Activation of the Interleukin-34 Inflammatory Pathway in Response to Influenza A Virus Infection

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Abstract: Interleukin 34 (IL-34) is a newly recognized cytokine that functions similarly to macrophage colony-stimulating factor. This study investigated the mechanism by which IL-34 is produced in response to exogenous pathogen infections in humans. The results showed that the IL-34 levels were higher in the serum and peripheral blood mononuclear cells (PBMCs) from 155 influenza A virus (IAV)-infected patients than in those from 145 healthy individuals. The expression level of IL-34 in IAV-infected PBMCs was blocked by IL-22-specific siRNA. This result indicated that IL-34 was induced by IL-22 in the inflammatory cascade. The mRNA and protein expression levels of IL-22 activated by IAV infection were significantly inhibited by IL-34 overexpression but induced by IL-34-specific siRNA. Thus, a feedback system most likely exists between IL-34 and IL-22. The IL-22 expression in T helper type 17 (Th17) cells of PBMCs was higher than IL-34 expression in Th17 cells of PBMCs, and there was IL-34 expression in IL-22+ Th17 cells. This result showed that the production of IL-22 and IL-34 is both from the same and different subset of cells, which indicated that the regulatory mechanism of IL-22/IL-34 is through the autocrine or paracrine systems. In conclusion, IL-34 is induced by IL-22 in the inflammatory cascade in response to IAV infection. Therefore, IL-34 is a promising target for the screening of anti-inflammatory medicines.

Key Indexing Terms: Influenza A virus; Interleukin 34; Interleukin 22; Inflammatory. **[Am J Med Sci 2015;349(2):145–150.]**

Influenza viruses of the *Orthomyxoviridae* family are highly contagious single-stranded RNA viruses whose genome consists of 8 distinct RNA segments that encode 11 to 12 proteins.[1,2] Seasonal strains (eg, influenza A viruses (IAVs), H3N2) that cause annual epidemics can also cause severe diseases among immunocompromised individuals, such as the young and the elderly.[3,4] A global H5N1 pandemic could have devastating effects, and H5N1 represents a serious public health threat.[5] The host cytokine immune response provides the first line of defense against IAV infection.[6] IAV infection induces excessive cytokine production (cytokine storm) and leukocyte recruitment, both of which are the principal causes of severe disease among humans.[7,8] However, the mechanism by which cytokine induction is increased during IAV infection remains unclear to date.

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Submitted July 14, 2014; accepted in revised form October 3, 2014.

This study was supported by National Natural Science Foundation of China (No 30872491/C160402, 81372552 and 81172349/H1617).

The authors have no conflicts of interest to disclose.

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Interleukin 22 (IL-22) of the IL-10 family is produced by $CD4^+$ T helper 17 cells, natural killer cells, $CD11c^+$ myeloid cells and lymphoid tissue inducer-like cells.[9,10] The IL-22 receptor is composed of both IL-22R1 and IL-10R2 subunits; this receptor is found on nonhematopoietic cells, such as those in the liver, lung, gut, kidney and skin.[11,12] IL-22 assumes different functions in inflammatory and autoimmune diseases; it potentially protects epithelial cells and hepatocytes on barrier surfaces.[13,14] However, uncontrolled IL-22 contributes to the development of inflammatory disorders, such as psoriasis, lupus erythematosus and rheumatoid arthritis.[14,15]

The cytokine interleukin 34 (IL-34) has been recently identified through the functional screening of a library of secreted proteins.[1,16] IL-34 is specifically expressed in splenic tissue, predominantly in the red pulp region; it supports human monocyte survival and promotes the formation of the colony-forming unit macrophage in human bone marrow cell cultures.[17,18] IL-34 is a dimeric glycoprotein that presents different amino acid sequences with colony-stimulating factor 1.[19] Murine IL-34 also promotes the survival of peritoneal macrophages and the proliferation of bone marrow progenitor cells.[20,21] Despite these studies, the role of IL-34 in the inflammatory network remains unknown.

