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Akirin2 expression in response to vaccine-induced immunity in chicken

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ABSTRACT. Akirin2 is a nuclear factor that plays an important role in the development and regulation of innate immune response. In this study, *akirin2* gene expression in several primary immune organs (liver, thymus, and bursa) of Hi-Line Brown chicken administered with the LoSota vaccine was analyzed during the various stages of increase in Newcastle disease virus antibody titer. The results revealed that *akirin2* expression was significantly higher in the liver ($P < 0.01$) and bursa ($P < 0.05$) of vaccinated chicken 7 and 14 days post-immunization, respectively. These results could serve as a foundation for further studies on the functions of *akirin2* in immune response.

Key words: Chicken; *Akirin2* expression; Immune response; Liver; Bursa

INTRODUCTION

Akirin2 is a strict nucleoprotein with functions in innate immune response and embryonic development (Goto et al., 2008). Akirin2 facilitates the interaction between Twist and the Brahma-containing chromatin remodeling complex to promote gene expression during *Drosophila* embryogenesis (Nowak et al., 2012). In mice, Akirin2 plays major roles in the Toll-like receptor (TLR), tumor necrosis factor (TNF), and interleukin (IL)-1 signalling pathways, and act in conjunction with nuclear factor kappa B (NF-κB) to induce the transcription of many immune-related genes, including the interleukin-6 (*IL-6*) gene (Goto et al., 2008; Tartey et al., 2014). Moreover, the recombinant Akirin2 protein can be used as a vaccine to partially protect animals against insect infestations (Galindo et al., 2009; Harrington et al., 2009; Moreno-Cid et al., 2010; de la Fuente et al., 2011; Carreón et al., 2012; Manzano-Román et al., 2012). Akirin2 plays an essential role in NF-κB activation; NF-κB is a multi-functional transcription complex that regulates the expression of many immune-response genes such as cytokines, chemokines, and microRNAs (Hayden and Ghosh, 2004, 2008; Bidère et al., 2009; Vallabhapurapu and Karin, 2009). Therefore, the role of *akirin2* in vaccine-induced immune response must be elucidated.

Despite the essential role of *akirin2* in the innate immune system, the possible role in acquired immune response mechanisms after vaccine administration in chicken remains unknown. The poultry industry is currently facing a host of major problems, including an increase in disease incidence, immune failure, and immunosuppression. Therefore, an in-depth analysis of the molecular mechanism of immune response to vaccines could provide valuable references to address these issues. In this study, we attempted to measure the variability in the expression of the *akirin2* gene during different stages of immune response, following the immunization of chicken with the LoSota vaccine.

MATERIAL AND METHODS

Ethics statement

The proposed study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Harbin Normal University (No. SYXKHEI2008006). All experiments in chicken were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council of China.

Animal model, plasmid, and vaccination

Hi-Line Brown chicken (N = 60) were used in this study. The chicken were randomly assigned into two groups (N = 30 each) and strictly isolated from the birds in the other group. Seven-day-old chicken in the experimental group were vaccinated with LaSota (Heilongjiang Biological Production Company, Harbin, China) according to the manufacturer instructions by the eye-drop method. Age-matched chicken in the control group were administered with PBS (20 mM phosphate, 150 mM NaCl, pH 7.4) by the same method.

Sample collection

Blood samples were collected weekly from veins in the wings of chicken in each group

for 7 weeks (on days 0, 7, 14, 21, 28, 35, and 42 post-vaccination). The serum was separated by centrifugation and stored at -20°C. Five chicken from each group were humanely euthanized by an anesthetic overdose of Sumianxin II (0.2 mL/kg body weight; Academy of Military Medical Sciences, Changchun, China) on the days 7 and 14 post-vaccination. Tissue samples were obtained from the liver, thymus gland, and bursa of Fabricius of all chicken, frozen in liquid nitrogen, and stored at -80°C.

RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from tissue samples using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer protocols. RNA samples were digested with DNase I (TaKaRa Bio Inc.) for 1 h at 37°C. RNA concentrations were measured using a NanoDrop Spectrophotometer (Nanodrop Technologies). One microgram total RNA from each sample was reversely transcribed into cDNA using an FSK-100 RT reagent kit (Toyobo, Osaka, Japan) according to the manufacturer instructions.

