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Cross-species association of quail invariant chain with chicken and mouse MHC II molecules

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ABSTRACT

There are different degrees of similarity among vertebrate invariant chains (Ii). The aim of this study was to determine the relationship between quail and other vertebrate Ii MHC class II molecules. The two quail *Ii* isoforms (*qIi-1*, *qIi-2*) were cloned by RACE, and qRT-PCR analysis of different organs showed that their expression levels were positively correlated with MHC II gene (*B-LB*) transcription levels. Confocal microscopy indicated that quail full-length Ii co-localized with MHC II of quail, chicken or mouse in 293FT cells co-transfected with both genes. Immunoprecipitation and western blotting further indicated that these aggregates corresponded to polymers of Ii and MHC class II molecules. This cross-species molecular association of quail Ii with chicken and mouse MHC II suggests that Ii molecules have a high structural and functional similarity and may thereby be used as potential immune carriers across species.

1. Introduction

The invariant chain (Ii), known as CD74, plays a critical role in the correct folding and functional stability of MHC II molecules and in the presentation of antigenic peptides (Warmerdam et al., 1996). Ii is distributed as a type II transmembrane protein in various immune and non-immune cells, and is associated with MHC II trimers (αβIi) (Saudrais et al., 1998; Ashman and Miller, 1999; Lindner, 2002; Roche, 2011). Ii is a cell-surface receptor for macrophage migration inhibitory factor (MIF) (Leng et al., 2003) and plays an important role in binding signaling molecules and transferring information during inflammation (Vera et al., 2008).

Ii exists in different isoforms generated by alternative splicing (Bikoff et al., 1998). The wild-type *Ii* isoform, p33 in humans or Ii-1 in chicken, enhances presentation of Ii-dependent MHC II-restricted epitopes (Gregers et al., 2003; Zhong et al., 2004), it has three functional domains: the cytosolic (Rudensky et al., 1994; Vogt et al., 1995; Xu et al., 2008), transmembrane (TM) (Frauwirth and Shastri, 2001; Dixon et al., 2006) and luminal domains (Ashman and Miller, 1999). The cytosolic domain contains an endosome-targeting signal for Ii that targets the endosomal compartment (Rudensky et al., 1994; Vogt et al., 1995). TM mediates Ii trimerization (Ashman and Miller, 1999). Ii trimers as well as MHC II-Ii complexes are diverted to the cellular surface (Benaroch et al., 1995) or enriched at the cell surface to form an Ii pool (Ashman and Miller, 1999). Luminal domain contains a class II associated Ii peptide (CLIP) and a trimerization region (Ashman and Miller, 1999). p41 or Ii-2, another isoform, includes an additional thyroglobulin (Tg), which appears to be mandatory for its inhibitory properties (Mihelic and Turk, 2007), and acts as a regulatory factor in antigen presentation (Lenarcic and Bevec, 1998; Turk et al., 1999). This is an important control mechanism for antigen presentation (Mihelic et al., 2008).

Ii is a chaperone protein of MHC II molecules, and different species have highly conserved structures and sequences (Chen et al., 2011). Similarly, highly conserved MHC structures have been found among different species. de Bellocq and Leirs (2009) reported a cross-species DNA sequence encoding MHC molecules in rodents, and Mulder et al. (2010) defined the amino acid sequence of swine MHC epitopes that cross-reacted with human MHC I monoclonal antibodies. We found conserved amino acid sequences in human and chicken MHC II (Chen et al., 2012) that recognize T cell receptor epitopes (Stadinski et al., 2011; Bremel and Homan, 2010).

However, how these conserved structures function in Ii interaction with MHC II of other species is not clear. Here, we present the cloning of quail *Ii* cDNA isoforms, the comparison of the structural similarity and the transcription levels between quail *Ii* isoforms and MHC II (*B-LB*) in several organs, and report cross-species association of quail Ii with the MHC class II molecules of chicken and mouse.

2. Materials and methods

2.1. Quails

Two-month-old quails were obtained from a breeding farm in Anhui Province, China.

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2.2. RNA extraction

Samples from the blood, liver, lung, muscle, kidney, heart, bursa of Fabricius, thymus, spleen, brain, skin, and stomach muscle of normal quail were collected and immediately frozen in liquid nitrogen. Total RNA was extracted using TRIzol reagent (Takara, Dalian, China), according to manufacturer's instructions. The DNase-treated RNA was subjected to reverse transcription polymerase reaction (RT-PCR) and quantitative real-time PCR (qRT-PCR), using a GeneAmp PCR system (Applied Biosystems, Foster City, CA, USA) and an ABI 7300 Real-Time system (Applied Biosystems), respectively.

2.3. Cloning of a quail Ii domains using degenerate primers

First-strand cDNA was synthesized from 1.0 μg of RNA isolated from peripheral blood cells using an RT-PCR kit (Takara, Dalian, China), according to the manufacturer's instructions. Based on highly conserved *Ii* sequences among poultry (chicken, AY597053 and duck, AY904336), the degenerate primers, qIi-d-f and qIi-d-r (Table 1 and Fig. 1), were used to amplify a segment of quail *Ii* cDNA. PCR was performed with LA Taq (Takara), as follows: 3 min at 94 °C, 30 cycles of denaturation for 40 s at 94 °C, annealing for 40 s at 52 °C, extension for 2 min at 72 °C and a final step of 10 min at 72 °C. The PCR product was subcloned into the vector pMD18-T (Takara) and sequenced by Sengon (Shanghai, China).