Viral infections stimulate IL-22 expression.[22] However, the mechanism by which IL-34 expression is regulated by IAV infection remains unclear. Considering that IL-22 and IL-34 are obligatory mediators of inflammation, we determined whether these cytokines act as independent effectors of host inflammatory response to viral infection or interact with each other. This study investigated the mechanism by which IAV infection regulates IL-34 expression. The results showed that IAV infection activated IL-22 and IL-34 by a previously unrecognized mechanism in which IAV stimulates IL-34 expression through IL-22 and IL-34 feedback inhibits IL-22 expression.

METHODS

Patients

A total of 155 patients with IAV were randomly selected from the Zhongnan Hospital of Wuhan University from February 2012 to February 2014. The blood samples were obtained from these patients during their first visit after onset of symptoms. Samples from healthy individuals were randomly collected as controls from the local blood donation center. The main clinical and demographic characteristics of the population are presented in Tables 1 and 2. Written informed consents were obtained from all patients. This study was approved by the Research Ethics Committee of Zhongnan Hospital of Wuhan University and was performed in accordance with the Declaration of Helsinki Principles.

Virus and Constructs

The influenza virus strain A/chicken/Hubei/327/2004 (H5N1) used in this study was provided by the China Center

TABLE 1. Baseline characteristics of IAV-infected patients and healthy individuals in Figures 1A and 1B

Characteristic	Healthy individuals (n = 47)	Patients (n = 51)
Age (y)	42.43 ± 13.42	39.53 ± 15.34
Gender (male/female)	20/27	24/27
HA-antigen positive	0 (0)	47 (43)
Anti-HA positive	0 (0)	47 (43)
Viral genotype A (H3/H1)	0/0	39/12
IL-22 (folds)	77.86 ± 34.85	657.17 ± 97.89[a]
IL-34 (folds)	46.29 ± 31.62	459.3 ± 85.2[a]

Data are presented as mean ± standard error of mean.
[a] $P < 0.01$ compared with healthy individuals group.

for Type Culture Collection. Stock virus was propagated in 10-day-old embryonated chicken eggs for 36 to 48 hours at 37°C. Allantoic fluid was harvested, and aliquots were stored at −80°C before testing. Mammalian expression plasmids for IL-22 and IL-34 were constructed using standard molecular biology techniques.

Peripheral Blood Mononuclear Cell Isolation and Transfection

Peripheral blood mononuclear cells (PBMCs) were isolated from the venous blood samples of healthy individuals. PBMC fractions were obtained through density centrifugation using Histopaque following the manufacturer's instructions (Sigma, St. Louis, MO). PBMCs were transfected with plasmid DNA through electroporation using an Amaxa Nucleofector II Device according to the manufacturers' protocols.

RNA Interference

Small interfering RNAs for both IL-22 and IL-34 (IL-22 RNAi and IL-34 RNAi) and their negative controls were synthesized using RiBo Biotech (GuangZhou RiBo Biotech, Guangzhou, China); siRNA was used following the manufacturers' instructions.

Quantitative RT-PCR Analysis

Total RNA was isolated using TRIzol (Invitrogen, Basel, Switzerland). Cellular RNA samples were reverse transcribed using random primers. Quantitative RT-PCR (qRT-PCR) was performed

TABLE 2. Baseline characteristics of IV-infected patients and healthy individuals in Figure 1C and 1D

Characteristic	Healthy individuals (n = 98)	Patients (n = 104)
Age (y)	38.45 ± 9.51	43.51 ± 11.08
Gender (male/female)	54/44	57/47
HA-Antigen positive	0 (0)	104 (100)
Anti-HA positive	0 (0)	104 (100)
Viral genotype A (H3/H1)	0/0	85/19
IL-22 (pg/mL)	61.75 ± 23	148.49 ± 45.02[a]
IL-34 (pg/mL)	66.85 ± 27.53	172.53 ± 39.33[a]

Data are presented as mean ± standard error of mean.
[a] $P < 0.05$ compared with healthy individuals group.

