

Distribution of the type I interferon in different organs of chicken digestive system

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Abstract

Objective: Distribution of the type I interferon in different organs of the chicken digestive system.

Material and methods: In order to obtain a certain length (274 bp) of a fragment, a pair of primers was designed according to the conserved nucleotide sequence of gallus IFNAR-1 (EU477527.1) fragment that was published by the GenBank. The fragment was cloned by pEASY-T1 and amplified by relative fluorescence quantitative PCR with SYBR Green I; according to the results, we made a standard curve. The experimental group took interferon orally, while the control group took equivalent physiological saline orally, then we used relative fluorescence quantitative PCR to detect the copies of the IFNAR-1 gene of the palate, tongue, esophagus, craw, glandular stomach, duodenum and rectum of the experimental group and control group. Copies of the IFNAR-1 gene of those organs were calculated by Ct value. Finally, all the chickens were infected with the Newcastle Disease Virus after 48 hours.

Results: The results showed that the IFNAR-1 gene had the most expression in the esophagus. In the experiment of interferon antiviral activity detection, the chickens which took interferon orally were healthier than the other group.

Conclusions: It is confirmed that the interferon receptor did exist in the digestive organs. However, according to the physical and chemical properties of interferon, interferon is easily inactivated in the acid and alkali environment, by pepsin and trypsin, so the absorption site for interferon exists in organs above the craw, especially in the esophagus and tongue.

Key words: relative fluorescence quantitative PCR, interferon receptor, interferon antiviral activity, oral interferon.

(Centr Eur J Immunol 2014; 39 (4): 455-460)

Introduction

In the 1930s, it was reported that when infected with a virus, the body can produce interference phenomena to another kind of virus. However, when Isaacs used chick chorioallantoic membrane to study a flu interference phenomenon in 1957, he learned that infected cells can produce a kind of factor, which could have an effect on other cells to interfere with the replication of the virus, namely interferon [1, 2]. Interferons, according to their bond principle and receptors, are divided into type I and type II. Type I interferon, also according to its different bond antigenicity of bond and receptors, can be divided into two classes, interferon α and interferon β and the original type II interferon named γ [3]. Molecules of interferon α and interferon β have sequence homology and share the same cell surface receptor – type I interferon receptor (IFNAR), and have similar features [4]. Interferon γ is a homologous dimers glycoprotein [5], the cell surface receptors for type II interferon receptor (IFNGR) [6]. Type I interferon receptor is composed of two strands, α chain (IFNAR-1) and

β chain (IFNAR-2). The biological function of IFNAR-1 gene is related with the combination of interferon and biological signal transduction [7, 8]. However, the biological function of IFNAR-2 is still not clear.

Interferon is a kind of glycoprotein with high biological, it is sensitive to acid and alkali, and it can be degraded by trypsin and pepsin into small peptide fragments, even amino acid residues [9]. At present, interferon is clinically mainly used in the injection way, but now the farming scale is very large, so the injection method is very troublesome. If the oral way can be used, it will make the breeding efficiency improved. Above the craw of the digestive organs, PH is near 7, and there is no trypsin and pepsin, so can the interferon receptor exist in organs above the craw?

In this experiment, we make the laboratory chicken take interferon orally, and use relative fluorescence quantitative PCR to detect the distribution of IFNAR in the digestive tract on a gene level. Then, we analyze the changes of IFNAR in the apparatuses detected after the chickens take interferon orally.

Material and methods

Material

Forty one-day-old specific pathogen-free (SPF) chickens were provided by professor Shuhong Sun. Interferon was purchased from Dalian SanYi Animal Medicine Co., Ltd. SYBR Premix Ex Taq, DNA Marker, EasyTaq DNA Polymerase, dNTPs, Reverse Transcriptase were purchased from Takara (Dalian, China). *Escherichia coli* DH5 α , pEASY-T1 vector and pEGFP-N1 vector were bought from Beijing TransGen Biotech Co., Ltd. RNA Extraction Kit and Plasmid Extraction Kit were purchased from Sangon Biotech (Shanghai) Co., Ltd. Tryptone and Yeast extracts were provided by Oxoid.