2.4. Rapid amplification of cDNA ends (RACE)

To obtain the 3′ and 5′ ends of quail *Ii* cDNA, RACE was performed using the RACE core set Version 2.0 (Takara) primers (Table 1), according to the manufacturer's instructions. PCR products were subcloned into vector PMD18-T and sequenced.

2.5. Amplification of full-length Ii cDNA and gene fragments

The full-length *Ii* cDNA was amplified by PCR with a pair of primers, qIi-F-f and qIi-F-r (Table 1 and Fig. 1), as follows: 3 min at 94 °C, 30 cycles of 40 s at 94 °C, 30 s at 50 °C, 2 min at 72 °C, and a final 10 min step at 72 °C. To determine isoforms (*qIi-1* and *qIi-2*) in different tissues, both fragments and control quail β-actin gene (GenBank: M26111), were amplified with primers qIi-Iso-f and qIi-Iso-r, and β-a-f and β-a-r (Table 1 and Fig. 1), respectively, as follows: 3 min at 94 °C, 30 cycles of 40 s at 94 °C, 30 s at 60 °C, 2 min at 72 °C, and a final step of 10 min at 72 °C.

2.6. qRT-PCR analysis of quail Ii expression levels in different tissues

qRT-PCR was performed on an ABI 7300 Real-Time system (Applied Biosystems). Gene-specific primers (Table 1 and Fig. 1) were designed using Primer Express 3.0 software (Applied Biosystems) to amplify 90–150 bp PCR products. Each reaction contained 25 μL of 2× SYBR Green premix Ex Taq™ II (Takara), 1 μg diluted cDNA sample, and 800 nM gene-specific primers in a final volume of 25 μL. The thermal cycling profile used was as follows: 3 min at 94 °C, 40 cycles of 15 s at 94 °C, and 1 min at 60 °C. Measurements were carried out at the end of the 60 °C extension phase. A melting curve was generated to determine primer specificity by increasing the temperature from 60 to 95 °C. Three technical replicates were performed for each gene. Quail Ii of blood was used as a reference for each reaction. Quail β-actin was used as an internal control. The relative expression level was calculated as $2^{-\Delta\Delta Ct}$ (ΔCT = CT, Target – CT, β-actin. ΔΔCT = ΔCT, treatment −ΔCT, blood). The

Table 1
Primers tested in this study.

Name	Sequence (5′ → 3′)	Amplified sequence
		In Fig. 1B
qIi-d-f(forward)	stgtaccagcagmrsggsca	Quail Ii degenerate sequence
qIi-d-r(reverse)	cagsagccamtkgtgcatcca	
qIi-3′R	tatggactggcaggattttgag	3′RACE outer sequence
qIi-5′R	acttgatcctcagtcttgttgccgc	5′RACE sequence
qIi-F-f	gagcagcagtggggcagccatg	Quail full-length Ii
qIi-F-r	agcagccatatgatcacccct	
qIi-Iso-f	gcctggatgcacaagtggctgc	Two isoforms segments
qIi-Iso-r	tgatcttctctggggtggaca	
qIi-q1-f	tgctgaaagccaagtaaaagagg	qIi-1 segment in qRT-PCR
qIi-q1-r	aagtgaggttagggcaggagtg	
qIi-q2-f	atgagaacggcgactacctg	qIi-2 segment in qRT-PCR
qIi-q2-r	gcatcctcttttacttggct	
		In Fig. 1C
qIi-S-f	ccgctcgagatatggccgaggagcagcggg	For q*Ii*-S1 and S4
qIi-S1-r	cgggatccctggtacacgtagtagatgg	For q*Ii*-S1
qIi-S2-f	ccgctcgagatatgagagtgggcagatcagc	For q*Ii*-S2
qIi-S3-f	ccgctcgagatatgaggactgtgctgtcagcg	For q*Ii*-S3
qIi-d-CL1	caccaacagaggggccaacttcttctgcag	For q*Ii*-S4 (to delete CLIP)
qIi-d-CL2	ctgcagaagaagttggccccctctgttggtg	
qIi-S-r	cgggatccttacttggctttcagcatgtcc	For q*Ii*-S2, S3 and S4
qLB-B-qf	gagatcgaggtgaagtggttc	For q*LB-B* segment in qRT-PCR
qLB-B-qr	agcacctggtacgtccagtc	
β-a-f	atgtggatcacaaagcaggga	For qβ-actin segment in qRT-PCR
β-a-r	acaagggtgtgggtgttgg	
qLB-B-f	cccaattcacatggggagcgggcgcgtcccg	For *Myc*/q*LB-B* in IP
qLB-B-r	ggaagatctactaattcagcatccctgga	
cLB-B-f	cccgaattcacatggggagcgggcgcgt	For *Myc*/c(chicken)*LB-B* in IP
cLB-B-r	ggaagatctactaattcagcatccct	
mH2-Ab-f	cccgaattcacatggctctgcagatcccc	For *Myc*/m(mouse)*H2-Ab* in IP
mH2-Ab-r	ggaagatctatcactgcaggagccct	
G-qLB-B-f	ccgctcgagatggggagcgggcgcgtcccg	For *GFP*/q*LB-B* in IP
G-qLB-B-r	gcgtcgacagctaattcagcatccctgga	