Molecular and functional characterization of chicken interleukin 1 receptor 2 (chIL-1R2)

Anh Duc Truong ,*[,1] Ha Thi Thanh Tran,*[,1] Huyen Thi Nguyen,* Nhu Thi Chu,* Yeong Ho Hong ,† Hyun S. Lillehoj,‡ Hoang Vu Dang ,* and Ki-Duk Song [§,2]

*Department of Biochemistry and Immunology, National Institute of Veterinary Research, Dong Da, Ha Noi, 100000, Vietnam; †Department of Animal Science and Technology, Chung-Ang University, Anseong, 17546, Republic of Korea; ‡Animal Biosciences and Biotechnology Laboratory, Agricultural Research Services, United States Department of Agriculture, Beltsville, MD 20705, USA; and §The Animal Molecular Genetics and Breeding Center & Department of Agricultural Convergence Technology, JeonBuk National University, Jeonju, 54896, Republic of Korea

ABSTRACT Interleukin-1 receptor type 2 (**IL1R2**) is a decoy receptor for exogenous IL-1. However, its functional role in chicken immunity is poorly understood. Herein, chicken IL-1R2 (**chIL-1R2**) was identified and functionally characterized in vivo and in vitro. The *chIL-1R2* coding sequence includes 1,236 nucleotides encoding 412 amino acids, is highly conserved, and has a close relationship with its mammalian counterpart. Its extracellular region has three Ig-like domains but no TIR domain for intracellular signaling. Using ELISA, the recombinant chIL-1R2 protein was demonstrated to specifically bind to the chicken IL-1β. Ch*IL-1R2* mRNA expression was shown to be higher in the spleen, lung, kidney, small intestine, and liver. The expression of *chIL-1R2* and *chIL-1R1* was significantly upregulated in DF-1 cells treated with poly (I:C), but significantly downregulated in the presence of NF-κB, JNK, and MEK inhibitors, indicating that the NF-κB, JNK, and MEK signaling pathways are required for the transcriptional regulation of ch*IL-1R1* and *chIL-1R2* expression. It is worth noting that while the p30 MAPK pathway was required for ch*IL-1R1* expression, it was not required for ch*IL-1R2* expression. Furthermore, ch*IL-1R2* expression increased as early as day 1, and then significantly decreased until day 3, while chIL-1R1 was dramatically upregulated in four organs of chickens infected with the highly pathogenic avian influenza virus (**HPAIV**). These findings indicate that ch*IL-1R1* and ch*IL-1R2* may play a crucial in innate and adaptive immune responses toward HPAIV infection. In summary the present study showed that chIL-1R2 binds to chIL-1β antibody. Ch*IL-1R2* expression can be induced by a viral infection, and may be regulated through NF-κB/JNK/MEK-mediated signaling pathways.

Key words: avian influenza, chicken, interleukin 1, receptor, signaling

2023 Poultry Science 102:102399
https://doi.org/10.1016/j.psj.2022.102399

INTRODUCTION

Interleukin-1β (**IL-1β**), a member of the interleukin-1 family (Awomoyi et al., 2005), plays an important role in inflammatory responses and autoinflammatory diseases (He et al., 2011). In mammals, IL-1 receptors (**IL-1R**) are divided into 2 types that include the type I IL-1 receptor (**IL-1R1**) and the type II receptor (**IL-1R2**). The biological activities of IL-1β are associated with IL-1R1, while IL-1R2 inhibits IL-1β activity (Dale and Nicklin, 1999; Liu et al., 2015). IL-1β is associated with and induces the expression of proinflammatory cytokine genes such as IL-1β, IL-6, and IL-8 by associating and activating the JAK-STAT, NF-κB, PI3K, and JNK signaling pathways (Tsukada et al., 1996; Oh et al., 2016). In contrast, IL-1β is overexpressed and induces IL-1β signaling inhibitors in T-cells, such as the IL-1 receptor antagonist (**IL-1Ra**), rilonacept (**IL-1 Trap**), and IL-1R2, which are endogenous inhibitors of IL-1β activity (Molto and Olive, 2010). In mammals, IL-1Ra can bind to IL-1R with similar affinity to IL-1α and IL-1β, resulting in IL-1 activity inhibition with reduced IL-1α, IL-1β, IL-2, IL-12, and IFNγ expression (Molto and Olive, 2010). In parallel, IL-1R2 competes with IL-1R1 as a potential decoy receptor of IL-1β because IL-1R2 lacks the Toll/interleukin-1 receptor (**TIR**) domain and

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received August 17, 2022.
Accepted December 5, 2022.

[1]ADT and HTTT contributed equally to this work.

[2]Corresponding author: kiduk.song@jbnu.ac.kr

cannot transduce signals after IL-1β binding (McMahan et al., 1991). IL-1R2 is expressed on the cell surfaces of neutrophils, monocytes, and B cells (McMahan et al., 1991) and is a soluble receptor produced by alternative splicing (Vambutas et al., 2009) or proteolysis (Dale and Nicklin, 1999). These findings support the role of IL-1R2 as a naturally occurring inhibitor of IL-1β activity in mammals.

In mammals, IL-1R2 is expressed in macrophages, B cells, neutrophils, and monocytes and is induced in keratinocytes and endothelial cells (McMahan et al., 1991; Lukiw et al., 1999). IL-1R2 expression is inhibited by pro-inflammatory agents, such as LPS (Penton-Rol et al., 1999) and INFγ (Chang et al., 2009), and is induced by anti-inflammatory or immunosuppressive agents, such as dexamethasone (Re et al., 1994), prostaglandins (Spriggs et al., 1992), glucocorticoids, IL-4 (Colotta et al., 1993), IL-13 (Colotta et al., 1994), IL-27 (Kalliolias and Ivashkiv, 2008), and aspirin (Daun et al., 1999). Although chicken IL-1R2 (**chIL-1R2**) expression appears to be related to viral infection, the structure and expression pattern of this gene have not been studied. Furthermore, the mechanism underlying chIL-1R2 signaling has not yet been identified. Herein, we analyzed the amino acid sequence encoded by the chIL-1R2 gene by comparing it with IL-1R2 amino acid sequences from other species. Gene expression was investigated in various chicken tissues, and the expression pattern of the chIL-1R2 gene was evaluated after infection with highly pathogenic avian influenza in chickens. In addition, we examined the signaling of chIL-1R2 using the NF-kB signaling pathway in chicken embryonic fibroblast DF1 cell lines.

MATERIALS AND METHODS

Virus

A/chicken/Vietnam/NA-01/2019 (H5N1), a highly pathogenic avian influenza virus (**HPAIV**) was used in this study. The viral isolate was propagated in 10-day-old embryonated chicken eggs at 37°C for 48 h. The allantoic fluid (**AF**) of the eggs was then harvested, and aliquots of the AF were stored at −80°C until use, according to the OIE guidelines (Chapter 3.3.4) (OIE, 2018). The infectious egg dose of 50% (**EID50**) of the influenza virus was determined as previously described (Huprikar and Rabinowitz, 1980). Briefly, serial 10-fold dilutions of the virus were prepared in phosphate-buffered saline (**PBS**), and 100 μL of each dilution was inoculated into the chorioallantoic cavities of 10-day-old embryonated chicken eggs. Five eggs were infected with each of the virus dilution and the eggs were incubated at 37°C for 96 h. Harvested AF was tested for haemagglutination test (HA) activity using 0.5% red blood cells (**RBCs**) according to the OIE guidelines (Chapter 3.3.4) (OIE, 2018). The EID50/mL of the virus suspension was calculated using the Reed and Muench mathematical method (Reed and Muench, 1938).

Chicken infected with HPAIV

Specific pathogen-free White Leghorn chickens (4 wk old) were purchased from the Poultry Research Centre of the National Institute of Animal Science, Ha Noi, Vietnam. Fifteen chickens (White Leghorn chickens per group) were intranasally inoculated with allantoic fluid (AF) containing 10^4 EID50 of A/chicken/Vietnam/NA-01/2019 (H5N1) in 200 μL. The control group consisted of 15 uninfected White Leghorn chickens. Following viral infection, the chickens were checked for clinical signs of disease and the samples were collected from the lung, spleen, small intestine, kidney, and trachea on d 1, 2, and 3 after infection, following the World Health Organization Manual on Animal Influenza Diagnosis and Surveillance (WHO, 2013).