Sample collection and tissue preparation

Twenty one-day-old specific pathogen-free (SPF) chickens were randomly divided into two groups and kept in the same conditions. Every group of 10 chickens (group division arrangement: 7-day-old chickens in group I were given interferon orally; group II chickens were given the equivalent physiological saline as the control). After 3 to 5 days, the palate, tongue, esophagus, craw, glandular stomach, duodenum and rectum were sampled. Samples were stored at -20°C for later use.

Total RNA extraction and synthesis of cDNA

Through the use of Trizol (TransGen), total RNA samples were extracted from the palate, tongue, esophagus, craw, glandular stomach, duodenum and rectum. Through using the PrimScript RT Reagent Kit (TaKaRa), the cDNA was obtained.

The preparation of standard curve

β -actin gene was chosen as a reference gene (the primer was supplied by our lab). A pair of primers was designed according to the conserved nucleotide sequence of gallus IFNAR-1 (EU477527.1) fragment that was published at the GenBank. The PCR reaction was performed at 94°C for 10 minutes, followed by 30 cycles of 94°C for 45 seconds, 59°C for 30 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. Two certain lengths (274 bp and 139 bp) of the fragment were obtained. The PCR products were detected by electrophoresing 5 μl aliquots through 0.8% agarose gels in $1 \times \text{TAE}$ (40 mM Tris-acetate [pH 8.0], 1 mM EDTA), and purified by Agarose Gel DNA Extraction Kit (Shanghai Sangon Biotech Co., Ltd). The products were cloned by Peasy-T1 (TransGen) and sent to Shanghai Sangon Biotech Co., Ltd. for sequencing. After the success of the sequencing, we amplified the plasmid DNA, determined the concentration, calculated the number of copies, as standard plasmid of PCR reaction, the plasmid DNA named pEASY-T1-IFNAR-1 and

pEASY-T1- β -actin. We diluted pEASY-T1-IFNAR-1 and pEASY-T1- β -actin according to the following concentrations: 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and then performed relative fluorescence quantitative PCR. The reaction was carried out as follows: initial denaturation at 94°C for 30 s; 40 cycles of denaturation at 94°C for 15 s; annealing at 59°C for 40 s. The fluorescence was collected and detected in the annealing stage of each cycle. The standard curve was drawn based on the obtained results.

Samples detection

According to reaction conditions of the standard curve, the organs that have been collected were detected by relative fluorescence quantitative PCR. According to the formula of the standard curve and the measured Ct value, we could calculate a copy number of IFNAR-1 gene and β -actin gene of those organs, and also calculated arelative expression of volume of IFNAR-1 of digestive organs depending on the copy number.

Oral interferon and interferon antiviral activity detection

Twenty 10-day-old SPF chickens were divided into two groups: the experimental group was given 1 ml of interferon; the control group was given the equivalent saline. After 48 hours, all the chickens were injected with the Newcastle Disease Virus. The morbidity and mortality were observed and recorded.

Results

IFNAR-1 and β -actin gene cloning amplification and identification

The target produced a specific amplification with a size of 274 bp (IFNAR-1 gene) and 139 bp (reference gene) (Fig. 1). The fragments were analyzed by electrophoresis in 0.8% agarose, and purified by the Agarose Gel DNA Extraction Kit. The products were cloned by pEASY-T₁ and identified by primer M13 with PCR. We separately obtained a 474 bp fragment and a 339 bp fragment (Fig. 2, 3).

Establishment of the standard curve of pEASY-T1-IFNAR-1 and pEASY-T1- β -actin

The standard curve was established when CT value was presented as the vertical axis and copy number of logarithmic as the horizontal axis. We found that it presented a good linear relation within $1.96 \times 10^2 \sim 1.96 \times 10^7$. We got S type amplification curves. By analyzing the melting curve, there was a single specific peak at $78.4 \pm 0.8^{\circ}\text{C}$ for pEASY-T1-IFNAR-1 gene and $82.7 \pm 0.8^{\circ}\text{C}$ for pEASY-T1- β -actin gene. There was no primer dimer and nonspecific product (Fig. 4).

