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Type III interferon gene expression in response to influenza virus infection in chicken and duck embryonic fibroblasts

Zhijie Zhang[a,b], Tingting Zou[a], Xiaotong Hu[a], Hong Jin[a,*]

[a] Department of Microbiology and Parasitology, China Medical University, Shenyang 110122, PR China
[b] The Clinical Laboratory of Shengjing Hospital, China Medical University, Shenyang 110003, PR China

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ABSTRACT

Type III interferons (IFN-λs) comprise a group of newly identified antiviral cytokines that are functionally similar to type I IFNs and elicit first-line antiviral responses. Recently, type III IFNs were identified in several species; however, little information is available about type III IFNs in ducks. We compared the expression of type III IFNs and their receptor in chicken embryonic fibroblasts (CEFs) and duck embryonic fibroblasts (DEFs) in response to influenza virus infection. The results showed that the expression of type III IFNs was upregulated in both DEFs and CEFs following infection with H1N1 influenza virus or treatment with poly (I:C), and expression levels were significantly higher in CEFs than in DEFs at each time point. The expression of the receptor for type III IFNs (IL-28Rα) was also upregulated following infection with H1N1 virus or treatment with poly (I:C) and was significantly higher in CEFs than in DEFs at each time point. The expression of the receptor for type III IFNs occurred from 8 hpi and remained at similar levels until 36 hpi in CEFs, but the expression level was elevated from 36 hpi in DEFs. These findings revealed the existence of distinct expression patterns for type III IFNs in chickens and ducks in response to influenza virus infection. The provided data are fundamentally useful in furthering our understanding of type III IFNs and innate antiviral responses in different species.

1. Introduction

Influenza A viruses (IAVs) are pathogens that cause an influenza epidemic every year and have high morbidity and mortality. The current vaccines largely aim to induce antibodies against viral glycoproteins, such as hemagglutinin (HA), but seasonal influenza viruses experience frequent mutations in the antigenic sites of the HA molecule, resulting in antigenic drift. As a result, the neutralizing activity of antibodies induced by previous infections or vaccination fails to eliminate the viruses (Hillaire et al., 2011). The creation of a universal flu vaccine has been a long-term goal of influenza researchers (Grebe et al., 2008).

Ducks do not typically show signs of disease after infection with influenza virus. The highly pathogenic avian influenza (HPAI) viruses are less pathogenic to ducks and rapidly adapt to maintain the balance between the virus and the host (Barber et al., 2010). In contrast, many of these same viruses cause 100% mortality in chickens within hours or days; thus, superior innate immunity might protect ducks during this critical period (Barber et al., 2010). The molecular basis of the natural resistance of ducks to influenza virus infection is unknown. Understanding the mechanism of this phenomenon is helpful for the creation of a universal flu vaccine.

The synthesis of antiviral cytokines, such as type I interferons, is the first cellular response to a viral infection. Recently, a new family of IFNs, type III interferons, was described in several species. Compared with type I IFNs, type III IFNs induce similar innate antiviral responses but signal through very different receptors (Perez-Martin et al., 2012; Sang et al., 2010). Epithelium-rich organs, such as the lungs and intestinal tract of chickens were found to express high levels of the transcript for the putative chicken type III IFN (IFN-λ3) receptor alpha chain (chIL28Rα) gene (Reuter et al., 2014).

To better understand how the responses differ between these two highly relevant species (i.e., the chicken and the duck), we compared the expression of type III IFNs and their receptor (IFN-λR) in chicken embryonic fibroblasts (CEFs) with that observed in duck embryonic fibroblasts (DEFs) in response to influenza virus infection.

* Corresponding author.
E-mail address: jinhongf@sina.com (H. Jin).

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2. Materials and methods

2.1. Ethics statement

The Ethics Committee for Experimental Animal of China Medical University approved all animal-related experiments and procedures. All experimental procedures conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Efforts were made to minimize the number of animals used and their suffering, and cultures of embryonic fibroblasts were made carefully and quickly from embryos from chickens and ducks.

2.2. CEF and DEF culture

CEFs and DEFs were generated from 10-day-old chicken embryos and 11-day-old duck embryos (Liaoning Yikang Biological Corporation Limited, Liaoyang,China). Cultures of embryo fibroblasts were made in triplicate for both types of cells. The CEFs and DEFs were grown overnight in DMEM supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin at 37 °C in 5% CO_2, with a cell density of approximately 1×10^6 cells/100 mm tissue culture dish.

2.3. Virus infection

Avian influenza viruses (A/FM/1/47) (H1N1) were propagated in the allantoic cavity of 10 day-old embryonated SPF chicken eggs at 34 °C, and allantoic fluid was harvested after 2–3 days. Fifty percent egg infectious dose (EID_{50}) titers were determined by serial titration of viruses in eggs and calculated using the method of Reed and Muench. Embryo fibroblasts were cultured in 100 mm tissue culture dishes in DMEM for 24 h. The culture medium was then removed, and $10^{4.8}$ EID^{50} of H1N1 influenza virus in a volume of 50 μl was added to each dish. Negative control embryo fibroblast cultures were set up identically but without the addition of virus. Positive control embryo fibroblast cultures were set up identically with the addition of Poly C (50 μg/ml). The culture plates were gently rocked every 15 min for 1 h, after which the medium was replaced with DMEM supplemented with 1% heat-inactivated fetal bovine serum. The cultures were incubated, and RNA was extracted from the cell monolayers at 0, 8, 24, and 36 h postinfection (hpi).