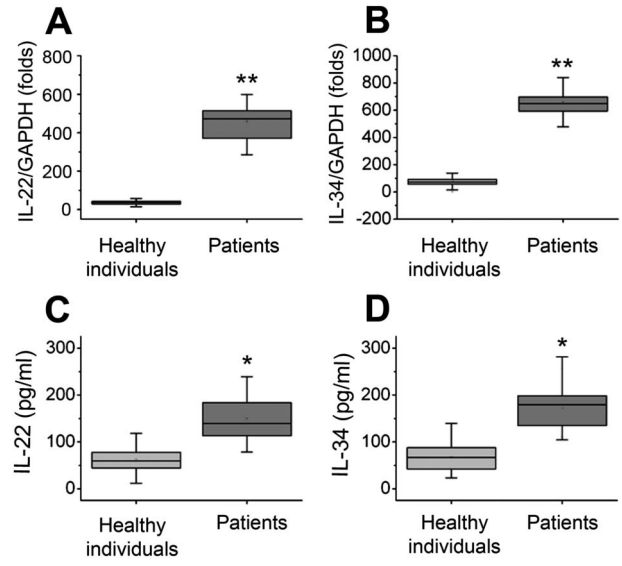

FIGURE 1. Expression levels of IL-22 and IL-34 in healthy individuals and patients with IAV. Total RNA was extracted from freshly isolated PBMCs from healthy individuals (n = 47) or patients with IAV (n = 51). The mRNA expression levels of IL-22 (A) and IL-34 (B) were detected by qRT-PCR. Data represent the mean ± standard error of mean of triplicate samples. Boxplots illustrate medians with 25% and 75% and error bars for 5% and 95% percentiles (**$P < 0.01$). Serum IL-22 (C) and IL-34 (D) levels in healthy individuals (n = 98) and patients with IAV (n = 104) were detected by ELISA. Samples were tested in duplicate, and concentrations were determined from standard curves. Data represent the mean ± SD. Boxplots illustrate medians with 25% and 75% and error bars for 5% and 95% percentiles (**$P < 0.01$).

using the LightCycler 480 (Roche, Nutley, NJ) system and the SYBR Green system (Applied Biosystems, Foster City, CA). GAPDH was amplified as an internal control. The following primers were used: IL-22, 5′-GAAGAAGTGCTGTTCCCT-CAATC-3′ and 3′-ATTCCTCTGGATATGCAGGTCAT-5′;

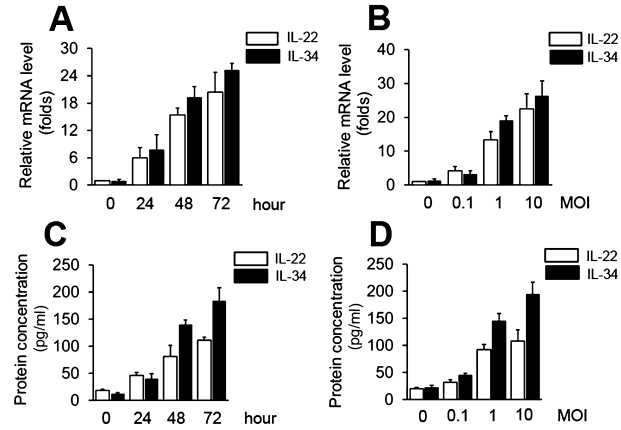

FIGURE 2. Effect of IAV on IL-22 and IL-34 expression levels. PBMCs were infected with IAV (MOI = 1) at indicated time points. The mRNA and protein levels of IL-22 and IL-34 were quantified by qRT-PCR (A) and ELISA (C), respectively. After PBMCs were infected with IAV for 48 hours, the mRNA and protein levels of IL-22 and IL-34 were quantified by qRT-PCR (B) and ELISA (D), respectively. Data represent the mean ± SD, n = 3 per group.