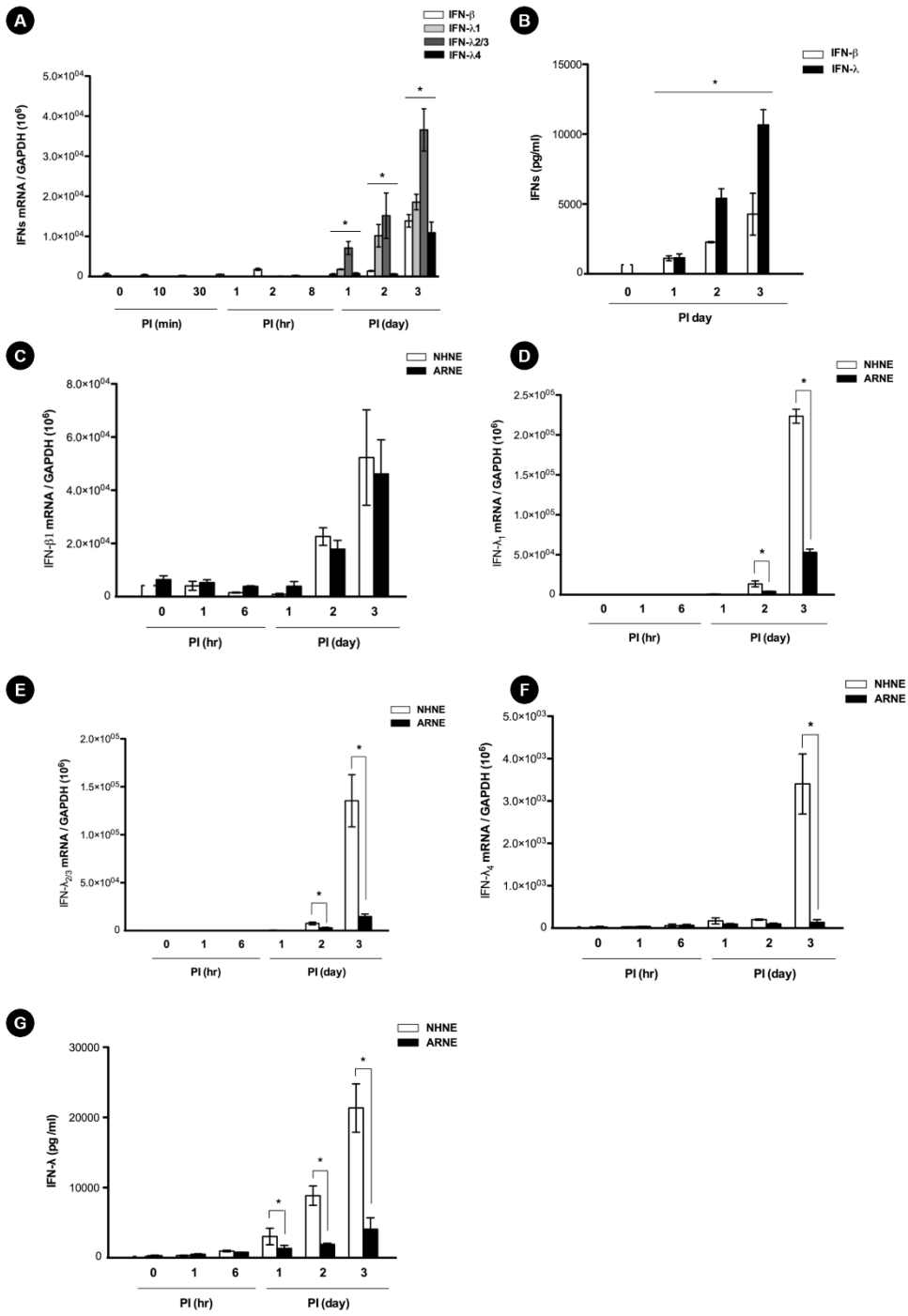
A
IFN-β
IFN-λ1
IFN-λ2/3
IFN-λ4
IFNs mRNA / GAPDH (10^6)
PI (min)
PI (hr)
PI (day)
B
IFN-β
IFN-λ
IFNs (pg/ml)
PI day
C
NHNE
ARNE
IFN-β1 mRNA / GAPDH (10^6)
PI (hr)
PI (day)
D
NHNE
ARNE
IFN-λ1 mRNA / GAPDH (10^6)
PI (hr)
PI (day)
E
NHNE
ARNE
IFN-λ2/3 mRNA / GAPDH (10^6)
PI (hr)
PI (day)
F
NHNE
ARNE
IFN-λ3 mRNA / GAPDH (10^6)
PI (hr)
PI (day)
G
NHNE
ARNE
IFN-λ (pg/ml)
PI (hr)
PI (day)

