Chinese goose (Anser cygnoides) CD8α: Cloning, tissue distribution and immunobiological in splenic mononuclear cells

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CD8 molecule is a cell membrane glycoprotein, which plays an important role in cell-mediated immunity. Here, we identified Chinese goose CD8α (goCD8α) gene for the first time. The full-length cDNA of goCD8α is 1459 bp in length and contains a 711 bp open reading frame. Phylogenetic analysis shows that the waterfowl CD8α formed a monophyletic group. Semi-quantitative RT-PCR analysis showed that transcripts of goCD8α mRNA were high in the immune-related organs and mucosal immune system in gosling, and high in thymus and spleen comparing to other immune-related tissues in goose. The obvious increase of CD8α expression was observed in spleen of acute new type gosling viral enteritis virus (NGEV) infected bird, while the increase of CD8α was observed in the thymus, bursa of fabricius, and cecum of chronic infected bird. The CD8α mRNA transcription level in spleen mononuclear cells was significantly up-regulated when stimulated by phytohemagglutinin, but not by lipopolysaccharide in vitro.

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1. Introduction

Cell-mediated cytotoxicity (CMC) is a crucial part of vertebrate immunity against intracellular pathogens. CD8, which works as a co-receptor with the T cell receptor (TCR), is mostly expressed on cytotoxic T lymphocytes (CTLs). CD8 interacts with the constant region of major histocompatibility complex (MHC) class I proteins that present peptides on the cell surface, stabilizing the interaction, enhancing TCR activation through the CD3 chain tyrosine phosphorylation pathway and function in transduction of regulatory signals in the course of T cell activation (Emmrich et al., 1987). CD8α+ T cells play an important role during virus infection, for example HCV (Sansonno, 2012), HIV-1 (Matthews et al., 2012), dengue virus (Sung et al., 2012) and LCMV (Kitchen et al., 2005). CD8+ T lymphocytes cause the death of infected cells either by direct lysis, or by inducing apoptosis through the activation of Fas receptor (Barr and Bleackley, 2002; Kagi et al., 1994). CD8+ T cell exhaustion results in widespread functional defects, thus leading to impaired immunity in mammal (Doering et al., 2012).

CD8 is expressed either as an αβ-homodimer or αβ-heterodimer (DiSanto et al., 1998; Norment and Litman, 1988; Zamoyska, 1994). Both α and β subunits are composed of a single N-terminal extracellular Ig superfamily (Igs) V-domain, a membrane-proximal hinge region, a single-pass transmembrane domain and a C-terminal cytoplasmic tail (Zamoyska, 1994). CD8αβ heterodimers are mainly expressed on CTLs, whereas selective CD8αα homodimers expression has been described in some Natural Killer cells (NKs) (De Tote et al., 1992), CD4+ T cell subpopulations (Reimann and Rudolphi, 2005), intestinal intraepithelial lymphocytes (IEL) (Jarry et al., 1990), the subsets of dendritic cells (Vremec et al., 2000) and monocytes (Gibbons et al., 2007). Most of these cells are involved in the immune response against bacterial and viral infections, thus CD8 is a useful marker for cellular immune responses detection and for some immunological toolbox development.

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CD8α chain sequences had been reported in duck (Kothlow et al., 2005), chicken (Tregaskes et al., 1995), and turkey (Powell et al., 2009). However, up to now, none of goose CD8α (goCD8α) was identified. Here, for the first time, the cDNA sequence of Anser cygnoides CD8α from the Sichuan White Goose (Chinese domestic goose) was identified; the cDNA and amino acid sequence, the structural and phylogenetic analysis of goCD8α, as well as the tissue distribution of CD8α in both gosling and adult goose were studied.

New type gosling viral enteritis (NGVE) causes an hemorrhagic, fibrinonecrotic, hyperaemic, and exudative enteritis in the small intestine of goslings less than 30 days of age (Cheng et al., 2001). Until now, the pathological characteristic (Cheng et al., 2001) and morphological observations of NGVE (Chen et al., 2008a) have been reported; however, little information is available on the interaction between the virus and the host immune system. In this paper, the tissue distributions of CD8α both in the acute and chronic NGVEV infected goslings were detected through semi-quantitative RT-PCR in vivo.

