Developmental and Comparative Immunology 60 (2016) 160–166

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Molecular analysis of the immunoglobulin genes in goose☆

Tian Huang [a, b], Kun Wu [b], Xiaoli Yuan [a, b], Shuai Shao [a, b], WenYuan Wang [a, b], Si Wei [a, b], Gengsheng Cao [a, b, *]

[a] School of Life Science, Henan University, Kaifeng 475004, PR China
[b] Institute of Bioengineering, Henan University, Kaifeng 475004, PR China

ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form
22 February 2016
Accepted 22 February 2016
Available online 24 February 2016

Keywords:
Goose
Immunoglobulin gene
Evolution

ABSTRACT

Immunoglobulins play an important role in adaptive immune system as defense molecules against pathogens. However, our knowledge on avian immunoglobulin genes has been limited to a few species. In this study, we analyzed goose (*Anser cygnoides orientalis*) immunoglobulin genes. Three IgH classes including IgM, IgA, IgY and λ light chain were identified. The IgM and IgA heavy chain constant regions are characteristically similar to their counterparts described in other vertebrates. In addition to the classic Ig isotypes, we also detected a transcript that encoded a truncated form of IgY (IgY(ΔFc)) in goose. Similar to duck, the IgY(ΔFc) in goose was generated by using different transcriptional termination signal of the same υ gene. Limited variability and only one leader peptide were observed in VH and VL domains, which suggested that gene conversion was the primary mechanism involved in goose antibody diversity. Our study provides more insights into the immunoglobulin genes in goose that had not been fully explored before.

1. Introduction

Immunoglobulins (Igs) are essential components of the adaptive immune system and are expressed only in jawed vertebrates (Flajnik, 2002). A typical molecular structure of Ig is composed of two identical heavy (H) chains and two light (L) chains, with the H chain and L chain being encoded by variable (V) and constant (C) regions. The classification of Ig is based on their heavy chain constant genes (Bengten et al., 2000). Most mammals produce five isotypes of Igs, IgM, IgD, IgG, IgA, and IgE. Both IgM and IgD are the most primitive IgH isotypes, as they have been identified in most jawed vertebrates (Ohta and Flajnik, 2006). However, the IgD is an immunoglobulin with a high degree of structural diversity, and the number of CH-encoding genes varies in different species (Sun et al., 2011). IgG and IgE are exclusively expressed in mammals. IgG efficiently opsonizes pathogens for engulfment by phagocytes, while the binding of IgE to allergens triggers the release of histamine in mast cells and basophils. IgA, which is predominantly induced by commensal intestinal microbes in mucosal areas, can act as a neutralizing antibody to pathogens and exotoxins (Macpherson et al., 2008). Previous studies showed that the IgA-encoding gene was found to be lost in a number of reptiles (Gambon-Deza et al., 2012; Wei et al., 2009; Xu et al., 2009). However, the identification of the α gene in crocodilians suggested that the IgA in reptile and the IgX in amphibians evolved from the same ancestor (Cheng et al., 2013). Additionally, distinct IgH genes encoding many other Ig classes, such as IgNAR, IgZ (IgT), IgY, IgF and IgO, have been identified in various species (Danilova et al., 2005; Dooley and Flajnik, 2006; Warr et al., 1995; Zhang et al., 2010; Zhao et al., 2006, 2009).

Birds are one of the most highly evolved vertebrates, and they have an immune system disparate from mammals. The bursa of Fabricius is the critical organ which is involved in avian B-cell development. The isotypes and genomic organization of Ig in birds also exhibit some unusual characteristics. It has been known that in birds there are only three different isotypes: IgM, IgA, IgY, encoded by Cμ, Cα and Cυ gene, respectively (Choi et al., 2010; Dahan et al., 1983; Lundqvist et al., 2006; Magor et al., 1998; Mansikka, 1992). The transcriptional orientation of Cα gene in the IgH locus is opposite to the Cμ and Cυ genes (Lundqvist et al., 2001; Magor et al., 1999; Zhao et al., 2000). IgY is a functional homolog of mammalian IgG and structurally closely related to IgE, thus it is considered to be

☆ The sequences reported in this study have been deposited in the NCBI GenBank under the following accession numbers: KT253939–KT253945.

* Corresponding author. School of Life Science, Institute of Bioengineering, Henan University, Kaifeng 475004, PR China.
E-mail address: gscao@henu.edu.cn (G. Cao).

http://dx.doi.org/10.1016/j.dci.2016.02.024
0145-305X/

the progenitor of IgG and IgE (Warr et al., 1995). In addition to the full length of IgY, duck also produces a truncated isoform of IgY(IgY(ΔFc))which lacks the last two CH domains of the heavy chain (Magor et al., 1994b). The expression level of such truncated antibody increases when repeatedly immunized by antigen (Grey, 1967; Humphrey et al., 2004). Another difference on Ig isotypes between birds and other jawed vertebrates is that only λ light chain-locus has been found in some birds (Das et al., 2010; Magor et al., 1994a; Reynaud et al., 1983).

In the avian IgH locus, multiple pseudo genes (φVH) are located upstream of limited functional VH gene segments (McCormack et al., 1991). Only one functional Vλ and Jλ gene have been identified in the chicken, duck and zebra finch genomes (Das et al., 2010; Magor et al., 1994a; McCormack et al., 1989). Somatic diversification of V region mainly depends on the intra-chromosomal gene conversion, a DNA recombination event that uses φVH as sequence donors to transfer part of sequences of the rearranged functional VDJ segment (Backstrom et al., 2013; McCormack et al., 1991).