Cloning and Expression of Recombinant chIL-1R2 Protein

To clone the full-length coding sequence of chIL-1R2, the predicted chIL-1R2 coding sequence (GenBank accession # XM_015277810) was amplified from the total RNA of the spleen using restriction enzyme-anchored primers *Eco*RI-anchored forward primer and *Hin*dIII-anchored reverse primer (restriction sites are underlined in Table 1). Total RNA was isolated from the spleen of White Leghorn chickens using TRizol reagent (Invitrogen, Carlsbad, CA) as described previously (Truong et al., 2015). Reverse transcription with random primers (Invitrogen) was conducted using MMLV Reverse Transcriptase (Promega, Madison, WI), according to the manufacturer's protocol.

Table 1. Similarity (top) and identity (grey) between chicken IL-1R2 and other IL-1R2 molecules.

	1	2	3	4	5	6	7	8	9	10	11	Acc.no
1. Zebrafish		42.69	26.94	27.66	30.24	29.08	30.50	30.30	31.76	31.90	31.90	AJA72724
2. Salmon	32.25		30.33	30.09	32.92	31.73	33.75	33.58	32.75	34.42	34.67	NP_001138892
3. Turkey	15.77	20.63		96.35	44.63	44.90	50.00	48.23	48.63	50.00	50.25	XP_010726144
4. Chicken	16.26	20.63	94.66		44.63	44.90	51.25	49.49	48.88	51.00	51.25	XP_416914
5. Mouse	19.02	21.46	36.58	36.58		92.19	67.25	65.65	66.50	68.34	68.84	CAJ18390
6. Rat	18.50	20.43	36.65	36.65	90.00		68.50	65.90	67.49	69.34	69.84	NP_446405
7. Cattle	17.25	22.75	39.50	39.75	57.75	58.75		94.44	77.00	77.38	78.14	NP_001039675
8. Sheep	17.42	22.47	38.38	38.63	57.32	57.07	93.18		73.98	75.50	76.26	XP_012029111
9. Horse	19.35	22.82	40.69	40.94	58.80	58.80	70.00	66.66		76.38	76.13	NP_001075285
10. Human	20.10	24.12	41.45	41.70	61.30	62.06	70.35	68.43	69.09		99.24	NP_004624
11. Chimpanzee	20.10	24.37	41.70	41.95	61.80	62.56	71.10	69.19	68.84	99.24		XP_009441272

Polymerase chain reaction (**PCR**) was performed to amplify the full-length coding sequences of chIL-1R2 cDNA under the following conditions: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 60 s; and a final extension at 72°C for 5 min. The correct size of the PCR product was inserted directly into the pCR2.1-TOPO vector (Invitrogen), followed by transformation into *Escherichia coli* (*E.coli*) TOP10 (Invitrogen). Transformed *E. coli* TOP10 cells were cultured overnight in Luria-Bertani medium (Difco and BBL, NJ) at 37°C. A transformant was selected using a combination of PCR screening and sequencing (First BASE, The Gemini, Singapore Science Park II, Singapore). The chIL-1R2-expressing plasmid was then digested with the endonucleases *Eco*RI (Invitrogen) and *Hin*dIII (Promega). The digested chIL-1R2 fragment was purified from an agarose gel using a QIAQuick gel extraction kit (Qiagen, Valencia, CA) and ligated into digested pET32a (Novagen, San Diego, CA). To express rchIL-1R2 in *E. coli*, purified chIL-1R2-pET32a plasmid was introduced into *E. coli* BL21 (Invitrogen).

Recombinant protein expression and purification have been described previously (Truong et al., 2017; Truong et al., 2018a; Truong et al., 2018b). Briefly, the expression of chIL-1R2 was induced by adding 1 mM isopropyl ß-D-1-thiogalactopyranoside (IPTG) (USB Corporation, Cleveland, OH), and bacteria were cultured at 37°C overnight at 250 rpm. The bacterial cells were collected and pelleted by centrifugation at 3,500 × *g* for 30 min at 4°C. Total protein from the cells was extracted using B-PER bacterial protein extraction reagent (Thermo Scientific, Waltham, MA) according to the manufacturer's protocol. Recombinant chIL-1R2 was purified using HisPur cobalt resin (Thermo Scientific) in the first step of purification according to the manufacturer's instructions. The purified chIL-1R2 recombinant protein was concentrated, and the buffer was changed by ultrafiltration using a 3,000-molecular-weight-cutoff membrane (Millipore, Burlington, MA). The chIL-1R2 protein was dialyzed in PBS (pH 7.2) overnight using SnakeSkin dialysis tubing (Thermo Scientific) with stirring and was analyzed by SDS-PAGE.

Western Blotting Analysis

Western blotting was performed as described previously (Truong et al., 2017; Truong et al., 2018a; Truong et al., 2018b). Recombinant proteins (100 ng/μL) were subjected to SDS-PAGE, and the separated proteins were transferred to a polyvinylidene difluoride (**PVDF**) membrane (Invitrogen) using a mini Trans-Blot System (Bio-Rad, CA) according to the manufacturer's protocol. The transferred PVDF membrane was blocked with 5% non-fat milk Sigma-Aldrich (St. Louis, MO) dissolved in PBS containing 0.05% Tween 20 (**PBST**). The membrane was incubated with the primary antibody overnight at 4°C, washed thrice with PBST, and further incubated with HRP-conjugated secondary antibodies for 1 h at room temperature (25°C). Immunoreactive bands were detected using Western Lightning Plus-ECL substrate (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions.

Cell Culture

The chicken DF-1 cell line was purchased from American Tissue Culture Collection (CRL-12203, Manassas, VA). DF-1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/mL each of penicillin and streptomycin (Thermo Scientific, Logan, UT) at 37°C in a humidified atmosphere of 5% CO_2.

The Signaling Pathway Potential of the IL-1 Receptors

Polyinosinic-polycytidylic acid (poly (I:C)) was purchased from Invitrogen, dissolved in endotoxin- and nuclease-free water, and stored at −20°C until use. DF-1 cells were treated for 24 h with 5 μg/mL poly (I:C) to evaluate the time kinetics of mRNA expression.

BAY 11−7085 (BAY, inhibitor of IκBα phosphorylation) was purchased from Sigma-Aldrich (St. Louis, MO). SB203580 (p38 MAPK inhibitor) and SP600125 (JNK inhibitor) were purchased from InvivoGen (San Diego, CA). PD98059 (MEK inhibitor) was purchased from MedChem Express (Monmouth Junction, NJ). BAY inhibits IκBα phosphorylation, resulting in the prevention of NF-κB activation and is used as an NF-κB inhibitor (Kogut et al., 2008).

BAY 11-7085 (5 μM) was applied to DF-1 cells for 3 h before being stimulated with poly (I:C) (5 μg/mL) for 6 h. Furthermore, MEK was inhibited in DF-1 cells by treating them with 10 μM PD98059 for 18 h, followed by 6 h of stimulation with 5 μg/mL poly (I:C). p38 MAPK was inhibited in DF-1 cells for 1 h with 10 μM SB203580, followed by 6 h with 5 μg/mL poly (I:C). JNK was inhibited by treating DF-1 cells with 25 μM SP600125 for 1 h, followed by 6 h of stimulation with 5 μg/mL poly (I:C). The concentrations and times of the inhibitors were determined from preliminary experiments in this study. *IL-8L2* was used as a positive control in all experiments with the chicken DF-1 cell line. Our previous study suggested that the expression of chicken *IL-8L2* can be used as a positive control for TLR3 signaling in DF-1 cells (Jang and Song, 2020).