Akirin2 mRNA expression in the different tissues was, analyzed by semi-quantitative RT-PCR. The β-actin housekeeping gene (GenBank accession No. NM205518) was utilized as the internal standard. The PCR primers used in this study were as follows: β-actin: 5'-CACCAACTGGG ATGATAT-3' and 5'-CGTACTCCTGCTTGCTGATC-3'; *akirin2* (GenBank accession No. HM357352): 5'-CACCAACTGGGATGATAT-3' and 5'-CGTACTCCTGCTTGCTGATC-3'. The PCR cycles were optimized to ensure product intensity within the linear phase of amplification. The 25-μL reaction system contained 1 μL cDNA (20 ng/μL), 10 pM each primer, 2 μL dNTP mix (2.5 mM), 2.5 μL 10X Ex Taq buffer, 1.0 U Ex Taq™ DNA polymerase (TaKaRa Bio Inc.), and sterile water, for a total volume of 25 μL. The PCR conditions were set as follows: initial denaturation at 94°C for 4 min; 25 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The reaction was terminated by exposing the reaction products to 4°C. The PCR products (3 μL) were electrophoresed on a 1% agarose gel.

Hemagglutination inhibition (HI) assay

The antibody titers of LaSota were detected by the HI test. Briefly, 25 μL PBS was added to each well of a V-bottom plate. An equal volume of serum was then added to the first well, and a two-fold serial dilution was ensured across the plate. Four hemagglutination units (HAU) NDV antigen in 25 μL PBS was added to each well, and the plates incubated at 37°C for 40 min; subsequently, 25 μL 1% (v/v) chicken red blood cells (RBC) was added to each well and mixed gently. The RBCs were allowed to settle for about 30 min at room temperature. All samples were tested in duplicate. Positive and negative serum controls were included on each plate. The HI titers were defined as the highest dilution of serum causing complete inhibition of 4 HAU antigen. Mean HI titers and standard deviations (SD) were calculated for each group.

Statistical analysis

All samples were subjected to three repetitions of the test. Mean values of the various parameters of each group were analyzed by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

HI titer in serum post-vaccination

Before vaccination, we observed no differences in the level of NDV antibodies between the 2 groups. However, chicken administered with the LoSota vaccine (experiment group) expressed significantly higher HI titers against NDV in their serum, compared to those administered with PBS (control group), 7 days post-immunization (PI). Additionally, the HI titers of the experiment group increased 14 days PI, and peaked on day 21 PI. However, the HI titers of the control chicken remained unchanged (Figure 1).

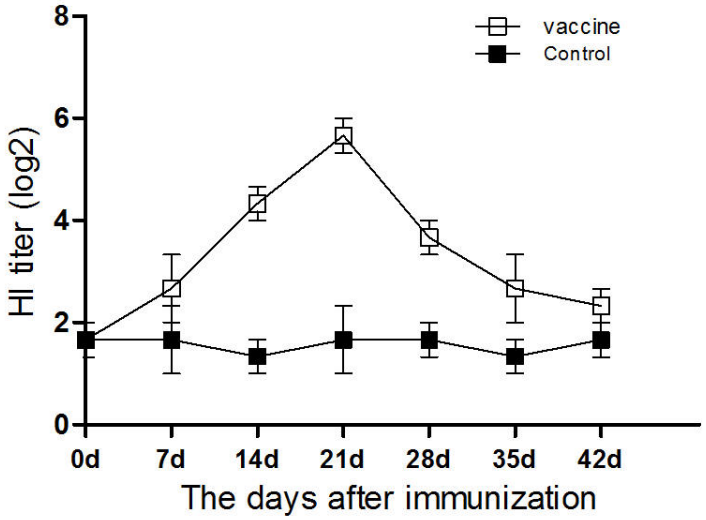

Figure 1. Newcastle disease virus-specific HI titer in serum collected from chicken on days 0, 7, 14, 21, 28, 35, and 42 post-vaccination. Seven-day-old chicken were vaccinated with the LaSota vaccine by the eye-drop method (according to the manufacturer instructions). The values are reported as means ± standard deviation (SD). All samples were subjected to three repetitions of the test.

RT-PCR analysis of the *akirin2* gene

RT-PCR was conducted to compare the expression of different immune tissues (liver, thymus, and bursa) in order to characterize the expression of *akirin2* gene post-vaccination. As the linear phase of the increase in antibody titer could reflect the dynamic molecular changes and gene expression/activities of immune response, the immune tissues obtained from chicken 7 and 14 days PI were analyzed by RT-PCR. The ratio of *akirin2* band intensity to that of β-actin represented the relative expression of *akirin2* gene. *Akirin2* gene expression was observed in all three tissue samples (liver, thymus, and bursa) obtained from young chicken (days 7 and 14 PI); additionally, *akirin2* gene expression profiles of different chicken from the same group were identical. However, the expression level differed significantly between different groups, with the strongest expression observed in the liver (7 days PI, $P < 0.01$) and bursa (14 days PI, $P < 0.05$) of chicken in the experiment group, compared to that in the chicken from the control group (Figure 2).