Duck lymphocytes can be artificially stimulated by phytohaemagglutinin (PHA) (Higgins and Teoh, 1988), which has also been described in mammal. To further characterize immune biological activity of CD8α in goose, the goose spleen mononuclear cells (MNCs) were chosen as the cell model, PHA and lipopolysaccharide (LPS) were chosen as the stimulators, and the CD8α mRNA expression of MNCs after stimulation was investigated by real-time quantitative PCR (qPCR) assay. These findings would shed lights on the role of CD8+ T cell against viral infection in cellular immune system of waterfowl and contribute to provide information for the development of novel immunological assay.

2. Materials and Methods

2.1. Animals and Virus Strain

All goslings (1 day old) and adult geese (the Chinese goose, A. cygnoides), used in this study were purchased from the farm of Sichuan Agricultural University (Ya’an City, Sichuan province, CH). One-day old goslings were maintained for 2 days and adult goose were maintained for 3 days in laboratory animal rooms for acclimatization prior to experimental processing and provided water and food ad libitum. The control goslings and NGVEV infected goslings were maintained in different laboratory animal rooms.

The NGVEV-CN strain (a high virulence field isolate) was provided by the Avian Diseases Research Centre of Sichuan Agricultural University. The median Lethal Dose (LD50) and Tissue Culture Infective Dose (TCID50) of the virus suspension were 10−5.5 ± 1.7/0.5 ml and 10−7.23 ± 0.8/ml respectively. This strain has been previously described (Chen et al., 2006, 2008a,b; Cheng et al., 2001).

2.2. Cloning of goCD8α cDNA

Total RNA was isolated from goose tissues by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. About 2 μg of RNA was subjected to reverse transcription with the use of Oligo(dT)18 as the primer and SuperScript II Reverse Transcriptase (Promega, USA). The partial sequence of goCD8α was amplified by the degenerate primers CD8-F1 and CD8-R1 (primer sequences listed in Table 1), which were based on regions of high homology in the full-length CD8α cDNA sequences of Muscovy duck, mallard, turkey, and chicken [GenBank: AY738735, AF378373, AY519197, and AM884251, respectively]. The fit resultant PCR products were purified using the universal DNA purification kit (Tiangen, Beijing, China), and subcloned into the pGEM-T vector (Promega, USA), which was followed by transformation of JM109 high-efficiency competent cells. Through blue-white screening, the positive clones were sequenced using the ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA). Subsequently, the full-length cDNA of goCD8α including the 3′- and 5′- untranslated regions (UTRs) was obtained by 3′- and 5′- RACE technique. Based on the partial sequence of goCD8α obtained, the Gene Specific Primers (GSPs) including 3′-GSP1, 3′-GSP2, 5′-GSP1, 5′-GSP2, and 5′-GSP3 were designed to pull out the full-length of goCD8α cDNA (all primer sequences were shown in Table 1). For 3′- RACE, the first strand cDNA was synthesized using the Adapter Primer (AP). The 3′-end of goCD8α was amplified by the method of nested PCR using the primers of 3′-GSP1 and 3′-GSP2 with AP1 and AP2, respectively. For 5′- RACE, the first-strand cDNA was synthesized by using the primer of 5′-GSP1 and SuperScript II Reverse Transcriptase (Promega, USA), and then a homopolymeric tail was added to the 5′-end of the cDNA using TdT and dCTP (Beyotime, China). The 5′-end of goCD8α was obtained by two rounds of nested PCR with the primers 5′-GSP2 and Abridged Anchor Primer (AAP), and the primers 5′-GSP3 and Abridged Universal Amplification Primer (AUAP). Then, the full-length cDNA sequence of goCD8α was confirmed by using KOD-Plus-DNA polymerase (Toyobo Co., Ltd., Japan).

2.3. Tissue Transcriptions of goCD8α mRNA

The CD8-F2 and CD8-R2 were designed (showed in Table 1) for detecting the mRNA transcriptions of CD8α in various tissues by a semi-quantitative RT-PCR in healthy goose. Thirteen-day-old gosling was sacrificed and the selected tissues were sampled including thymus, spleen, bursa of fabricius (BF), cervical tonsil (CT), hardesian gland (HG), small intestine, cremast, brain, skin, pancreas, heart, lung, trachea, gizzard, and proventriculus. The RNA extraction and reverse transcription of samples were prepared as described above. To optimize the cycle number, PCR amplification was performed by using the mixture of cDNAs of all selected tissues as the template. Finally, the PCR cycle number was optimized, which was found to be in the mid phase of the PCR amplification. Amplification of β-actin was used as the control.