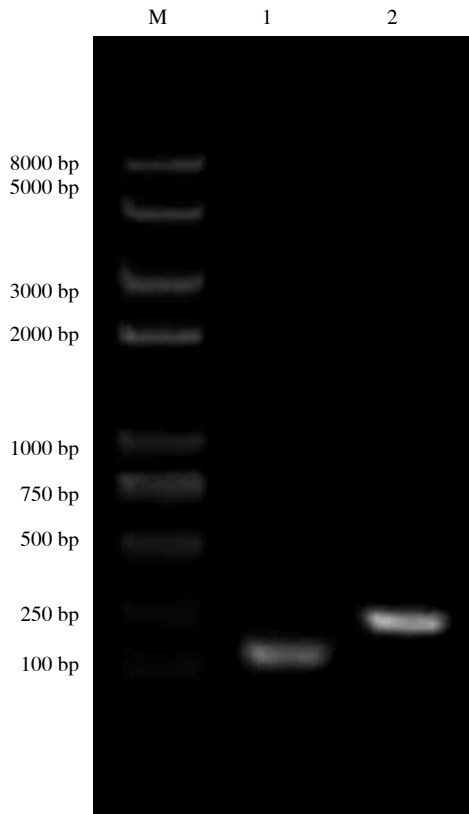


Fig. 1. Amplification of IFNAR-1 and β -actin gene. M: 2000 plus II marker; 1: β -actin; 2: IFNAR-1

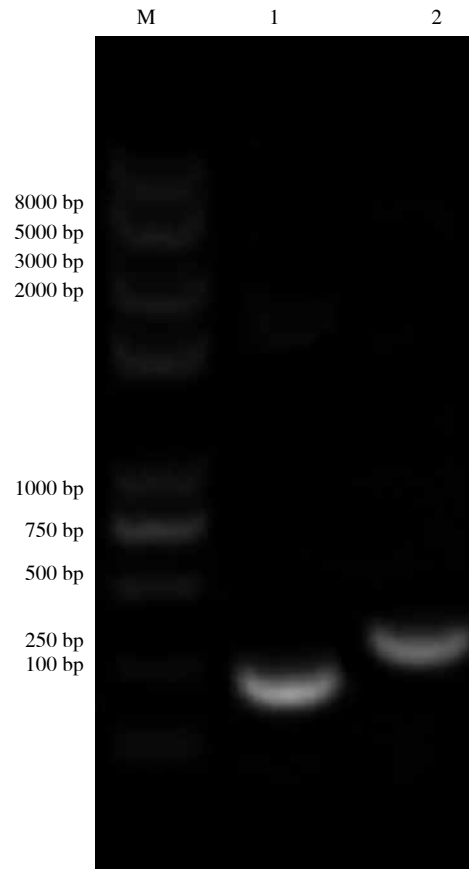


Fig. 2. The identification of pEASY-T1-IFNAR-1 and pEASY-T1- β -actin by primer M13. M: 2000 plus II marker; 1: pEASY-T1- β -actin; 2: pEASY-T1-IFNAR-1. There was 200 bp fragment, from Peasy-T1, which also was amplified by primer M13. It made the target fragment long 200 bp than the original fragment

Detection of the samples

After relative quantitation PCR for each organ, we could get the melting curve which had only one single peak and no other impurity peak (Fig. 5). We got S type amplification curves for IFNAR-1 gene and β actin gene (Fig. 6). According to the formula of the standard curve (IFNAR-1: $y_1 = -4.101x + 41.524$; β -actin: $y_2 = -3.334x + 35.694$) and the measured Ct value, we could calculate a copy number of IFNAR-1 gene and β -actin gene of those

organs, and also calculated a relative expression of volume of IFNAR-1 of digestive organs depending on the copy number (Tables 1, 2).