Figure 3. IFN-λ was preferentially induced to control IAV infection in NHNE cells.

NHNE cells were inoculated with WS/33 (H1N1) for 0, 1, and 6hr and 1, 2, and 3 days at an MOI of 1. (A) The mRNAs levels of IFN-β, IFN-λ_1, IFN-$\lambda_{2/3}$, and IFN-λ_4 were measured using real-time PCR. (B) Supernatant was assayed by ELISA to measure the secreted IFN-β and IFN-λ concentrations after IAV infection (∗: p-value<0.05 when comparing mRNA and protein levels of IFN-λ with those of IFN-β). (C) IFN-β mRNA levels in IAV-infected NHNE cells were not different from those of IAV-infected ARNE cells. However, the mRNAs level of IFN-λ_1 (D), IFN-$\lambda_{2/3}$ (E), and IFN-λ_4 (F) were significantly lower in IAV-infected ARNE cells and secreted protein levels of IFN-λ in supernatant of IAV-infected ARNE cells were also attenuated compared to IAV-infected NHNE cells from 1dpi (G). Results are presented here as the mean±SD from five independent experiments (∗: p-value<0.05 when comparing mRNA and protein levels of IAV-infected ARNE cells with those of NHNE cells). NHNE, normal human nasal epithelial; ARNE, allergic rhinitis nasal epithelial; IFN, interferon; IAV, influenza A virus; MOI, multiplicity of infection; SD, standard deviation.

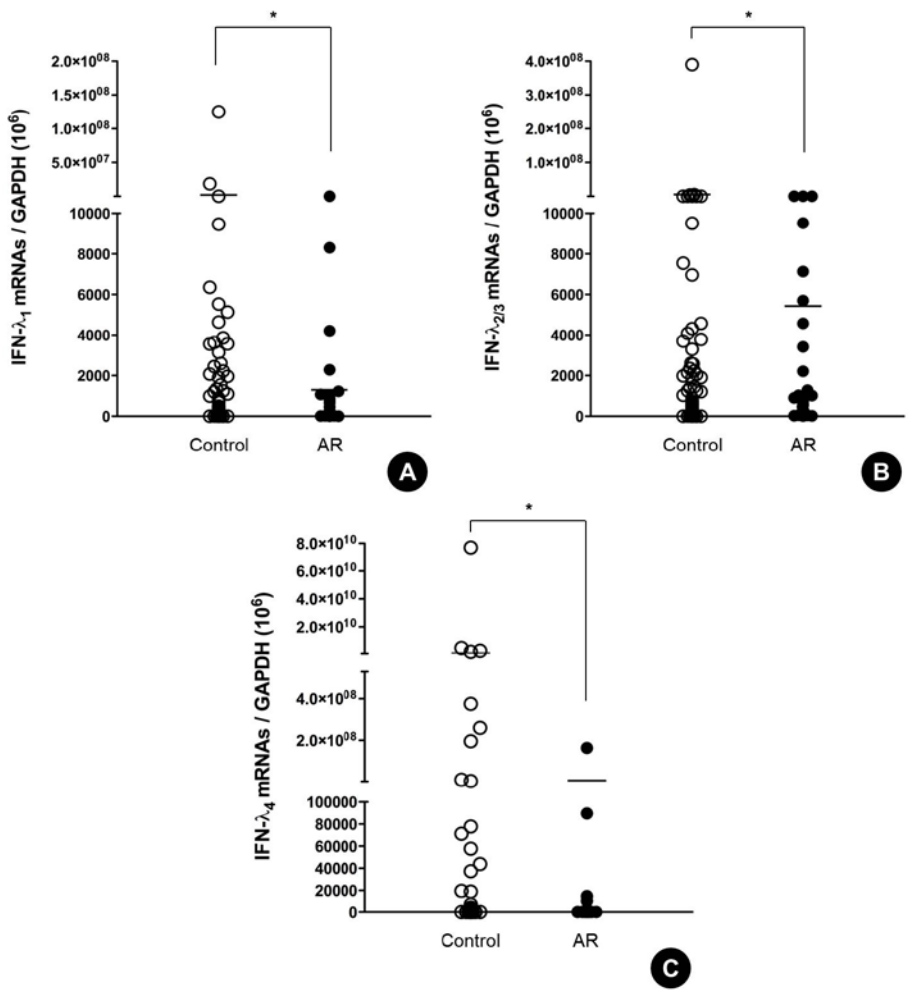
IFN-λ1 mRNAs / GAPDH (10^6)
2.0×10^08
1.5×10^08
1.0×10^08
5.0×10^07
10000
8000
6000
4000
2000
0
Control
AR
*
A
IFN-λ2/3 mRNAs / GAPDH (10^6)
4.0×10^08
3.0×10^08
2.0×10^08
1.0×10^08
10000
8000
6000
4000
2000
0
Control
AR
*
B
IFN-λ4 mRNAs / GAPDH (10^6)
8.0×10^10
6.0×10^10
4.0×10^10
2.0×10^10
4.0×10^08
2.0×10^08
100000
80000
60000
40000
20000
0
Control
AR
*
C