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2.4. The transcriptions of CD8α in the immune-related tissues of goose and gosling

The CD8-F2 and CD8-R2 were used for detecting the mRNA transcripts of CD8α in the immune-related tissues of healthy goose and gosling by a semi-quantitative RT-PCR method. Goose and 13-day-old gosling were sacrificed and the immune-related tissues were sampled including thymus, spleen, BFT, HT, HG, small intestine, and cecum. Amplification of β-actin was used as the control. Total RNA extraction and reverse transcription of samples, as well as the optimization of PCR cycle number were prepared as described above.

2.5. Relative transcription levels of CD8α in healthy and NGVEV infected goslings

To analyze CD8α mRNA transcription in the immune-related tissues of viral infected goslings, 3-day-old goslings were artificially infected with NGVEV. Three goslings served as the control group and each bird was injected with 0.7 ml PBS (sterile, pH 7.4), and seven goslings were injected intramuscularly with 0.7 ml NGVEV virus suspension containing 10 LD50 each served as the infected group. One virus-infected gosling was injected with 0.7 ml PBS (sterile, pH 7.4), and seven goslings with NGVEV. Three goslings served as the control group and each bird was injected 8 h and 48 h, respectively. Total RNA of cells were extracted by TRIzol Reagent (Invitrogen, Aldrich, USA). Cells were collected at 8 h and 48 h, respectively. Real-time qPCR using the Bio-Rad CFX96 Real-Time Detection System and reverse transcription was performed using SuperScript II Reverse Transcriptase (Invitrogen, USA). The CD8-F2 and CD8-R2 were used for detecting the mRNA transcription in the immune-related tissues of healthy goose and gosling by a semi-quantitative RT-PCR method. Goose and 13-day-old gosling were sacrificed and the immune-related tissues were sampled including thymus, spleen, BFT, HT, HG, small intestine, and cecum. Amplification of β-actin was used as the control. Total RNA extraction and reverse transcription of samples, as well as the optimization of PCR cycle number were prepared as described above.

2.6. Preparation of goose spleen MNCs

Spleens from adult geese were collected aseptically and rinsed with PBS adding 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen/GIBCO), minced with a pair of scissors and then passed through a 100 stainless steel screen to obtain a homogeneous cell suspension. The cells were collected after centrifugation at 600 × g for 10 min at 20 °C and re-suspended in RPMI1640 (GIBCO, USA). Then, cell suspension was added on equal volume of Histopaque-1077 (Sigma, St. Louis, MO, USA). An interface rich in mononuclear cells was recovered after centrifugation at 2000 rpm for 25 min at room temperature. Cells were washed twice in RPMI1640. The population of cell was counted and planted into forty-eight-well cell culture plates in RPMI 1640 medium containing 10% serum at a concentration of 1 × 10⁷ cells/ml. Finally, cells were cultured at 37 °C in 5% CO₂ incubator (Thermo Fisher scientific).

2.7. Real-time qPCR amplification of goCD8α

After recovering at 37 °C under 5% CO₂ and saturated humidity for 24 h, the cells were stimulated with LPS (15 μg/ml) ([L4391, Sigma-Aldrich, USA]) and PHA (15 μg/ml and 25 μg/ml) ([L1668, Sigma-Aldrich, USA]). Cells were collected at 8 h and 48 h, respectively. Total RNA of cells was extracted by TRIzol Reagent (Invitrogen) and reverse transcription was performed using SuperScript II Reverse Transcriptase (Promega, USA). The mRNA levels were determined by real-time qPCR using the Bio-Rad CFX96 Real-Time Detection System (Bio-Rad) with the primers of CD8-F3 and CD8-R3 (listed in Table 1). Goose β-actin was amplified as the control. qPCR treatment was carried out in quadruplicates in a total volume of 20 μl containing 10 μl SensiFast™ SYBR® Green Supermix (Bio-Rad), 1.5 μl of cDNA, 0.6 μl of each primers (10 μM), and 7.3 μl of sterile water. The amplification program was 94 °C for 3 min, followed by 40 cycles of 94 °C for 10 s, 64.1 °C (CD8α) for 30 s (60 °C for β-actin). After the amplification phase, a melting curve program (65 °C to 95 °C with a heating rate of 0.5 °C per second and a continuous fluorescence measurement) was routinely performed to confirm the presence of a single PCR product. To estimate amplification efficiency, a standard curve was generated for each target molecule from 10-fold serial dilutions (from 1 × 10⁻¹ to 10⁻⁶ femtomole) of a plasmid containing the target gene sequences. Data were analyzed with the CFX manager and normalized to β-actin after correcting for variation in amplification efficiency as recommended in CFX manager package (Bio-Rad). PBS-treated cells were used as controls and performed equally.