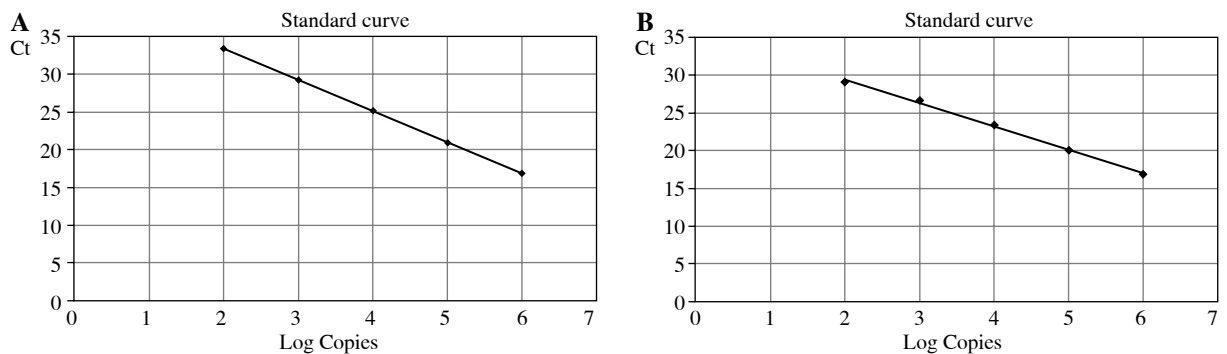


Fig. 3. The standard curve of pEASY-T1-IFNAR-1 and pEASY-T1- β -actin the gene. **A)** standard curve of pEASY-T1-IFNAR-1, formula: $y = -4.101x + 41.524$; the correlation index was 0.9986 ($R^2 = 0.9986$). **B)** the standard curve of pEASY-T1- β -actin, formula: $y = -3.334x + 35.694$; the correlation index was 0.9954 ($R^2 = 0.9954$)

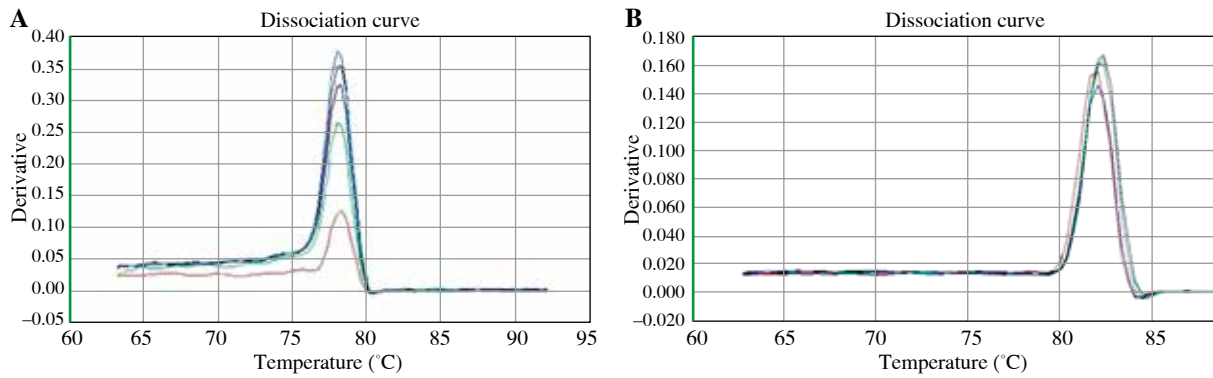


Fig. 4. The melting curve of IFNAR-1 and β -actin gene. **A)** the melting curve of IFNAR-1 gene. **B)** the melting curve of β -actin gene