Figure 4. The mRNA levels of IFN-λs were considerably lower in human nasal mucosa of AR patients.

The mRNA levels of IFN-λ_1 (A), IFN-$\lambda_{2/3}$ (B) and IFN-λ_4 (C) were measured in human nasal mucosa of healthy volunteers (Control, N=72) and AR subjects (AR, N=29). Real-time PCR results showed that the mRNA levels of IFN-λs were significantly lower in human nasal mucosa of AR subjects compared to healthy volunteers (*: p-value<0.05 when comparing the levels between healthy volunteers and AR subjects). IFN, interferon; AR, allergic rhinitis.

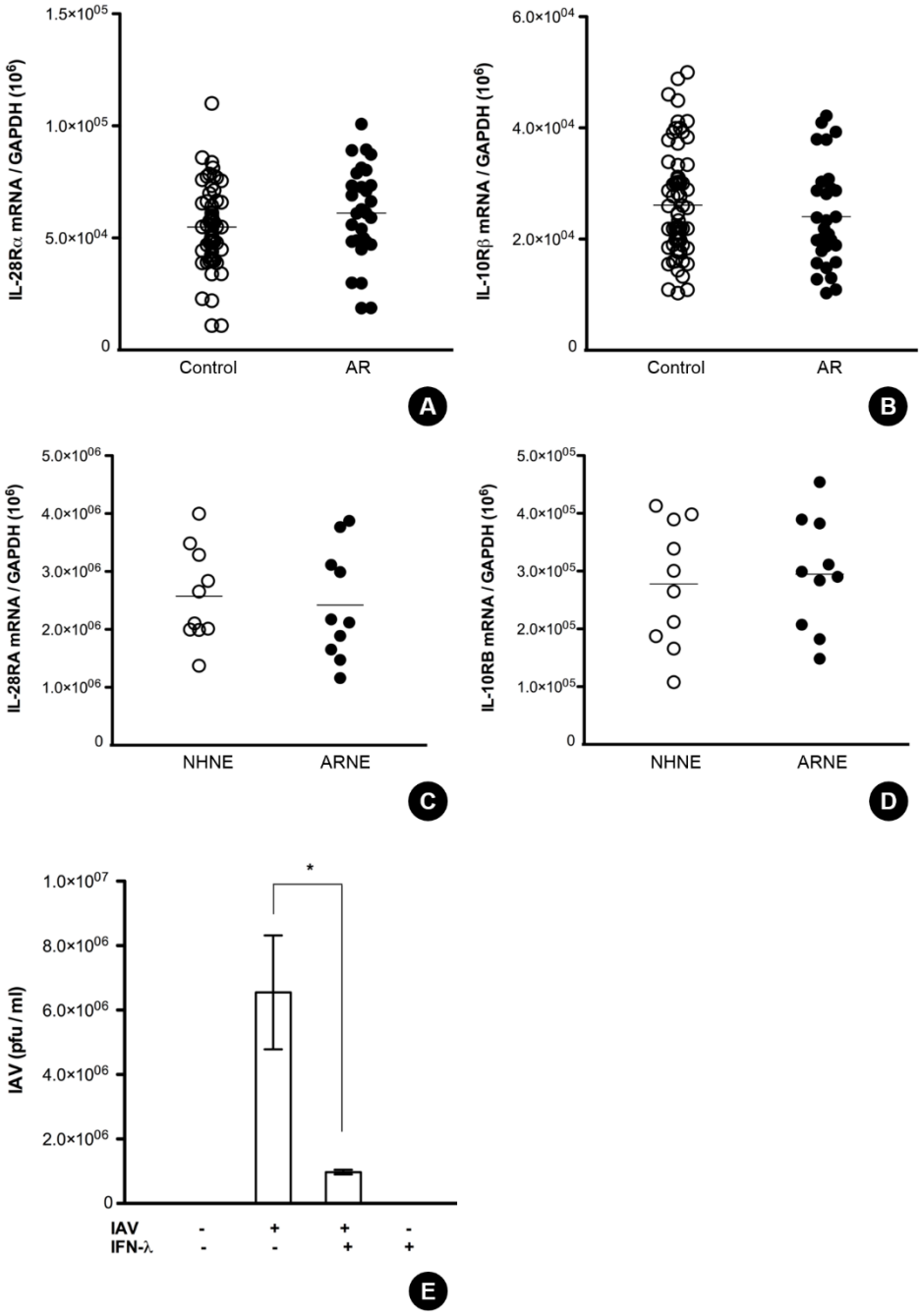
IL-28Rα mRNA / GAPDH (10^6)
1.5×10^05
1.0×10^05
5.0×10^04
0
Control
AR
A
IL-10Rβ mRNA / GAPDH (10^6)
6.0×10^04
4.0×10^04
2.0×10^04
0
Control
AR
B
IL-28RA mRNA / GAPDH (10^6)
5.0×10^06
4.0×10^06
3.0×10^06
2.0×10^06
1.0×10^06
0
NHNE
ARNE
C
IL-10RB mRNA / GAPDH (10^6)
5.0×10^05
4.0×10^05
3.0×10^05
2.0×10^05
1.0×10^05
0
NHNE
ARNE
D
IAV (pfu / ml)
1.0×10^07
8.0×10^06
6.0×10^06
4.0×10^06
2.0×10^06
0
*
IAV - + + -
IFN-λ - - + +
E

Figure 5. The mRNA levels of IFN-λ-specific receptors did not differ between ARNE cells and human nasal mucosa of AR patients.

The mRNA levels of IL28Rα and IL10Rβ were measured in human nasal mucosa of healthy volunteers (Control, N=72) (A) and AR subjects (AR, N=29) (B) using real-time PCR (*: p-value<0.05 when comparing the levels between healthy volunteers and AR subjects). The receptors' gene expressions were also compared in NHNE (N=10) and ARNE (N=10) cells (C, D) (*: p-value<0.05 when comparing the levels between NHNE and ARNE cells). ARNE cells were treated with recombinant IFN-λs (IFN-λ_1: 10 ng/㎖ and IFN-λ_2: 10 ng/㎖) simultaneous with WS/33 (H1N1) inoculation. A plaque assay (E) showed that the increased viral titers of IAV at 2dpi was completely attenuated after treatment with recombinant IFN-λs. Results are presented here as the mean±SD from five independent experiments (*: p-value<0.05 when comparing the levels of IAV-infected cells). NHNE, normal human nasal epithelial; ARNE, allergic rhinitis nasal epithelial; IFN, interferon; IAV, influenza A virus; dpi, day(s) post of infection; SD, standard deviation.