2.8. Bioinformatic analysis

The potential open reading frames (ORF) were analyzed and translated into the corresponding amino acids using DNAMAN. The similarity was analyzed with the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The signal peptide cleavage site was predicted using the SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP) and the transmembrane region with TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) servers. Multiple alignment of deduced amino acid sequence was performed by using DNAMAN. Phylogenetic tree was constructed by MEGA 4 (Tamura et al., 2007). N-glycosylation sites were analysis with the software online (http://www.cbs.dtu.dk/services/NetNGlyc/). The qPCR data were analyzed on a personal computer using Bio-Rad CFX Manager Software and Excel 2003. Effects were considered to be significant if P < 0.05.

3. Results

3.1. Cloning and sequence analysis of goCD8α cDNA

The full-length cDNA of goCD8α [GenBank: KC476104] is 1459 bp in length and contains a 29 bp 5’-untranslated region (UTR), a 711 bp single ORF, and a 719 bp 3’-UTR (Fig. 1). A eukaryotic polyadenylation signal (AATAAA) was found in the 3’-UTR. Conceptual translation of the ORF showed a protein of 236 amino acids, including a 23 amino acids signal peptide, a 160 amino acid immunoglobulin super family V domain and a stalk/hinge region, a 23 amino acid transmembrane region, and a 30 amino acid positively charged cytoplasm tail. The sequence may not contain N-glycosylation sites that were characterized by analysis.

The deduced amino acid sequence of goCD8α was compared with those of other avian and mammalian species. The deduced amino acid sequence of CD8α was conserved in avian (Fig. 2). The multiple sequence alignment analysis showed that goCD8α shared 89.79%, 89.23%, 75.45% and 74.23% homology with the CD8α of Muscovy duck [GenBank: AY738735], mallard [GenBank: AF378373], turkey [GenBank: AM884251] and chicken [GenBank: AY519197], and shared 83.12%, 79.32%, 60.08% and 59.92% homology with CD8α of Muscovy duck, mallard, turkey, and chicken in amino acid sequence, less than 35% homology to mammal.

To clarify the evolutionary relationship between goCD8α and that of avian and other species, the phylogenetic tree with the amino acid sequence of CD8α was shown in Fig. 3. The results suggested that these amino acid sequences were clustered into three groups including mammalian, avian and fish. In addition, it showed that avian CD8α was subdivided into two monophyletic lineages as fowl and waterfowl. The goose and duck CD8α were located at the same monophyletic group which is distinct from chicken CD8α and turkey CD8α, indicating the similarity of genetic evolution between duck and goCD8α genes. However, goCD8α was the farthest distant from fish CD8α molecules.

3.2. Tissue distribution of CD8α in gosling

The spatial transcripts of goCD8α were assessed by semi-quantitative RT-PCR in gosling. The optimized PCR cycle number was 30 cycles,
and a specific PCR production of 544 bp was consistently amplified. β-Actin was amplified as the control for cDNA quantity and quality.

In gosling, the highest expression of CD8α was observed in thymus and BF, which was followed by brain, skin, spleen, CT, small intestine and lung. In addition, goCD8α transcript was hardly detected in some
tissues including HG, pancreas, heart, trachea, gizzard and proventriculus (Fig. 4).