Birds are the most species-rich class in tetrapod vertebrates. However, the structure, expression and genetics of Igs are not clear for most avian species. In the present study, we cloned IgM, IgA, IgY, λ light chain genes in goose (*Anser cygnoides orientalis*) and analyzed their phylogenetic relationships with Igs in other vertebrates.

2. Materials and methods

2.1. Animals, RNA and DNA extraction

Geese were purchased from a local farm. Total RNA from different tissues was prepared using TRIzol kit (Tiangen Biotech, Beijing, China). Genomic DNA was isolated from liver according to the routine protocol. Reverse transcription was conducted using M-MLV reverse transcriptase, following the manufacturer's instructions (Invitrogen, USA). All animal studies and procedures were approved by the Animal Care and Use Committee of the Henan University.

2.2. Amplification of IgA, IgM, IgY constant regions

Degenerate primers were designed on the basis of previously published variable region and constant region of α, μ, υ and λ genes from different species including chicken, duck and ostrich. VHs and IgAas for IgA; IgMs and IgMas; IgYs and IgYas for IgY; CLs and CLas for λ. RT-PCR was carried out with these primers using spleen cDNA. S and AS refer to sense and antisense primers, respectively. The resultant PCR products were cloned into the pMD19-T vector (Takara) and sequenced. Primers used in the present study are listed in Table S1(Supplementary data).

2.3. 5′RACE, 3′RACE and construction of spleen and intestine cDNA libraries

We used the 5′ RACE System for Rapid Amplification of cDNA Ends (Invitrogen). Primers derived from Cμ and Cλ were used for 5′RACE amplification (MGSP1, MGSP2, LGSP1, LGSP2). The 650bp PCR products were cloned into the pMD19-T vector (Takara) and sequenced. 3′RACE was performed using JH and JL specific primers (JHs and JLs) with spleen and intestine cDNA as templates. The 1.5 kb products were inserted into pMD19-T vectors (Takara) to generate Ig cDNA mini-libraries. Universal primers including M13F and M13R were used to screen positive clones that containing correct insertions; IgA1 and IgA2 for IgA positive clones; IgY1 and IgY2 for IgY positive clones; IgM1 and IgM2 for IgM positive clones. Cμ and Cυ gene specific 3′RACE were performed using sense primers MCH3s and YCH2s (Table S1). F and R, and 1 and 2 refer to sense (forward) and antisense (reverse) primers, respectively.

2.4. Northern blotting detection of the goose IgH gene expression in different tissues

Total RNA was extracted from various tissues, including the heart, liver, spleen, lung, kidney, large intestine, small intestine and stomach. Seven micrograms of RNA from each tissue were separated on an agarose/formaldehyde gel and transferred to a nylon membrane. Plasmids containing Cμ, Cα and Cυ full-length cDNA were used for labeling probes with a PCR DIG Probe Synthesis kit (Roche, Germany). The primers used to amplify the probes were as follows: IgM-F and IgM-R for μ gene; IgA-F and IgA-R for α gene; IgY-F2 and IgY-R2 for υ gene (Table S1). The hybridization and detection were performed following the manufacturer's instructions. The goose EIF1A1 gene was chosen as the control gene for normalization.

2.5. DNA and protein sequence computations

The sequence editing was performed using software DNAStar. The phylogenetic tree was built in MrBayes3.1.2 (Ronquist and Huelsenbeck, 2003) and viewed in TreeView (Page, 1996). Multiple sequence alignments were performed using *ClustalW*. The accession numbers for the sequences used are as follows: μ gene: *X. laevis*, BC084123; axolotl, AM419754; lizard, EF690357; gecko, EU287911; crocodile, JQ417423; Chinese soft-shelled turtle, ACU45376.1; chicken, X01613; duck, AJ314754; ostrich, AFA41926; human, X14940; mouse, V00818; cow, AAN60017; horse, AAU09792. α or χ gene: *X. laevis*, BC072981; axolotl, AM774592; *Xenopus tropicalis*, BC157650; Crocodile, AFZ39174; ostrich, AFA41928; chicken, S40610; duck, AJ314754; human, J00220; mouse, J00475; platypus, AY055778; cow, AF109167; rabbit, S09264. δ gene: human, BC021276; mouse, J00449; platypus, ACD31540; crocodile, AFZ39207. υ gene: *X. laevis*, X15114; *X. tropicalis*, BC089679; axolotl, X69492; lizard, EF690360; gecko, EU827594; Chinese soft-shell turtle, FJ605148; crocodile, AFZ39169; ostrich, AFA41930; chicken, X07175; duck, X78273; γ gene: human, J00228; mouse, J00453; platypus, AY055781; horse, AJ302055; giant panda, AAX73307. ε gene: human, J00222; mouse, X01857; horse, AAA85662; pig, AAC48776; rabbit, AY386696.

3. Results

3.1. Ig isotypes expressed in goose

In order to analyze the IgH genes expressed in the goose, we first amplified a cDNA fragment of α gene using degenerate primers based on the conserved IgA VH and CH genes. The identity of the amplified fragment was confirmed by sequencing and Blast analysis. Then we performed 3′RACE using sense primers derived from JH gene segment and constructed spleen and intestine Ig-mini libraries. Analysis of 331 clones in the libraries containing the 1.5-kb inserts revealed most were IgA-positive (305) and 26 clones were non-Ig sequences. We therefore designed reverse degenerate primers based on the conserved IgM and IgY CH regions from other species, and performed PCR using the JH gene as the sense primer. The resultant products were cloned and sequenced. Then we conducted 3′RACE of Cμ and Cυ gene using primers designed according to the partial sequence of IgM and IgY CH regions. Consequently, we obtained secretory form of IgM, IgY and membrane-bound form of IgM heavy chain transcripts.