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Construction and functional test of a chicken MHC-I (BF2*15)/peptide tetramer

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Abstract

The major histocompatibility complex class I (MHC class I) peptide tetramer is a sensitive and valuable tool to evaluate antigen-specific cytotoxic T lymphocytes (CTLs) of many animal species. To date, no chicken MHC class I peptide tetramer has been reported. In this report, we describe construction and functional evaluation of a chicken MHC-I (BF2*15)/peptide tetramer. To construct the chicken MHC class I peptide tetramer, genes of the chicken MHC-I α chain (BF2*15) and β2 microglobulin (Chβ2m) were synthesized by RT-PCR from the total RNA of PBMCs and the signal sequences were deleted. The BF2*15 was then fused with the BirA substrate peptide (BSP) sequence at the C terminus. Next, the synthesized PCR products of BF2*15 and Chβ2m were cloned into the expression vector pET-28a (+) and expressed in *Escherichia coli* strain BL21 (DE3). Highly purified BF2*15-BSP heavy chain and Chβ2m were obtained by a Ni^{2+} NTA column affinity purification, yielding approximately 1.6 mg of BF2*15-BSP and 2.4 mg of Chβ2m per 1 g of the pelleted bacteria. The purified BF2*15-BSP heavy chain and Chβ2m were refolded with synthetic peptide originated from infectious bronchitis virus nucleoprotein (IBV $N_{71–78}$) in refolding buffer to generate the monomer of BF2*15/peptide complex. The monomer was then biotinylated and tetramerized using PE-labeled streptavidin. Upon functional evaluation of the construct by using flowcytometry, we observed that 3.65% of CTLs were specific to IBV nucleoprotein. This demonstrates that the CTL response of IBV-infected chicks could effectively be evaluated using the prepared MHC-I BF2*15/peptide tetramer.

Keywords: Chicken MHC class I; BF2*15-BSP and β2 microglobulin genes; BF2*15/peptide tetramer

1. Introduction

The classical MHC class I is a membrane surface protein found on virtually all cells in the body, and its main function is to bind antigenic peptides and present them on the surface of virally infected or tumor cells (Kindt et al., 2007). CTLs recognize antigenic peptides, in the context of MHC class I molecules on the cell surface, viatheir TCR and, upon recognition, lyse these target cells. Therefore, it is important to quantitatively measure antigen-specific T-cells accurately and promptly. Until the development of MHC-I tetramer technology, the main techniques for functional and quantitative measurement of antigen-specific T-cells were limiting dilution assay (LDA), enzyme-linked immunospot assay (ELISPOT), and intracellular cytokine staining (ICS) in conjunction with flow cytometry (Taswell, 1981; Jung, 1993; Scheibenbogen et al., 1997, 2000).

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Altman et al. (1996) first described the use of HLA (human leucocyte antigen)-peptide tetrameric complexes to directly visualize antigen-specific CTLs by flow cytometry. This technique is not dependent on proliferation of the cells and therefore allows the direct quantification of antigen-specific CTLs without *in vitro* manipulation (Meidenbauer et al., 2003). To date, this technique has been applied successfully to study cell-mediated immunity on human (HLA), mouse (H-2, mouse MHC), macaque (MAMU, macaque MHC), chimpanzee (PATR, chimpanzee MHC), horse (ELA, equine leucocyte antigen), and pig (SLA, swine leucocyte antigen) (Dunbar et al., 1998; Donahoe et al., 2000; Kalergis et al., 2000; Oleksiewicz et al., 2002; Skinner et al., 2000; Meidenbauer et al., 2003; Mealey et al., 2005).