3.3. The transcription of CD8α in the immune-related tissue of adult goose and gosling

The transcription of CD8α in the immune-related tissue of adult goose and gosling were showed in Fig. 5. Both optimized PCR cycles number were 31. Either gosling or goose, the relatively high abundance of CD8α was detected in thymus and spleen, while the extremely low abundance of CD8α was found in HG. Interestingly, the highest expression of CD8α was observed in BF of gosling, however it was hardly observed in BF of goose (Fig. 5). Compared with gosling, the higher abundances of CD8α were detected in the small intestine and cecum in goose (Fig. 5).

3.4. The transcription of CD8α in the immune-related tissue of NGVEV infected gosling

The CD8α transcripts in the immune-related tissue of NGVEV infected gosling were showed in Figs. 6 and 7. Two (2/7) goslings died at 8 d PI, and the sick goslings were listlessness and had no appetite for 2 or 3 days until death. The optimized PCR cycle number for NGVEV infected gosling at 5 d PI and 19 d PI were 34 and 30, respectively. The optimized PCR cycle number for BF, cecum, small intestine, CT and HG of NGVEV infected gosling were 35 cycles, and for thymus and spleen were 27 and 34, respectively.

After infection, the relatively high abundance of CD8α was detected in the thymus and BF, and the relatively low abundance of CD8α was observed in the HG, cecum and small intestine of the acute (5 d PI) and chronic (19 d PI) NGVEV infected goslings (Fig. 6). While the high abundances of CD8α were also detected in the spleen of NGVEV infected gosling (19 d PI) (Fig. 6).

In order to know the changes of goCD8α expression profile triggered by NGVEV, the ratio was calculated by the expression of CD8α to the expression of house-keeping gene β-actin using ImageJ software (Fig. 7). The increases of CD8α expression were observed in spleen, thymus, BF and cecum both during the acute and chronic NGVEV infection (5 d and 19 d PI). However, the results of small intestine and HG were different from other immune-related organs. The obvious decreased expression of CD8α was observed in the small intestine and HG during the acute NGVEV infection (5 d PI), however, it recovered and raised to the high level during the chronic infection (19 d PI).

3.5. Effects of LPS and PHA on the transcription of CD8α mRNA in goose spleen MNCs

To further characterize the immune biological activity of goCD8α, effects of LPS and PHA on the transcription of CD8α mRNA in goose spleen MNCs were determined by qPCR (Fig. 8). In this case, the mRNA expression of goCD8α was significantly up-regulated by 15 μg/ml and 25 μg/ml PHA after incubation for 8 h and 48 h, respectively (Fig. 8). However, LPS cannot affect the mRNA expression of goCD8α in goose spleen MNCs (the inserted picture of Fig. 8).

4. Discussion

Study of T cell responses to pathogen infection has progressed significantly in the past decades (Harty et al., 2000). The T cell response following virus infection typically consists of two major populations of cells: virus-specific CD8+ T cells and CD4+ T cells. CD4+ T cells respond to antigen in association with MHC class II molecules and CD8+ T cells...
respond to antigen in association with MHC class I molecules peptide complex as the TCR (König and Ronald, 1992; Salter et al., 1990). CD8+ T cells appear to be particularly important in the immune response to acute infections and in the long-term control of latent and reactivating viruses (Hwang et al., 2004). Comparatively little is known about the immune system of waterfowl at the molecular and cellular level other than chicken. Waterfowl as a natural reservoir of many avian viruses, for example the duck is the natural reservoir of influenza viruses (Kida et al., 1980). However, up to date, little is known about the sequence and immune biological activity of T cell surface proteins CD8α in goose.

Here, the full-length cDNA of goCD8α was obtained for the first time. The protein sequence of goCD8α was highly conserved with other avian especially duck (more than 75%) (Figs. 1 & 2), which further indicated that goCD8α was conserved in the evolution of waterfowl (Fig. 3). Moreover, the extracellular domains were the least conserved among avian species. The sequence of goCD8α may not contain N-glycosylation sites, which is consistent with the results of chicken (Tregaskes et al., 1995).