Table 1. Relative expression quantity of IFNAR-1 of the control group

Organ	β -actin gene		IFNAR-1 gene		Relative expression quantity
	CT value	copy number	CT value	copy number	
palate	15.589 \pm 0.585	6.03 \pm 0.070	30.779 \pm 0.867	2.62 \pm 0.018	0.435 \pm 0.019
tongue	14.889 \pm 0.35	6.24 \pm 0.005	26.514 \pm 0.685	3.66 \pm 0.021	0.587 \pm 0.029
esophagus	13.723 \pm 0.776	6.59 \pm 0.083	25.366 \pm 0.239	3.94 \pm 0.013	0.598 \pm 0.031
craw	16.323 \pm 0.327	5.81 \pm 0.095	29.097 \pm 0.791	3.03 \pm 0.037	0.522 \pm 0.027
glandular stomach	15.023 \pm 0.134	6.20 \pm 0.025	30.779 \pm 0.046	2.62 \pm 0.028	0.423 \pm 0.015
duodenum	17.823 \pm 0.791	5.36 \pm 0.094	35.237 \pm 0.799	1.53 \pm 0.016	0.286 \pm 0.036
rectum	15.289 \pm 0.91	6.12 \pm 0.052	38.311 \pm 0.194	0.78 \pm 0.014	0.128 \pm 0.026

Table 2. Relative expression quantity of IFNAR-1 of the experimental group

Organ	β -actin gene		IFNAR-1 gene		Relative expression quantity
	CT value	copy number	CT value	copy number	
palate	14.823 \pm 0.750	6.26 \pm 0.046	27.047 \pm 0.342	3.53 \pm 0.020	0.564 \pm 0.024
tongue	15.757 \pm 0.521	5.98 \pm 0.032	26.245 \pm 0.468	3.72 \pm 0.024	0.623 \pm 0.036
esophagus	14.389 \pm 0.778	6.39 \pm 0.078	24.280 \pm 0.291	4.20 \pm 0.015	0.658 \pm 0.021
craw	15.656 \pm 0.228	6.01 \pm 0.025	27.376 \pm 0.369	3.44 \pm 0.029	0.574 \pm 0.035
glandular stomach	16.020 \pm 0.603	5.90 \pm 0.041	29.764 \pm 0.351	2.86 \pm 0.036	0.486 \pm 0.014
duodenum	16.123 \pm 0.829	5.87 \pm 0.059	34.013 \pm 0.619	1.83 \pm 0.041	0.312 \pm 0.018
rectum	14.289 \pm 0.104	6.42 \pm 0.061	35.705 \pm 0.986	1.42 \pm 0.052	0.221 \pm 0.017

Based on the data of the experimental group and the control group (Tables 1 and 2), we knew that the number of mRNA of interferon receptor was the most in the esophagus, the second in the tongue, the third in the craw. The number was the least in the duodenum (there was little in the rectum from the data of the control group). We could see the increase of the interferon receptors after the chickens took interferon.

Interferon antiviral activity detection

On day 3 post-infection with Newcastle Disease Virus, 10 chickens of the control group were in a state of illness with symptoms of depression, heads and wings dropping, coughing and dyspnea. On day 5 post-infection, the legs were paralyzed and death appeared. On day 7 post-infection, the rest of the chickens began to recover, finally, six chickens died in the control group.

On day 4 post-infection with NDV, 10 chickens of the experimental group began to be in mental distress, limped, and stood unstably. On day 6 post-infection, death appeared. On day 9 post-infection, they began to recover, and finally, two chickens died in the experimental group.

Discussion

There are no relevant studies and reports about the distribution of interferon receptor in the digestive tract, recently it has been reported that interferon can still keep its effect on antiviral activity and immunoregulation effect after being taken orally [10–12], related studies have shown that interferon has a broad spectrum antiviral activity, interferon has a significant effect on the prevention of many diseases, such as Newcastle [13]. In this experiment, the oral interferon group's mortality rate is lower than in the control group, which can also confirm the conclusion. Besides, Kozlowski [14] has shown that interferon can be used to treat hepatitis C. Giannelli [15] using recombinant interferon alpha treatment in patients with chronic hepatitis, found that the body can produce antibodies.

