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Short Communication

Chinese goose (*Anser cygnoides*) CD8a: Cloning, tissue distribution and immunobiological in splenic mononuclear cells

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ABSTRACT

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 (NGVEV) infected bird, while the increase of CD8α were 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.

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). $CD8^+$ 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 (Barry and Bleackley, 2002; Kägi 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 αα-homodimer or αβ-heterodimer (DiSanto et al., 1988; Norment and Littman, 1988; Zamoyska, 1994). Both α and β subunits are composed of a single N-terminal extracellular Ig superfamily (IgSf) 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 Totero 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 (Gibbings 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.

Abbreviations: CMC, cell-mediated cytotoxicity; CTLs, cytotoxic T lymphocytes; TCR, T cell antigen receptor; MHC, major histocompatibility complex; IEL, intestinal intraepithelial lymphocytes; cDNA, complementary to RNA; goCD8α, goose CD8α; NGVEV, new type gosling viral enteritis virus; PHA, phytohemagglutinin; MNCs, monocolor cells; LPS, lipopolysaccharide; qPCR, quantitative PCR; LD_{50}, lethal dose; TCID50, tissue culture infective dose; RT-PCR, reverse transcription-polymerase chain reaction; AAP, abridged anchor primer; AP, Adapter Primer; AUAP, abridged universal amplification primer; GSP, gene specific primer; RACE, rapid amplification of cDNA ends; BF, bursa of fabricius; CT, cecal tonsil; HG, harderian gland; PI, post infection; OFR, open reading frame; UTR, untranslated region; BLAST, Basic Local Alignment Search Tool; CHCD8α, chicken CD8α; TuCD8α, turkey CD8α; CMI, cell-mediated immune; MIS, mucosal immune system; CIS, cutaneous immune system.

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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 cygnoide* 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 NGVEV (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 geese 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 ($TCID_{50}$) of the virus suspension were $10^{-6.5 \pm 1.3}$/0.5 ml and $10^{-7.23 \pm 0.8}$/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 CD8α including the 3′- and 5′-untranslated regions (UTRs) was obtained by 3′- and 5′-RACE technique. Based on the partial sequence of CD8α obtained, the Gene Specific Primers (GSPs) including 3-GSP1, 3-GSP2, 5GSP1, 5-GSP2, and 5GSP3 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).

Table 1
List of primers and sequences.

Reverse transcription	$Oligo(dT)_{18}$	TTTTTTTTTTTTTTTT
CD8α		
For partial sequence	CD8-F1	TGCACTGCTCCT CCT GCTC
	CD8-R1	ACCCAGATGAAAATCTCACAG
3RACE	3-GSP1	TTCCCAGTCACCACGACAGCG
	3-GSP2	GAGACGAGCAAGGAGAAGGAGC
	3-AP	GCTGTCAACGATACGCTACGTAAC GGCATGACAGTG(T)18
	3-AP1	GCTGTCAACGATACGCTACGTAACG
	3-AP2	CGCTACGTAACGGCATGACAGTG
5RACE	5-GSP1	TTCGCAGGTCTCTT
	5-GSP2	CCAGGGCAATGAGAAGCAAGGA
	5-GSP3	TCCTTGCTCGTCTCTGCCTCGT
	5-AUAP	GGCCACGCGTCGACTAGTAC
	5-AAP	GGCCACGCGTCGACTACGGGIIGGG IIGGGIIGGGIIG
Tissue distribution	CD8-F2	TGCACTGCTCCTCCTGCTC
	CD8-R2	ACCCAGATGAAAATCTCACAG
β-actin		
Tissue distribution	β-actin-F1	AGATGACGCAGATCATGTTTG
	β-actin-R1	GAAGGATGGCTGGAAGAGG
Real-time PCR		
	CD8-F3	AGAGACGAGCAAGGAGAA
	CD8-R3	GACCAGGGCAATGAGAAG
	β-actin-F2	CCGTGACATCAAGGAGAA
	β-actin-R2	GAAGGATGGCTGGAAGAG

2.3. Tissue transcriptions of gosling CD8α 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 gosling. Thirteen-day-old gosling was sacrificed and the selected tissues were sampled including thymus, spleen, bursa of fabricius (BF), cecal tonsil (CT), harderian gland (HG), small intestine, cecum, brain, skill, 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-action was used as the control.