The chicken MHC class I (BF2 and Rfp-Y) gene sequences have been previously reported (Kaufman et al., 1992; Briles et al., 1993; Miller et al., 1994). Moreover, the genomic structure of Leghorn chicken MHC has also been reported by Kaufman et al. (1999). Within a 44 kb DNA segment, there are two loci-encoding class I heavy chains named BF1 and BF2. The BF2 locus is dominantly expressed, whereas the BF1 locus is less expressed and has a lower mRNA transcript (Kaufman et al., 1999; Miller et al., 2004). However, despite this knowledge of the MHC-I sequence and genomic structure, no chicken MHC-I tetramer has been reported. In this communication, we report the first construction and functional evaluation of a chicken MHC-I/peptide tetramer.

2. Materials and methods

2.1. Peptide

Boots et al. (1991) reported the first defined coronavirus T-cell epitope which is located in the amino acid sequence 71–78 of the infectious bronchitis virus nucleoprotein (IBV $N_{71–78}$). The IBV $N_{71–78}$ (WRRQARYK) derived from IBV H52 strain was synthesized at GL Biotech Co. (Shanghai, China) purified to purity >95% by FPLC.

2.2. Chickens

All BF2*15 white Leghorn SPF (specific pathogen free) chickens used in this study were obtained from the Experimental Animal Center of Harbin Veterinary Research Institute, and housed in isolator cages.

2.3. Virus

The IBV H52 strain was titrated and stocked in our laboratory.

2.4. Cloning the BF2*15 and Chβ2m gene from chicken PBMCs

Total RNA was extracted from PBMCs (peripheral blood mononuclear cells) with TRIZOL (Invitrogen, San Diego, CA) from a SPF chicken. The full-length BF2*15 and Chβ2m cDNA were cloned by RT-PCR. Oligonucleotide primers were designed according to the entire coding region of BF2*15 and Chβ2m cDNA sequences reported in GenBank (accession numbers: **L28958** and **M84767**); the sequences of forward and reverse primers of the BF2*15 and Chβ2m were as follows: BF2*15 forward: 5′-TGC AGC GGT GCG AGG CGA T-3′, BF2*15 reverse: 5′-TTA TTT CAC AGG AAG CAG TGC-3′; Chβ2m forward: 5′-ACA GCG GAG CCA TGG GGA A-3′, Chβ2m reverse: 5′-ATC CCG GGC ACA GCT CAG A-3′. Reverse transcription reaction was conducted at 42 °C for 2 h using AMV Reverse Transcriptase XL (TaKaRa, Dalian, China). PCR amplifications were performed using the LA-Taq DNA polymerase system (TaKaRa, Dalian, China) under the following conditions: starting at 95 °C for 5 min for denaturation, followed by 35 cycles at 94 °C for 1 min, at 60 °C for 1 min, at 72 °C for 1 min, and then at 72 °C for 10 min for extension. The PCR products were inserted into the pMD18-T vector (TaKaRa, Dalian, China) according to the manufacturer's instructions. The positive clones with the correct sequence were named as pMD-BF2*15 and pMD-Chβ2m. The signal peptides of BF2*15 and Chβ2m genes were predicted with SignalP (Bendtsen et al., 2004) and the sequence of the signal peptide in the BF2*15 gene was deleted. In addition, a 15-amino acid residue substrate peptide for BirA-dependent biotinylation was fused to the COOH terminus of BF2*15 through the *Eco*RI and *Hin*dIII site adapters with the forward primer 5′-GGA ATT CAT GGG GCC GTG CGG GG-3′ and the reverse primer 5′-GCG CAA GCT TTT AAC GAT GAT TCC ACA CCA TTT TCT GTG CAT CCA GAA TAT GAT GCA GGA TGG AGG GGT TGC TCC CGG G-3′, using the pMD-BF2*15 plasmid as the template. The signal peptide of the Chβ2m gene was also deleted using *Eco*RI and *Hin*dIII site adapters with the forward primer 5′-GGA ATT CAT GGG GAA GGC GGC GGC-3′ and the reverse primer 5′-GCG CAA GCT TTT AGA ACT CGG GAT CCC A-3′, using the pMD-Cβ2m plasmid as the template. The amplified DNAs were digested with