Quantitative Real-Time PCR (qRT-PCR) Analysis

Total RNA was extracted using TRIzol reagent (Invitrogen) as described above. For cDNA synthesis, up to 3 μg of RNA was treated with 1 unit of DNase I and 1 μL of 10 × reaction buffer (Thermo Scientific) and incubated for 30 min at 37°C. Subsequently, 1.0 μL of 50 mM EDTA was added and heated to 65°C for 10 min

Table 2. Primer sequences for cloning and real-time PCR analyses of cytokine transcription.

Genes	F/R	The nucleotide sequence (5′-3′)	Accession no
GAPDH	Forward	TGCTGCCCAGAACATCATCC	NM_204305
	Reverse	ACGGCAGGTCAGGTCAACAA	
IL-1R2	Forward	CGGAATTCATGGTTGAAAGGATGCAGAA	XM_416914
Cloning	Reverse	CCAAGCTTTTCCTTTGCGATTATGTATCC	
IL-8L2	Forward	CCAAGCACACCTCTCTTCCA	NM_205498
	Reverse	GCAAGGTAGGACGCTGGTAA	
IL-1R1	Forward	GAATGCAACGTATCAAGTGGTGTG	NM_205485
	Reverse	TGTTCCCTGTAAGTGCTGTCAAAG	
IL-1R2	Forward	AACTGCCCAGATCACACCAT	XM_416914
	Reverse	TCAAGGCAGAAAAATCCACAT	
IL-1β	Forward	TCGGGTTGGTTGGTGATG	NM_204524
	Reverse	TGGGCATCAAGGGCTACA	

to inactivate DNase I and then reverse-transcribed using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's recommendations. Primers were designed using Lasergene software (Table 2), and qRT-PCR was performed using 2 × Power SYBR Green Master Mix (Roche, Indianapolis, IN) with the LightCycler 96 system (Roche). Chicken *GAPDH* was used as an internal control to normalize the cytokine expression. The level of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method after normalization to *GAPDH* (Livak and Schmittgen, 2001). All qRT-PCR experiments were performed in triplicate.

Enzyme-Linked Immunosorbent Assay (ELISA)

Quantitative ELISA was used to analyze the binding of the chIL-1β (Bio-Rad) with rchIL-1R2. Briefly, increasing concentrations (from 25 nM to 1,000 nM/well) of rchIL-1R2 or Trx-Tag protein (control) were coated onto a 96-well plate (Sigma-Aldrich, Egham, UK) at 4°C overnight. After blocking with 5% bovine serum albumin at room temperature for 3 h, the chIL-1β protein (100 nM/well) was added to each well, and incubated at 4°C overnight. The wells were washed with PBST (0.05% Tween-20 in PBS) for 3 times and then only the chIL-1β antibody was added to each well (1:1000) and incubated at room temperature for 2 h. The wells were washed with PBST thrice and then incubated with HRP-conjugated (1:1000) at room temperature for 1 h. After washing, positive signals were developed with the substrate buffer (1 mg/ml of 3,3′,5,5′-Tetramethylbenzidine, TMB) (Sigma-Aldrich, Egham, UK) at room temperature for 15 to 20 min. The reactions were terminated by adding 2M H_2SO_4, and the results were analyzed using an Elx800 microplate reader (Bio-Rad, Hercules, CA) at 450 nm. All samples were run in triplicates.

Ethics Statement

The study was conducted in compliance with the institutional rules for the care and use of laboratory animals and using a protocol approved by the Ministry of Agriculture and Rural Development of Vietnam (TCVN 8402:2010 and TCVN 8400-26:2014).

Bioinformatics Analysis

Protein identification was performed using the Expert Protein Analysis System (ExPASy; http://www.expasy.org/tools/) and multiple sequence alignments were performed using Lasergene software. Phylogenetic analyses of amino acid sequences of the IL1-R2 groups were performed using the neighbor-joining method with a bootstrap value of 1,000 in the MEGA6 program (Tamura et al., 2013). Signal peptides were predicted using SignalP v.4.1 software (Mitchell et al., 2015), and glycosylation motifs were constructed using NetOGlyc v.4.0 (Steentoft et al., 2013). The immunoglobulin (Ig) domains, a transmembrane domain, and cytoplasmic region were analyzed using InterPro v.56.0 (Mitchell et al., 2015).

Statistical Analyses

Statistical analysis was performed using the IBM SPSS software (SPSS 25.0, IBM Corp., Armonk, NY). The results are expressed as mean values ± standard error (SE) of 3 independent experiments for each group (n = 3) and were compared between groups using Duncan's multiple comparison method.

RESULTS AND DISCUSSION

IL-1R2 was first identified and characterized in mammals (McMahan et al., 1991), bovines (Yu et al., 1997), and fish (Yang et al., 2013), and it may act as a prototypical decoy receptor (Mantovani et al., 2001). Recently, the family of decoy receptors has been identified and characterized as IL-18, TNF, and IL-27 (Mantovani et al., 2001). In mammals, such as humans and mice, the transmembrane regions were similar in IL-1R2 and IL-1R1 but only having a 28% homology in their extracellular domains (Dale and Nicklin, 1999). The structure of IL-1R2 is similar to that of IL-1R1, which includes a signal peptide, 3 Ig-like extracellular domains,

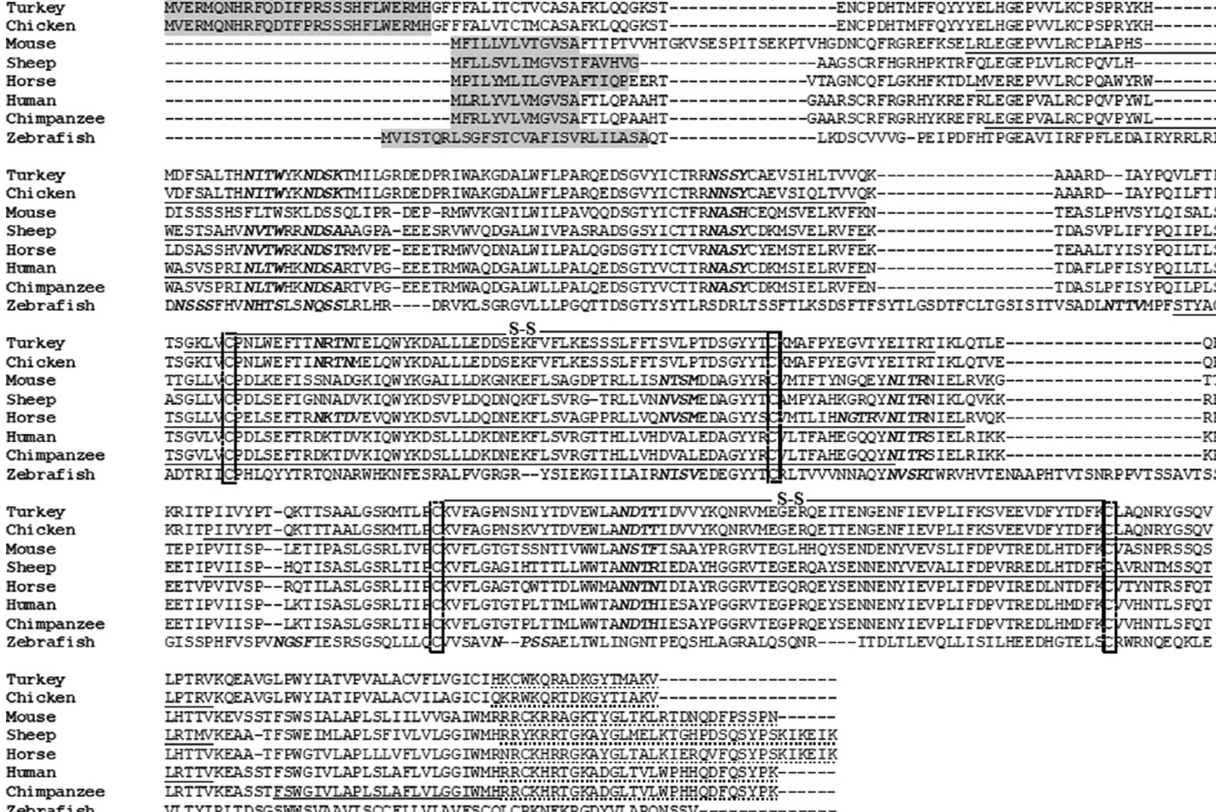

Figure 1. Multiple sequence alignment of the predicted chicken IL-1R2 with selected vertebrate mammalian IL-1R2 sequences was performed using the ClustalX program. The signal peptide, Ig domain, N-linked glycosylation sites, transmembrane, and cytoplasm domain are indicated in dark black, underline, bold, bold-underline, and dashed-underline, respectively. GenBank accession number NP_004624 for human, XP_010726144 for turkey, NP_446405 for mouse, AJA72724 for zebrafish, XP_009441272 for chimpanzee, NP_001075285 for horse, and XP_012029107 for sheep.