2.4. Quantitative real-time PCR

Real-time RT-PCR was performed using the SYBR® PrimeScript® RT-PCR kit (Perfect Real Time, TaKaRa), and conventional RT-PCR was carried out using the extracted RNA. Primers were developed for type III IFNs (IFN-λ) and the receptor chIL28Rα molecule based on published sequences. The predicted product sizes are shown in Table 1. A 20 μl reaction volume was prepared according to the manufacturer's instructions, and the cycling conditions used for real-time RT-PCR were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 3 s, 58 °C for 30 s, and 72 °C for 30 s. Real-time RT-PCR was conducted using an ABI 7500, and the data were analyzed using ABI software. The concentration of the RT-PCR product was determined by measuring the OD at 260 nm using a spectrophotometer (Eppendorf). Serial 10-fold dilutions of the product DNA in EASY dilution (TaKaRa) were used for real-time PCR, and a standard curve was generated from the amplification plot.

2.5. Genome analysis

Primers were synthesized based on the predicted sequences (Table 1) to amplify the full-length coding region of the chIFN-λ and duIFN-λ genes by PCR. The amplified PCR products were extracted from agarose gels using a TIANgel mini purification kit (Tiangen). The purified PCR fragments were then cloned into the pMD-18-T vector (TaKaRa, D102A) for sequencing. Sequencing was performed by Beijing Huada Gene Research Center Co., Ltd. Multiple alignments of the amino acid sequences of chIFN-λ and duIFN-λ were compared according to our sequencing results. In addition, we compared the amino acids sequences of IFN-λ with a group of other vertebrates in GenBank using BLASTP, and homologous contigs were retrieved. The GenBank IDs for the IFN-λ amino acid sequences used in this study are as follows: Human: IFN-λ3, XP_005258822.1, Pig: IFN-λ3, XP_005657547.1, Mouse: IFN-λ3, NP_796370.1, Platypus: IFN-λ3, XP_001517931.2, and Frog: IFN-λ3, NP_001165237.1. A phylogenetic tree was generated with MEGA 4 using the neighbor-joining method.

2.6. Calculations and statistics

Statistical analyses were carried out using the SPSS 17.0 software. Differences between values were evaluated using a one-way analysis of variance (ANOVA) followed by pair-wise comparison with the Student–Newman–Keuls test. $p < 0.05$ was considered to be statistically significant, and all values are expressed as the mean ± SEM.

3. Results

3.1. Expression of type III IFNs in DEFs and CEFs

We compared the expression of type III IFNs (IFN-λ3) in response to infection with H1N1 influenza virus in DEFs and CEFs. The results showed that the expression of type III IFNs was upregulated following infection with H1N1 virus or treatment with poly (I:C), and the peak expression of type III IFNs occurred from 8 hpi for both DEFs and CEFs. However, the expression of type III IFNs was significantly higher ($p < 0.05$) in CEFs than in DEFs at each time point following infection with H1N1 virus or treatment with poly (I:C) (Fig. 1A). Viral infection and treatment with poly (I:C) yielded similar results ($p > 0.05$) (Fig. 1C).

3.2. Expression of the receptor for type III IFNs (IFN-λR) in DEFs and CEFs

We compared the expression of the receptor for type III IFNs in response to infection with H1N1 virus in DEFs and CEFs. The results showed that the expression of IFN-λR was upregulated following infection with H1N1 virus or treatment with poly (I:C). The expression of IFN-λR occurred from 8 hpi for CEF and was maintained at similar levels until 36 hpi, but the expression levels of IFN-λR were elevated from 36 hpi in DEFs. The expression of IFN-λR was significantly higher ($p < 0.05$) in CEFs than in DEFs at each time point following infection with H1N1 virus or treatment with poly (I:C) (Fig. 1B), and viral infection and treatment with poly (I:C) yielded similar results ($p > 0.05$) (Fig. 1D).

3.3. Comparison of the type III IFN (IFN-λ3) genes of chicken and duck

The identification of duIFN-III provides the basis for further investigation of the role of type III IFNs in duck antiviral immunity, which is relevant to animal and human health. Few previous data revealed the duIFN-III sequences. We performed a phylogenetic analysis of duIFN-III with chicken type III IFNs sequences based on nucleic acid alignments of our sequencing results (Fig. 2). We also performed a phylogenetic analysis of duIFN-III with a group of other vertebrate sequences based on nucleic acid alignments in GenBank (Fig. 3). Multiple sequence alignment results showed that the type