In this experiment, mRNA levels of the IFNAR gene in chicken digestive organs was detected by using the relative fluorescence quantitative PCR method. It is confirmed that the interferon receptor does exist in the digestive organs. The number of interferon receptors of different organs from the highest to the lowest is as follows: esophagus, tongue, craw, palate, glandular stomach, duodenum. There was little in the rectum from the data of the control group. In the interferon antiviral activity detection experiment, as compared to the control group, the experimental group had lower morbidity and mortality rates. This could confirm that interferon could still exert its biological activities after it was given by oral administration. However, interferon is easily inactivated when it is affected by acid and alkali, degraded by pepsin and trypsin, so the absorption site for interferon exists in organs above the craw, especially in the

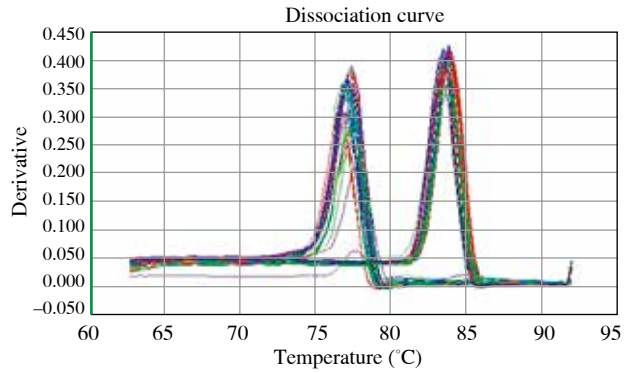


Fig. 5. The melting curve of IFNAR-1 and β -actin gene from the samples

esophagus and tongue. In addition, the expression of volume of the IFNAR-1 gene in the digestive organs has obviously increased after taking interferon orally, it indirectly confirmed that the interferon receptor does exist in the digestive organs. It is known to us that interferon is easily inactivated in organs below the glandular stomach and it is right that the quantity of interferon receptors should not be improved; on the contrary, the expression of volume of the IFNAR-1 gene in those organs is also increased. We think that one of the reasons may be that the function of interferon after being absorbed in the esophagus and tongue is that it could increase not only the expression of the IFNAR-1 gene in the corresponding organs but also the expression of interferon in those organs. The interferon that those organs themselves produce enter blood circulation, and reach some organs such as glandular stomach, and increase the expression of IFNAR-1 after being combined with the receptor on the face of the cell. This may be similar to the mechanism of interleukin [16–18].

The study confirmed that the interferon receptor does exist in the digestive organs by the relative fluorescence

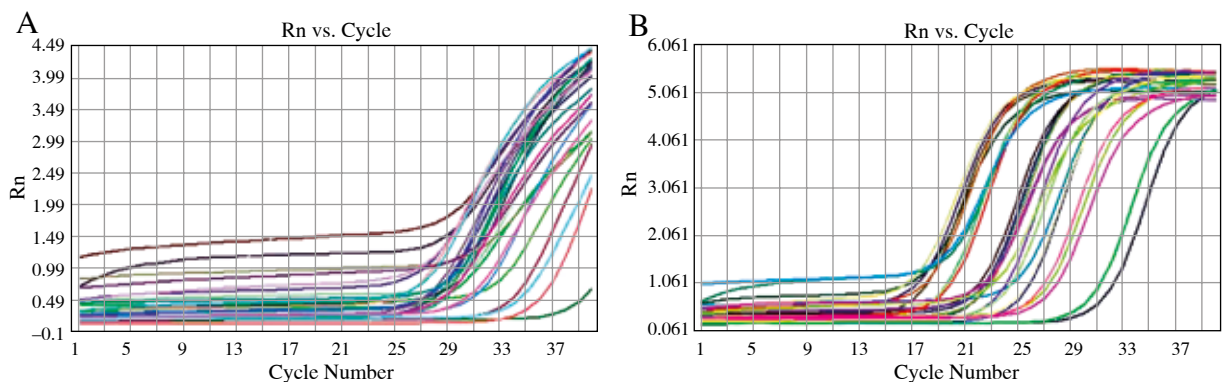


Fig. 6. The amplification curve of the relative quantitation PCR for IFNAR-1 and β -actin
A): The amplification curve of IFNAR-1 gene; **B):** The amplification curve of β -actin gene

quantitative PCR and interferon antiviral activity detection. We can say that the absorption for interferon exists in organs above the craw, especially in the esophagus and tongue, and interferon could still exert its biological activities after interferon was given by oral administration. This provided a laboratory basis for the clinical oral interferon.

The authors declare no conflict of interest.

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