and a single helical transmembrane domain and cytoplasmic domain; however, the length of the cytoplasmic domain in IL-1R2 is shorter than that of IL-1R1, which is approximately 200 amino acids, critical to the TIR domain (Slack et al., 2000). Moreover, it has been shown that that most decoy receptors including IL-1R2, can not transduce signals because of the lack of an intracellular TIR domain, a conserved region shared by IL-1R1 and Toll-like receptors (**TLRs**) as part of the IL-1/TLR superfamily (Colotta et al., 1993). In our study, chicken IL-1R2 (chIL-1R2) encoded 412 amino acids (aa), including 27 aa of the signal peptides, 3 aa of the Ig-like extracellular domains, 22 aa of the transmembrane domains, and 17 aa of the cytoplasmic domains (Figure 1). These results suggest that the structure of the chIL-1R2 gene is similar to that of mammals, such as humans and mice.

When this sequence was compared with other known sequences of IL-1R2, IL-1R1, and IL-18R1 in a phylogenetic tree, we observed a higher degree of amino acid similarity to the IL-1R2 sequences than to the IL-1R1 sequences (Figure 2A). The chIL-1R2 protein was most closely related to chicken and turkey IL-1R2 with 94.7% and 96.4%, respectively (Table 1). The predicted amino acid sequence of the chIL-1R2 was approximately 42% identical to that of chimpanzee and human IL-1R2; 39% identical to sheep and cattle; 36.5% identical to rat and mouse IL-1R2; and 16.3% and 20.6% identical to zebrafish and salmon IL-1R2, respectively (Table 1). When conservative amino acid substitutions were included in the analysis, the similarity values increased to 51% with chimpanzee and human IL-1R2, about 49% to 51% with the ruminant IL-1R2, 44% with the rodent (mouse and rat); about 27.6% to 30% identical to fish IL-1R2 (Table 1). The domain organization of chIL-1R2 further confirmed that this receptor corresponds to the mammalian IL-1R2 ortholog, which has three Ig-like domains in the extracellular region but lacks an intracellular signaling TIR domain (Figure 1). In the extracellular membrane region, six cysteine residues were conserved in chicken and all mammals compared at positions C^{75}, C^{135}, C^{177}, C^{232}, C^{284}, and C^{352}. Among the six conserved cysteine residues, the last four backbone residues formed interchain disulfide bonds in the Ig-like (C-x*-C) domain. In addition, four proline residues at positions P^{124}, P^{128}, P^{262}, and P^{267} related to signal transduction in IL-1R2 were conserved among all species examined (Kuno et al., 1993). The phylogenetic tree showed that chIL-1R2 sequences formed a cluster with those of mammalian IL-1R2, whereas IL-1R1 and IL-18R1 proteins formed a separate branch (Figure 2A). The chIL-1R2 cDNA was subcloned into the pET32a (+) vector, expressed in *E. coli* BL21, and purified using HisPur cobalt with a polyhistidine tag. A single protein band with an apparent molecular mass of approximately 70.0 kDa was observed (Figure 2B). The increased size

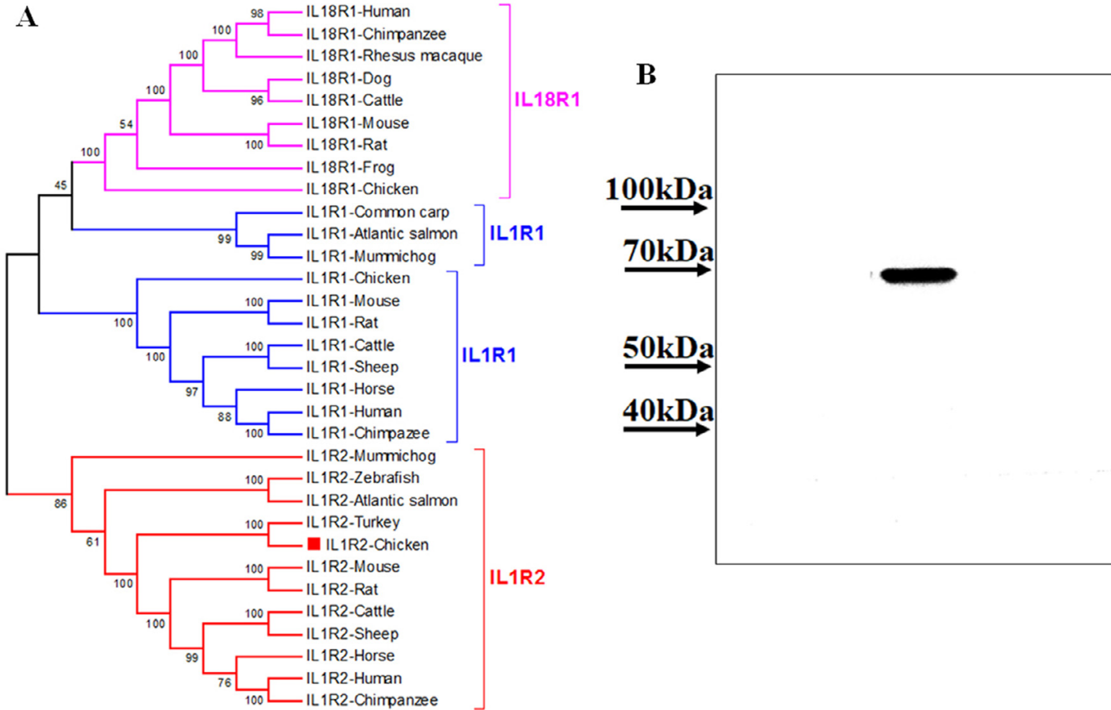

Figure 2. Phylogenetic and western blot analysis. (**A**) Phylogenetic tree illustrating the relationships between interleukin (IL)-1 receptor 2 (IL-1R2) cytokine family members in chickens and other vertebrates. (**B**) Western blot analysis of chIL-1R2 recombinant protein using anti-His (C-Term)-HRP antibody.

over the predicted 47.5 kDa was due to several epitope tags, such as polyhistidine, S-protein, and thioredoxin in the recombinant protein.

Human IL-1R2 could be used as a biomarker and plays a pivotal role in human diseases, such as arthritis, endometriosis, sepsis/sickness behavior, organ transplantation, diabetes, atherosclerosis, Alzheimer's disease, autoimmune inner ear disease (AIED), and ulcerative colitis (Vambutas et al., 2009; Peters et al., 2013; Liu et al., 2015; Chen et al., 2016). In this study, the gene expression pattern of chIL-1R2 was investigated in 17 healthy chicken tissue samples (Figure 3) using qRT-PCR analysis and showed that the chIL-1R2 gene was highly expressed in the chicken kidney, lung, spleen, small intestine, and liver under normal conditions (Figure 3A). Contrastingly, chIL-1R2 was also moderately expressed in chicken pancreas, trachea, ceca, cloaca, duodenum, thymus, and heart tissues. In addition, chIL-1R2 was expressed in other tissues, including the crop, gizzard, and muscle (Figure 3A). In mammals, IL-1R2 is expressed in all healthy adult tissues and cell types, and is especially highly expressed in immune cells and tissues (Peters et al., 2013). In our study, we showed that ch*IL-1R2* was highly expressed in immune tissues, such as the lung, spleen, kidney, and small intestine. At present, information on the physiological role of chIL-1R2 is limited. Interestingly, the expression of ch*IL-1R2* is the highest in the lung, spleen, kidney, and small intestine, and further studies are warranted to understand its role in normal conditions as well as inflammatory conditions caused by viral pathogens.