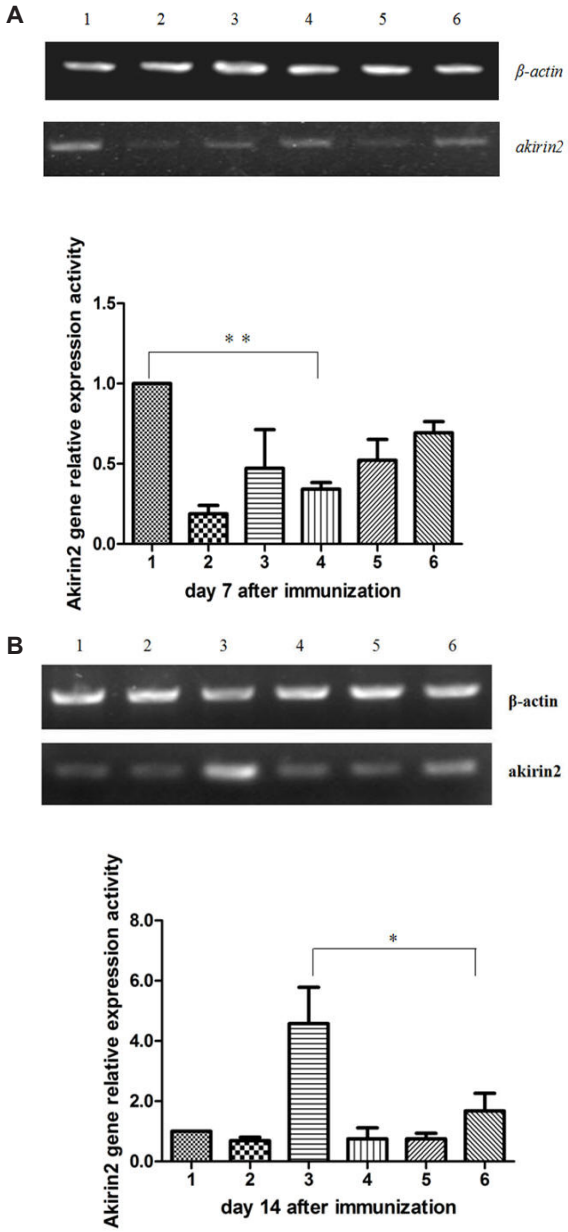

Figure 2. Analysis of *akirin2* gene expression by semi-quantitative RT-PCR. **A.** Levels of expression of *akirin2* 7 days after vaccination. **B.** Levels of expression of the *akirin2* gene 14 days post-vaccination. *Lanes 1*, *2*, and *3* represent the expression levels in the liver, thymus, and bursa of chicken from the experimental group, respectively; *lanes 4*, *5*, and *6* indicate the levels of expression of *akirin2* in the liver, thymus, and bursa of chicken from the control group, respectively (chicken from the experimental and control groups were vaccinated with the LaSota vaccine and PBS, respectively). The β-actin expression was determined as the internal standard. The PCR products of *akirin2* and β-actin were 576 and 860 bp in length, respectively. The quantity of expression of *akirin2* in the liver of chicken from the experimental group was normalized to 1. * or ** indicate differences that are statistically significant at $P < 0.05$ or $P < 0.01$, respectively.

Interestingly, *akirin2* was also observed to induce an immune response to vaccine. The liver is a frontline immunological organ, which contains numerous innate and adaptive immune cells that regulate the immune response (Crispe, 2009; Jenne and Kubes, 2013). In this study, *akirin2* gene was shown to be highly expressed in the liver of experimental chicken during the earlier stages of increase in antibody titer (7 days PI, $P < 0.01$) (Figure 2A). Thus, *akirin2* could play a key role in promoting the induction of an immune response in chicken liver.

The bursa of Fabricius and thymus are the primary lymphoid organs of the chicken immune system; in fact, the bursa is known to produce B cells and antibodies (Kendall, 1980; Miller, 1990; Masteller and Thompson, 1994; Ratcliffe, 2006). In this study, we observed a significantly high level of *akirin2* gene expression in the bursa of experimental chicken (14 days PI, $P < 0.05$) (Figure 2B), which indicated the possible role of *akirin2* in acquired immune response.

In conclusion, *akirin2* may play an important role in the regulation of immune response to vaccine, including innate and acquired immune response. The results of this study could act as a reference for the screening of candidate genes of the genetic adjuvant in chicken immunology.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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