국문 초록

알레르기 비염 비강상피세포에서 A형 인플루엔자 바이러스 감염 감수성을 결정하는 주요 숙주 인자로서의 제3형 인터페론에 대한 연구

서울대학교 대학원

의학과 이비인후과학 전공

전 영 진

배 경: 인터페론 (interferon, IFN)은 호흡상피세포에서 선천성 면역 기전의 중요 요소이며 바이러스 감염에 대한 첫 번째 방어기전을 담당한다. 바이러스 감염 후 분비된 IFN이 각각의 특이 수용체에 결합하고 300개가 넘는 IFN 자극 유전자를 활성화 시켜서 바이러스를 사멸시키는 역할을 수행한다. 이러한 IFN의 분비 능력이 감소하면 결국 바이러스 감염에도 취약한 양상을 보이게 된다. 대표적인 호흡기 알레르기 질환인 알레르기 비염은 제 1형 과민 반응이 주요한 발생기전으로 생각되는데 특히 2형 T helper cell (Th2 cell) 반응이 병태 생리에 중요하다. Th2 반응은 Th1 반응을 약화시켜 항 바이러스 면역력 저하를 초래하고 호흡상피세포를 바이러스 감염에 취약하게 만든다고 추정된다.

목 적: 본 연구에서는 알레르기 비염 비강 점막에서 정상인에 비해 A형 인플루엔자 바이러스 (influenza A virus, IAV) 감염에 더 취약한지 알아보고, IFN과

관련한 선천성 비강 점막 면역력의 차이를 비교하고자 시행되었다.

방 법: 정상인과 알레르기 비염 환자의 생체 외 배양 시스템 (air-liquid interface culture system)을 이용하여 배양된, 정상 비강상피 (normal human nasal epithelial, NHNE) 세포 (N=10) 와 알레르기 비염 비강상피 (allergic rhinitis nasal epithelial, ARNE) 세포 (N=10) 간의 IAV mRNA 수치, 바이러스 역가 (viral titer), IFN 발현 정도 및 IFN 특정 수용체 발현 정도를 비교하였다. 또한 72명의 정상인과 29명의 알레르기 비염 환자의 비강 점막에서도 IFN 발현 정도 및 IFN 특정 수용체 발현 정도를 비교하였다.

결 과: 배양된 비강상피세포에 IAV를 감염 시킨 후 IAV mRNA 수치와 바이러스 역가가 ARNE 세포에서 NHNE 세포보다 유의하게 증가되었다. IFN-β 와 -λ은 IAV 감염 후 3일째까지 NHNE 세포와 ARNE 세포에서 발현이 증가되었다. IFN-β 발현은 ARNE 세포에서도 NHNE 세포와 차이 없이 증가되었지만 IFN-λ 발현은 NHNE 세포에 비해 ARNE 세포에서 굉장히 낮았다. 알레르기 비염 환자의 비강 점막에서도 IFN-λ 발현이 정상군에 비해 유의하게 낮았다. 한편 ARNE 세포에서 IFN-λ 특이 수용체의 발현 정도는 NHNE 세포와 비슷함을 알 수 있었고 ARNE 세포에 IFN-λ를 전처치하고 IAV를 감염시키면 IAV mRNA 수치를 현저히 저하시킬 수 있었다.

결 론: 본 연구를 통해 알레르기 비염 환자의 비강 점막에 IAV를 감염시키면 IFN-β 발현은 정상군과 차이 없으나 IFN-λ의 발현이 현저히 감소되어 있음을 알 수 있었다. 이러한 차이를 미루어 볼 때 알레르기 비염 환자에서 비강 점막

의 약화된 선천성 면역 기전이 IAV 감염 감수성 증가와 관련이 있을 것으로 생각된다. 그러나 알레르기 비염 환자의 비강 점막에서 IFN-λ 특이 수용체의 발현은 정상군와 유사하므로 외부에서 미리 IFN-λ를 처치해주면 정상군과 유사한 바이러스 사멸 능력을 보일 수 있음을 알 수 있었다.

주요어: A형 인플루엔자 바이러스, 인터페론 람다, 알레르기 비염, 비강상피세포

학 번: 2015-23222