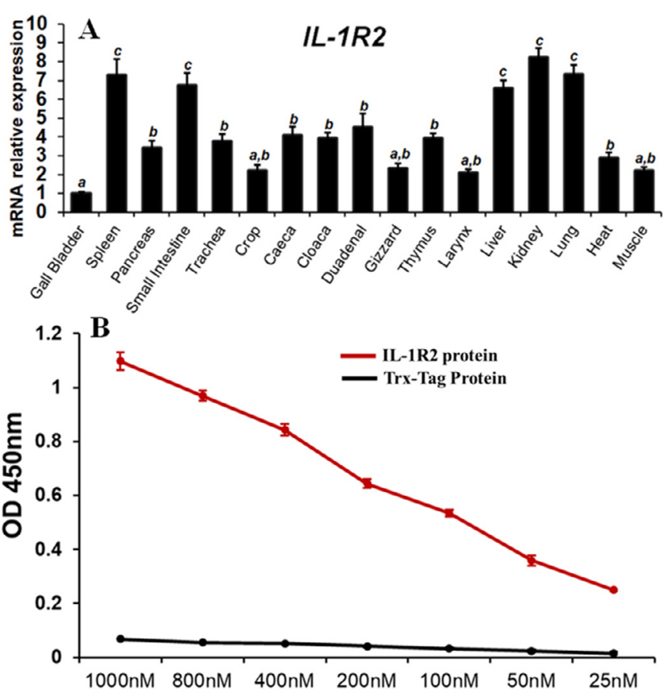

Figure 3. (**A**) The expression of chicken *IL-1R2* mRNA in healthy chicken tissues. Gene expression levels were normalized to *GAPDH*. Data are presented as the mean ± standard error (SEM) of three independent experiments, and the values with different superscript characters (a, b, and c) indicate significant differences between the control and treatment groups determined by one-way ANOVA ($P < 0.05$). (**B**) Reactivity of recombinant chIL-1R2 with IL-1β. Microtiter plates were coated with serial dilutions of recombinant chIL-1R2 (red line) and Trx-Tag protein (black line), then incubated with 20 pmol/well of chIL-1β protein, and sequentially incubated with chIL-1β antibody, and then the HRP-goat anti-chicken IgG and TMB substrate were added.

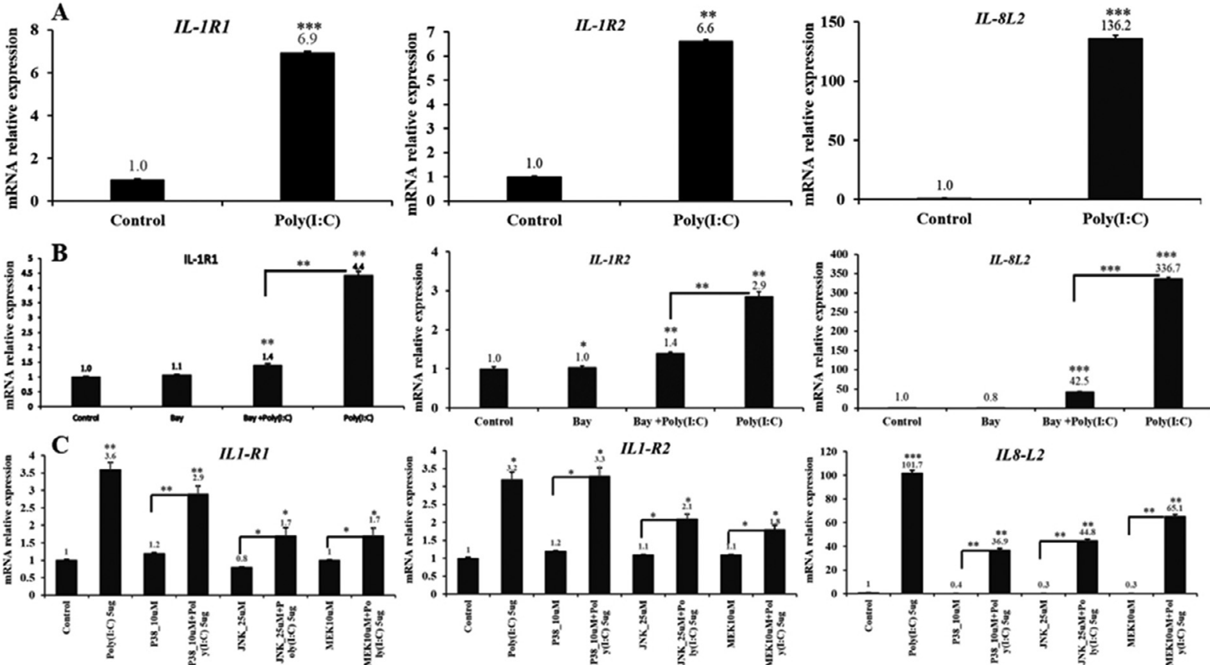

Figure 4. The expression of *chIL-1R1* and *chIL-1R2* mRNA in DF-1 cell line stimulated with Poly (I:C) in the presence of chemical inhibitors/inhibitors. (**A**) Chicken DF-1 cells were treated with 0.5 μg/mL of poly (I:C) for 24 h, and the expression of *chIL-1R2* and *chIL-1R1* were analyzed by qRT-PCR. (**B**) Effects of NFκB inhibitor (BAY11-7085; BAY) on the expressions of *chIL-1R1* and *chIL-1R2* after stimulation with poly (I:C) in chicken DF-1 cells. The cells were treated with or without 5 μM of BAY for 3 h in the presence or absence of 5 μg/mL of poly (I:C) for 3 h. (**C**) Effects of p38, JNK and MEK inhibitors on the expressions of *chIL-1R1* and *chIL-1R2* after stimulation with poly (I:C) in chicken DF-1 cells. The cells were treated with or without p38 (10 μM), JNK (25 μM), MEK (10 μM) in the presence of 5 μg/mL of poly (I:C) for 6 h. The expression levels of *chIL-1R1* and *chIL-1R2* were analyzed by qRT-PCR. Chicken *IL-8L2* was used as a positive control. Each experiment was repeated thrice (n = 3). The expression is shown as the fold induction compared with untreated control ± standard error. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ between the infected and control cells.

Engagement of IL-1β and its receptors, that is,IL-1R1 and IL-1R2, play an important role in mediating inflammation and initiating the immune response against virus infections in humans (Liu et al., 2013), mice (Schmitz et al., 2005; Szretter et al., 2007), dogs (Kang et al., 2013), ducks (Cornelissen et al., 2012), and chickens (Gao et al., 2017). Recent studies have demonstrated that IL-1β, IL-1R1, and IL-1R2 are highly upregulated in infected organs such as the lungs, intestine, trachea, spleen, bursa of Fabricius, gut, brain, thymus, and blood, which is considered a host defense response to influenza virus infection (Schmitz et al., 2005; Szretter et al., 2007; Cornelissen et al., 2012). Moreover, the binding of the IL-1β and IL-1R1 have already been shown to play a critical role in inducing the expression of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, and trypsin in mouse lungs and human alveolar A549 cells in the early phase of infection (Schmitz et al., 2005). Furthermore, it has been demonstrated that the engagement of IL-1β and IL-1R1 play essential roles in viral infectious diseases (Schmitz et al., 2005). Moreover, recent research has shown that IL-1R2 may serve as a local regulatory point involved in IL-1β-mediated immune responses, thereby controlling the inflammatory process in humans, mice, and teleost fish (Peters et al., 2013; Yang et al., 2013; Liu et al., 2015). In this study, the receptor-binding specificity of the recombinant protein of chIL-1R2 binding to IL-1β was evaluated using quantitative ELISA, which showed that the affinity of rchIL-1R2 binding to IL-1β was much higher than that of the control group with IL-1β (Figure 3B). This not only helps to understand the functional role of chIL-R2 but also suggests additional applications for targeting chicken IL-1β signal transduction pathways.