Semi-quantitative RT-PCR analysis revealed that CD8α most abundantly expressed in the immune-related tissues and peripheral immune-related organs of gooseling (Fig. 4). Notably, goCD8α was detected to be predominantly expressed in thymus and spleen, which is similar to the tissue expression pattern of chicken (Tregaskes et al., 1995) and human (Kavathas et al., 1984). It is worthy to note that the obviously difference with our previous study in goose CD4 (Yan et al., 2013) was that the highest transcription of goCD8α was found in BF. Kothlow et al., described that 90% of duck bursa cells were identified with CD8α expression on the cell surface, which is consist with our results (Kothlow et al., 2005). Therefore, it was suggested that BF plays an essential role in the cell-immediate response of waterfowl immune system, especially in young bird. In addition, the relative higher expression was also seen in the peripheral immune-related organs, such as small intestine, CT, cecum, skin, and lung. Small intestine, cecum and lung belong to the mucosal immune system (MIS), which are important protective humoral immune factors to hinder most pathogens enter the host (Brandtzæg, 1989; McGhee et al., 1992). The high expression of goCD8α in immune-related organs is consistent with that of goCD4 (Yan et al., 2013). The skin, namely the mucosa, is an active, and in many ways unique, immunological microenvironment quite different from the other primary interfaces between the body and the environment (Bos and Kapsenberg, 1993). Some studies have extended the finding that lymphocytes can migrate to lung (Bromley et al, 2005). These lymphocytes might execute cell-mediated cytotoxicity (CMC) against infection inducing by the pathogen invaded through the respiratory. Moreover, the expression of CD8α observed in brain also had been reported (Johnson et al., 2012).

In the present study, we profiled CD8α gene distribution involved in immune-related tissues of goose and gooseling (Fig. 5). In BF and CT, the higher and stronger expression was found in goose than gooseling. Given that BF is a unique primary lymphoid organ for generation of the immunoglobulin repertoire in young birds (Cooper et al., 1999). However, in other immune-related tissues, the expression of CD8α was broader in the goose than gooseling. Perhaps, in adult birds, the more perfect cellular immune function was established than in gooseling. This may explain why the goslings are more susceptible to many viruses and bacteria, while the adult birds have the power to resist diseases by recovery or carrying the pathogens all the time.

CTLs are key components of the host immune system that control viral infections by acting through the granule secretory pathway and/or the Fas-mediated pathway (Pardo et al., 2009). Here, the anti-viral defense efforts of goCD8α were further explored after NGVEV infection. Our results showed that T cell activation occurred and underwent extensive proliferation in immune-relative tissues after infection (Fig. 6). The transcripts of goCD8α were obviously upregulated during the chronic infection (19 d PI) compared to the acute infection (5 d PI) (Fig. 6). It was also worth mentioning that the transcription of CD8α in the small intestine during NGVEV infection was noticeable difference from other organs (Fig. 7), the expression of which was obviously decreased during the acute infection (5 d PI), and then it recovered and rose back to the high level during the chronic infection (19 d PI). This change had been identified in the expression of goCD4 in the intestine of NGVEV infected goose (Yan et al., 2013). In the present study, partial necrosis of the small intestine was observed in NGVEV infected goose and the direct viral infection may account for the severe depletion of intestinal CD8+ T cells, interpreting the low CD8α mRNA level of the small intestine during acute NGVEV infection. The slightly increase of CD8α expression in the small intestine at 19 d post-NGVE infection was detected, which suggested that intestinal CD8+ T cells may play an important role in chronic NGVEV infection. However, in BF and CT, the distinct up-regulation of CD8α wasn’t detected, which may be because of the migration of CD8+ T lymphocyte from the primary immune organ into the peripheral immune organs during viral infection.

In vitro, PHA promotes CD8+ T cell survival, proliferation in mammals, chicken, and duck (Bertram et al., 1997). LPS promotes the full activation of mature B cells, leading to proliferation and differentiation to plasmocytes (Andersson et al., 1977). To verify whether this also holds true for the goose immune system, freshly isolated goose spleen MNCs were chosen as cell model, and PHA and LPS were chosen as stimulators. In our study, LPS has no effect on the expression of CD8α in MNCs, which is in accordance with the previous research (Xu et al., 2011). PHA significantly up-regulated the expression of goCD8α mRNA that was detected in vitro (Fig. 8). The data documented that goCD8α could also program a biological activity as mammal in vitro.

Together, these findings may provide new insights into goCD8α molecule and its immunological characteristics, as well as shed lights on the role of CD8+ T cell against viral infection in cellular immune system of waterfowl.

**Conflict of interest**

The authors declare no conflict of interest.
Acknowledgments

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