Poly (I:C), a synthetic analog of double-stranded RNA (dsRNA), is one of the classes of pathogen-associated molecular patterns associated with viral infections (Maeda et al., 2019). Poly(I:C) activates the antiviral pattern recognition receptors TLR3, RIG-I/MDA5, and PKR, thereby inducing multiple inflammatory pathways including NF-κB and IRF (Maeda et al., 2019). To understand the expression pattern of this gene, the transcriptional profiles of chIL-1R1 and chIL-1R2 were investigated in chicken DF1 cells stimulated with the TLR3 agonist poly (I:C). To verify whether the expression of ch*IL-1R1* and ch*IL-1R2* genes is regulated by TLR3 signaling, the expression of ch*IL-8L1*, which is known to be induced by TLR3 signaling, was also examined in addition to ch*IL-1R1* and *chIL-1R2* expression. Stimulation with 5 μg/mL poly (I:C) after 24 h significantly upregulated the expression of both ch*IL-1R1* and ch*IL-1R2* (Figure 4A). Research in other species, such as humans, mice, and fish, demonstrated that the regulation of IL-1 receptors, such as IL-1R1 and IL-1R2, is possibly dependent on JNK, p42/44, and NF-κB signaling pathways (Dunne and O'Neill, 2003; Seo et al., 2012; Peters et al., 2013; Yang et al., 2013). In contrast, the regulation of chIL-1R1 or chIL-1R2 signaling pathways

in chickens remains to be determined whether the transcription factor NF-κB is responsible for ch*IL-1R1* and ch*IL-1R2* transcription. DF-1 cells were pretreated with NF-κB inhibitor before poly (I:C) stimulation (Figure 4B). The expression of both ch*IL-1R2* and ch*IL-1R1* was upregulated after treatment with 5 μg/mL poly (I:C) in the absence of inhibitors. However, in the presence of a transcriptional NF-κB inhibitor, the expression of ch*IL-1R2* and ch*IL-1R1* was significantly downregulated compared with that in the poly (I:C)-only treated control, in which ch*IL-1R1* expression was completely inhibited (more than 69%) by BAY as an NF-κB inhibitor only compared to poly (I:C)-only treated control ($P < 0.05$). The results demonstrated that the regulation of ch*IL-1R1* was mediated by the IκBα subunit of NF-κB (Figure 4B). In contrast, the expression of ch*IL-1R2* was significantly decreased up to 48% induced by NF-κB inhibitor compared to poly (I:C)-only treated control (Figure 4B), indicating that the expression of chicken IL-1R1 is regulated by one of the other signaling pathways. In contrast, recent research has shown that the expression of chIL-1β is suppressed by inhibitors of the transcriptional factors, which is BAY for NF-κB (Lee et al., 2019). These results indicated that poly (I:C) can induce the expression of chIL-1β and chicken IL-1 receptors, including *chIL-1R1* and *chIL-1R2*, through the activation of NF-κB transcriptional factors. Therefore, for the typical TLR3 signaling pathway, the expression of ch*IL-1R1* and ch*IL-1R2* can be induced by NF-κB in response to poly (I:C) treatment, and the expression of ch*IL-1R1* and ch*IL-1R2* is regulated by the TLR3 signaling pathway.

To further investigate the mechanism of signaling pathway of the chIL-1 receptors in the DF-1, we examined the expression of chIL-1 receptors in DF-1 cells treated with p38, JNK, and MEK inhibitors alone or with poly (I:C). Our results showed that the expression of ch*IL-1R1* was significantly decreased by poly (I: C) treatment in the presence of p38 (10 μM), JNK (25 μM), and MEK (10 μM) inhibitors (Figure 4C). In contrast, the expression of chicken *IL-1R2* was significantly downregulated in the presence of JNK (25 μM) and MEK (10 μM) inhibitors with poly (I:C) treatment and was not changed by p38 (10 μM) inhibitor stimulation (Figure 4C). Recent research in other species indicated that the mechanism of IL-1 receptors was mediated by JNK/MEK signaling pathways (Yang et al., 2013; Freiberger et al., 2015) while other research showed that IL1-R1, but not IL-1R2 expression, was dependent on p38 MAPK signaling pathways (Dunne and O'Neill, 2003). Our results indicated that the regulation of ch*IL-1R1* and ch*IL-1R2* mRNA is mediated by the JNK and MEK subunit signaling pathways. Moreover, the chIL-1R1 signaling pathway, but not chIL-1R2, is regulated by the p38 MAPK signaling pathway.

Moreover, to gain insight into the regulation of ch*IL-1β*, ch*IL-1R1*, and ch*IL-1R2* expression, we examined their expression in chickens that were infected with highly pathogenic avian influenza virus (HPAI, H5N1; Figure 5). As shown in Figure 5, the expression levels of

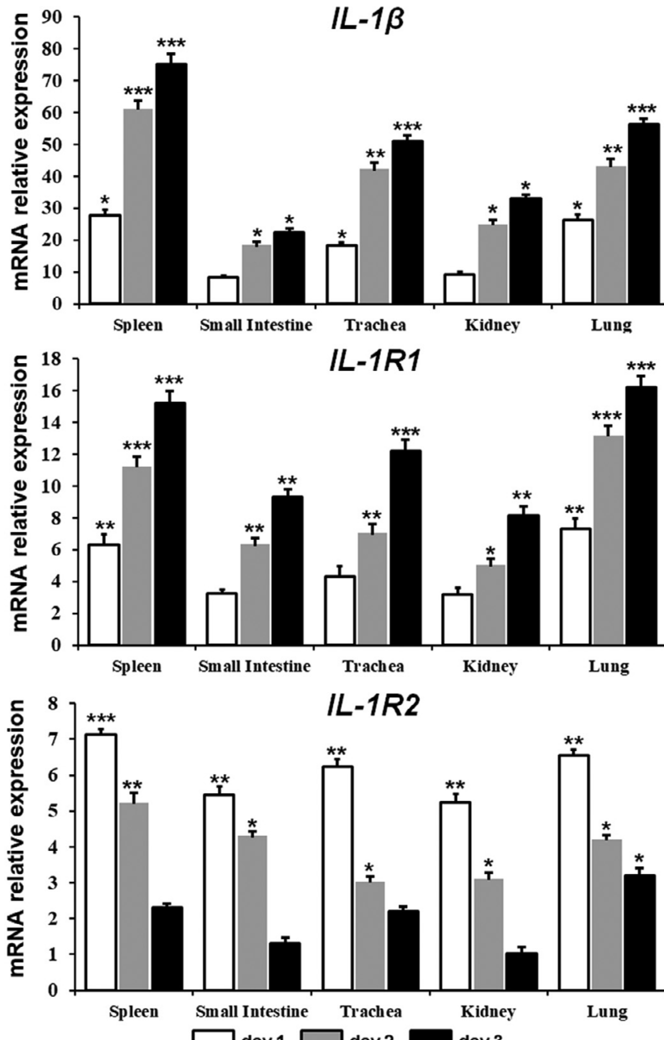

Figure 5. The expression levels of *IL-1β*, *IL-1R1*, and *IL-1R2* mRNA in tissues of high pathogenic avian influenza (HPAI)-infected chickens were analyzed by qRT-PCR. The White Leghorn chickens were infected with 10^4 EID50 of A/chicken/Vietnam/NA-01/2019 (H5N1) and the samples from lung, spleen, small intestine, kidney, and trachea were collected at d 1, d 2, and d 3 postinfection. The expression is shown as the fold induction compared with uninfected birds ± standard error. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ between infected and control chickens.

ch*IL-1β* and ch*IL-1R1* in the four organs of chickens infected with HPAIV significantly increased from day 1 to day 3 post infection (Figure 5). The expression level of ch*IL-1R2* was significantly increased as early as day 1, and considerably decreased further until day 3 in the four organs of HPAI-infected chickens (Figure 5). However, previous studies have shown that the IL-1R2 is mainly involved and plays an essential role in the host response to influenza virus (Szretter et al., 2007; Chen et al., 2016) because it regulates the expression of cytokines and immune-related genes through the JAK-STAT, NF-κB, PI3K, and JNK signaling pathways (Tsukada et al., 1996; Oh et al., 2016). To the best of our knowledge, this study is the first to report that ch*IL-1R2* mRNA is expressed in healthy tissues and tissues infected with HPAI in chickens. However, recent research has demonstrated a novel function of IL-1R2 as a positive or negative regulator of transduction signaling pathways that

activate IL-6/VEGF-A, IFNγ, IL-12, and IL-17 synthesis (Szretter et al., 2007; Peters et al., 2013), which are essential proinflammatory cytokines and angiogenic factors. Overall, these results suggest that ch*IL-1R2* expression is specifically associated with anti-viral host response and may thus be correlated with IL-1β-mediated cellular responses.

CONCLUSION

In summary, our results are the first to characterize the structure and regulation of IL-1R2 expression in chickens. The phylogenetic tree showed that chIL-1R2 sequences formed a cluster with those of mammalian IL-1R2, whereas IL-1R1 and IL-18R1 proteins formed a separate branch. In contrast, structural analysis demonstrated that chIL-1R2 is a conserved region in mammals that includes a signal peptide, three Ig-like extracellular domains, a single helical transmembrane, cytoplasmic domain, and lacks an intracellular signaling TIR domain, raising the question of whether chicken IL-1R2 can bind to IL-1β. Moreover, the receptor-binding specificity of chIL-1R2 binding to the IL-1β antibody provided a molecular basis for understanding the function of chIL-1R2 in buffering IL-1β actions. In addition, the upregulation of ch*IL-1R2* and ch*IL-1R1* mRNA after stimulation with poly (I:C) demonstrated that these genes might be related to viral infection in chickens, and the significantly lower expression levels of ch*IL-1R1* and ch*IL-1R2* in the presence of NF-κB, JNK, and MEK transcriptional inhibitors, which are involved in the NF-κB, TLR3, JNK, and MEK signaling pathways. In contrast, p30 MAPK regulated ch*IL-1R1* but not ch*IL-1R2* expression. Furthermore, the upregulation and downregulation of ch*IL-1R2* after HPAI infection in chickens suggests that this gene might be related to viral infection in chickens and that the chIL-1R2 gene is a promising biomarker candidate for infectious diseases in chickens. Future studies focusing on the local naturally occurring inhibitors involved in IL-1β signaling activity by chIL-1R2 could enhance our understanding of would be helpful to elucidate this issue.

ACKNOWLEDGMENTS

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.02-2019.01 (PI: Anh Duc Truong), the “Cooperative Research Program for Agriculture Science and Technology Development” (Project No. PJ015612), Rural Development Administration, Republic of Korea. The authors would like to acknowledge the staffs of Poultry Research Centre of the National Institute of Animal Science, Vietnam for them contribution during the in vivo study.

Authors’ contributions: The authors made the following contributions: ADT, KDS, HLH and YHH conceived and designed the experiments; ADT, KDS, and YHH provided the reagents, materials, and analysis tools; ADT, HTTT, TNC, THN, and HVD performed the experiments; ADT, HTTT, and HDV analyzed and interpreted the data; and ADT, HTTT, HDV, KDS, HLH, and YHH wrote the manuscript.

DISCLOSURES

The authors declare that they have no conflict of interest.

REFERENCES

Awomoyi, A. A., M. Charurat, A. Marchant, E. N. Miller, J. M. Blackwell, K. P. McAdam, and M. J. Newport. 2005. Polymorphism in IL1B: IL1B-511 association with tuberculosis and decreased lipopolysaccharide-induced IL-1beta in IFN-gamma primed ex-vivo whole blood assay. J. Endotoxin Res. 11:281–286.

Chang, J. H., Y. J. Kim, S. H. Han, and C. Y. Kang. 2009. IFN-gamma-STAT1 signal regulates the differentiation of inducible Treg: potential role for ROS-mediated apoptosis. Eur. J. Immunol. 39:1241–1251.

Chen, Y. C., T. W. Chen, M. C. Su, C. J. Chen, K. D. Chen, C. W. Liou, P. Tang, T. Y. Wang, J. C. Chang, C. C. Wang, H. C. Lin, C. H. Chin, K. T. Huang, M. C. Lin, and C. C. Hsiao. 2016. Whole genome DNA methylation analysis of obstructive sleep apnea: IL1R2, NPR2, AR, SP140 methylation and clinical phenotype. Sleep 39:743–755.

Colotta, F., S. K. Dower, J. E. Sims, and A. Mantovani. 1994. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol. Today 15:562–566.

Colotta, F., F. Re, M. Muzio, R. Bertini, N. Polentarutti, M. Sironi, J. G. Giri, S. K. Dower, J. E. Sims, and A. Mantovani. 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 261:472–475.

Cornelissen, J. B., J. Post, B. Peeters, L. Vervelde, and J. M. Rebel. 2012. Differential innate responses of chickens and ducks to low-pathogenic avian influenza. Avian Pathol. 41:519–529.

Dale, M., and M. J. Nicklin. 1999. Interleukin-1 receptor cluster: gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL18R1 (IL-1Rrp) on human chromosome 2q. Genomics 57:177–179.

Daun, J. M., R. W. Ball, H. R. Burger, and J. G. Cannon. 1999. Aspirin-induced increases in soluble IL-1 receptor type II concentrations in vitro and in vivo. J. Leukoc. Biol. 65:863–866.

Dunne, A., and L. A. O'Neill. 2003. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci. STKE 2003:re3.

Freiberger, S. N., P. F. Cheng, G. Iotzova-Weiss, J. Neu, Q. Liu, P. Dziunycz, J. R. Zibert, R. Dummer, K. Skak, M. P. Levesque, and G. F. Hofbauer. 2015. Ingenol mebutate signals via PKC/MEK/ERK in keratinocytes and induces interleukin decoy receptors IL1R2 and IL13RA2. Mol. Cancer Ther. 14:2132–2142.

Gao, S., Y. Kang, R. Yuan, H. Ma, B. Xiang, Z. Wang, X. Dai, F. Wang, J. Xiao, M. Liao, and T. Ren. 2017. Immune responses of chickens infected with wild bird-origin H5N6 avian influenza virus. Front. Microbiol. 8:1081.

He, B., Y. Zhang, Y. Pan, Y. Xu, L. Gu, L. Chen, and S. Wang. 2011. Interleukin 1 beta (IL1B) promoter polymorphism and cancer risk: evidence from 47 published studies. Mutagenesis 26:637–642.

Huprikar, J., and S. Rabinowitz. 1980. A simplified plaque assay for influenza viruses in Madin-Darby kidney (MDCK) cells. J. Virol. Methods 1:117–120.

Jang, H. J., and K. D. Song. 2020. Expression patterns of innate immunity-related genes in response to polyinosinic:polycytidylic acid (poly[I:C]) stimulation in DF-1 chicken fibroblast cells. J. Anim. Sci. Technol. 62:385–395.

Kalliolias, G. D., and L. B. Ivashkiv. 2008. IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38. J. Immunol. 180:6325–6333.

Kang, Y. M., H. M. Kim, K. B. Ku, E. H. Park, J. Yum, and S. H. Seo. 2013. H3N2 canine influenza virus causes severe

morbidity in dogs with induction of genes related to inflammation and apoptosis. Vet. Res. 44:92.

Kogut, M. H., K. J. Genovese, H. He, and P. Kaiser. 2008. Flagellin and lipopolysaccharide up-regulation of IL-6 and CXCLi2 gene expression in chicken heterophils is mediated by ERK1/2-dependent activation of AP-1 and NF-kappaB signaling pathways. Innate Immun. 14:213–222.

Kuno, K., S. Okamoto, K. Hirose, S. Murakami, and K. Matsushima. 1993. Structure and function of the intracellular portion of the mouse interleukin 1 receptor (type I). Determining the essential region for transducing signals to activate the interleukin 8 gene. J. Biol. Chem. 268:13510–13518.

Lee, S., R. H. Lee, S. J. Kim, H. K. Lee, C. S. Na, and K. D. Song. 2019. Transcriptional regulation of chicken leukocyte cell-derived chemotaxin 2 in response to toll-like receptor 3 stimulation. Asian-Australas. J. Anim. Sci. 32:1942–1949.

Liu, X., L. Min, H. Duan, R. Shi, W. Zhang, S. Hong, and C. Tu. 2015. Short hairpin RNA (shRNA) of type 2 interleukin-1 receptor (IL1R2) inhibits the proliferation of human osteosarcoma U-2 OS cells. Med. Oncol. 32:364.

Liu, Y., S. Li, G. Zhang, G. Nie, Z. Meng, D. Mao, C. Chen, X. Chen, B. Zhou, and G. Zeng. 2013. Genetic variants in IL1A and IL1B contribute to the susceptibility to 2009 pandemic H1N1 influenza A virus. BMC Immunol. 14:37.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif.) 25:402–408.

Lukiw, W. J., J. Martinez, R. P. Pelaez, and N. G. Bazan. 1999. The interleukin-1 type 2 receptor gene displays immediate early gene responsiveness in glucocorticoid-stimulated human epidermal keratinocytes. J. Biol. Chem. 274:8630–8638.

Maeda, A., E. Digifico, F. T. Andon, A. Mantovani, and P. Allavena. 2019. Poly(I:C) stimulation is superior than Imiquimod to induce the antitumoral functional profile of tumor-conditioned macrophages. Eur. J. Immunol. 49:801–811.

Mantovani, A., M. Locati, A. Vecchi, S. Sozzani, and P. Allavena. 2001. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. Trends Immunol. 22:328–336.

McMahan, C. J., J. L. Slack, B. Mosley, D. Cosman, S. D. Lupton, L. L. Brunton, C. E. Grubin, J. M. Wignall, N. A. Jenkins, C. I. Brannan, et al. 1991. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J. 10:2821–2832.

Mitchell, A., H. Y. Chang, L. Daugherty, M. Fraser, S. Hunter, R. Lopez, C. McAnulla, C. McMenamin, G. Nuka, S. Pesseat, A. Sangrador-Vegas, M. Scheremetjew, C. Rato, S. Y. Yong, A. Bateman, M. Punta, T. K. Attwood, C. J. Sigrist, N. Redaschi, C. Rivoire, I. Xenarios, D. Kahn, D. Guyot, P. Bork, I. Letunic, J. Gough, M. Oates, D. Haft, H. Huang, D. A. Natale, C. H. Wu, C. Orengo, I. Sillitoe, H. Mi, P. D. Thomas, and R. D. Finn. 2015. The InterPro protein families database: the classification resource after 15 years. Nucleic. Acids. Res. 43:D213–D221.

Molto, A., and A. Olive. 2010. Anti-IL-1 molecules: new comers and new indications. Joint Bone Spine 77:102–107.

Oh, K., O. Y. Lee, Y. Park, M. W. Seo, and D. S. Lee. 2016. IL-1beta induces IL-6 production and increases invasiveness and estrogen-independent growth in a TG2-dependent manner in human breast cancer cells. BMC Cancer 16:724.

OIE. 2018. Chapter 3.3.4: Avian influenza (infection with avian influenza viruses) (NB: Version adopted in May 2015). Accessed Dec. 2022. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.03.04_AI.pdf.

Penton-Rol, G., S. Orlando, N. Polentarutti, S. Bernasconi, M. Muzio, M. Introna, and A. Mantovani. 1999. Bacterial lipopolysaccharide causes rapid shedding, followed by inhibition of mRNA expression, of the IL-1 type II receptor, with concomitant up-regulation of the type I receptor and induction of incompletely spliced transcripts. J. Immunol. 162:2931–2938.

Peters, V. A., J. J. Joesting, and G. G. Freund. 2013. IL-1 receptor 2 (IL-1R2) and its role in immune regulation. Brain Behav. Immun. 32:1–8.

Re, F., M. Muzio, M. De Rossi, N. Polentarutti, J. G. Giri, A. Mantovani, and F. Colotta. 1994. The type II "receptor" as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. J. Exp. Med. 179:739–743.

Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27:493−497.

Schmitz, N., M. Kurrer, M. F. Bachmann, and M. Kopf. 2005. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. J. Virol. 79:6441–6448.

Seo, H., Y. Choi, J. Shim, Y. Choi, and H. Ka. 2012. Regulatory mechanism for expression of IL1B receptors in the uterine endometrium and effects of IL1B on prostaglandin synthetic enzymes during the implantation period in pigs. Biol. Reprod. 87:31.

Slack, J. L., K. Schooley, T. P. Bonnert, J. L. Mitcham, E. E. Qwarnstrom, J. E. Sims, and S. K. Dower. 2000. Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. J. Biol. Chem. 275:4670–4678.

Spriggs, M. K., P. J. Nevens, K. Grabstein, S. K. Dower, D. Cosman, R. J. Armitage, C. J. McMahan, and J. E. Sims. 1992. Molecular characterization of the interleukin-1 receptor (IL-1R) on monocytes and polymorphonuclear cells. Cytokine 4:90–95.

Steentoft, C., S. Y. Vakhrushev, H. J. Joshi, Y. Kong, M. B. Vester-Christensen, K. T. Schjoldager, K. Lavrsen, S. Dabelsteen, N. B. Pedersen, L. Marcos-Silva, R. Gupta, E. P. Bennett, U. Mandel, S. Brunak, H. H. Wandall, S. B. Levery, and H. Clausen. 2013. Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. EMBO J. 32:1478–1488.

Szretter, K. J., S. Gangappa, X. Lu, C. Smith, W. J. Shieh, S. R. Zaki, S. Sambhara, T. M. Tumpey, and J. M. Katz. 2007. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. J. Virol. 81:2736–2744.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725–2729.

Truong, A. D., C. T. Hoang, Y. Hong, J. Lee, K. Lee, H. S. Lillehoj, and Y. H. Hong. 2017. Functional analyses of the interaction of chicken interleukin 23 subunit p19 with IL-12 subunit p40 to form the IL-23 complex. Mol. Immunol. 92:54–67.

Truong, A. D., Y. Hong, J. Lee, K. Lee, D. Y. Kil, H. S. Lillehoj, and Y. H. Hong. 2018a. Interleukin-34 regulates Th1 and Th17 cytokine production by activating multiple signaling pathways through CSF-1R in chicken cell lines. Int. J. Mol. Sci. 19:1–19.

Truong, A. D., Y. Hong, D. Rengaraj, J. Lee, K. Lee, and Y. H. Hong. 2018b. Identification and functional characterization, including cytokine production modulation, of the novel chicken Interleukin-11. Dev. Comp. Immunol. 87:51–63.

Truong, A. D., Y. H. Hong, and H. S. Lillehoj. 2015. RNA-seq profiles of immune related genes in the spleen of necrotic enteritis-afflicted chicken lines. Asian-Austral. J. Anim. Sci. 28:1496–1511.

Tsukada, J., W. R. Waterman, Y. Koyama, A. C. Webb, and P. E. Auron. 1996. A novel STAT-like factor mediates lipopolysaccharide, interleukin 1 (IL-1), and IL-6 signaling and recognizes a gamma interferon activation site-like element in the IL1B gene. Mol. Cell. Biol. 16:2183–2194.

Vambutas, A., J. DeVoti, E. Goldofsky, M. Gordon, M. Lesser, and V. Bonagura. 2009. Alternate splicing of interleukin-1 receptor type II (IL1R2) in vitro correlates with clinical glucocorticoid responsiveness in patients with AIED. PLoS One 4:e5293.

WHO. 2013. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO (Accessed Mar. 13). http://www.who.int/influenza/human-animal-interface/H5N1-cumulative-table-archives/en/.

Yang, X., S. Wang, L. Du, K. Yang, X. Wang, A. Zhang, and H. Zhou. 2013. Molecular and functional characterization of IL-1 receptor type 2 in grass carp: a potent inhibitor of IL-1beta signaling in head kidney leukocytes. Dev. Comp. Immunol. 41:738–745.

Yu, P. W., H. T. Chen, C. J. Czuprynski, and L. A. Schuler. 1997. Molecular characterization of the bovine type II IL-1 receptor. Cytokine 9:1–8.