

## New Phenomenon

# A novel gene encoding goose immunoglobulin $\lambda$ light chain

Yongli Guo, Mingchun Gao\*, and Junwei Wang\*

Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China

\*Correspondence address. Tel: +86-451-55190385; Fax: +86-451-55191672; E-mail: gaomingchun@163.com (M.G.)/Tel: +86-451-55190385; Fax: +86-451-55191672; E-mail: jwwang@neau.edu.cn (J.W.)

Immunoglobulin (Ig) is an important effector molecule of humoral immunity, which plays an important role in immune regulation, representing the primary component of the adaptive immune system in all jawed vertebrates. The shorter and less genetically complex light chains represent the basic unit structure of the individual Ig. At present, it is believed that birds express only a single class of Ig light chain (IgL), which is most closely related to the  $\lambda$ -type chain of the mammalian IgLs [1–5]. Studies of chicken (*Gallus gallus*) and duck species (*Anas platyrhynchos*)  $\lambda$ IgLs have provided strong evidence to support this theory. However, the IgL of goose has yet to be identified.

In order to obtain the gene encoding goose IgL, primers were designed according to the published gene sequences of duck  $\lambda$ IgL (GenBank accession No. X82069.1) and chicken  $\lambda$ IgL (GenBank accession No. M33050.1). Single-strand cDNA was synthesized from total goose splenic RNA, and then two cDNAs were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR. The spliced cDNA extended from the 5' end of the start codon to the poly(A) tail of the 3'-untranslated region (UTR). It was found to have only one stop codon (TAA), different from two stop codons in duck  $\lambda$ IgL (TAG, TAA) and chicken  $\lambda$ IgL (TAA, TAG). The total length of spliced sequence encompassed 920 bp and has been submitted to the GenBank databases under the accession No. HQ852946 (Fig. 1). Compared with the known sequences of duck and chicken IgLs [1,2,5], an open reading frame (702 bp) is identified, containing the leader region, a variable region (VL), a join segment (JL), and a constant region (CL). The translated protein with 233 amino acids was obtained with a molecular mass of 24.882 kDa and a theoretical pI of 4.90.

Four lines of evidence indicated that the cDNA cloned here was an authentic  $\lambda$ IgL. First, the cDNA cloned here had 87% and 73% similarity with the duck and chicken  $\lambda$ IgLs, and there was 79% and 68% identity in the deduced amino acid sequences, respectively. Second, the

goose sequence cloned here was found to contain cysteines and tryptophans at the same positions as those in the duck and chicken  $\lambda$ IgLs (Fig. 1) [1,2], which may play an important role in maintaining the secondary structure of goose immunoglobulin. Third, comparisons of the goose constant region of IgL (IgCL) amino acid sequences with those of the chicken ( $\lambda$  isotype), duck ( $\lambda$  isotype), and mouse (both  $\lambda$  and  $\kappa$  isotypes) showed substantial conservation among the three kinds of avian  $\lambda$ IgCLs (Fig. 1) and confirmed that the avian light chains so far cloned were more similar to the mammalian  $\lambda$  isotype than the  $\kappa$  isotype. Fourth, the polyclonal antibody prepared with the recombinant protein rGoCL containing the goose IgCL expressed in Rosetta<sup>TM</sup>(DE3)pLysS (Novagen, Gibbstown, USA) according to the sequence cloned here had a clear reaction with goose IgL from serum and bile (Supplementary Fig. S1), which showed that rGoCL has the same antigenicity as the native goose IgL, and it also confirms that the sequence cloned here are goose IgL.

A 21-amino acid signal peptide sequence was predicted in goose  $\lambda$ IgL, and the most likely cleavage site of the leader sequence was between amino acid positions 21 and 22. Two *N*-glycosylation sites were predicted in goose  $\lambda$ IgL, which may play an important role in maintaining effector functions by contributing to the processes of folding, oligomerization, and stability [6–8], raising the possibility for goose  $\lambda$ IgL to form a glycoprotein. Phylogenetic analysis showed that goose had a closer genetic relationship to duck and *Cairina moschata* (the so-called Muscovy duck) than to any other birds analyzed in the evolution of  $\lambda$ IgL, suggesting that the  $\lambda$  clusters of goose, duck, and *C. moschata* may have arisen during evolution by duplication of an ancestral cluster. Homology modeling of goose  $\lambda$ IgL showed the characteristic immunoglobulin-like fold that was consistent with the division of goose  $\lambda$ IgL.

In conclusion, we present the first evidence of an encoded cDNA of goose  $\lambda$ IgL and this is a novel finding



**Figure 1** Sequence characteristics and homologous alignment of goose  $\lambda$ IgL (A) Nucleotide sequence and deduced amino acid sequences of the cDNA encoding goose  $\lambda$ IgL. The nucleotide and amino acid sequences are numbered on the left and right sides, respectively. The conserved cysteines and tryptophans are separately indicated by circles and boxes. The stop codon is indicated with an asterisk. The poly(A) signal sequences in the 3'-UTR are italicized and underlined. (B) Alignment of the goose IgCL with those of the duck (CAA57568), chicken (AAA48906), and mouse with  $\lambda$  and  $\kappa$  isotypes (AAC52488 and AAC98955). The homology of them with goose IgCL is numbered on the right sides.

that has not been reported, but further work needs to be done on the basis of our preliminary results including: (i) Where is the location of VL, JL, and CL in genome? (ii) Whether goose expresses the non- $\lambda$ IgL? (iii) When does the  $\lambda$ IgL appear during the development of B lymphocyte? (iv) How many copy numbers of  $\lambda$ IgL and its functional components exist in genome? (v) Whether the somatic mutation or gene rearrangements generate diversity of the limited functional V region. These will be the extension for further study of goose  $\lambda$ IgL, which will be beneficial for the research of goose humoral immunity.

## Supplementary data

Supplementary data are available at *ABBS* online.

## Funding

This work was supported by the grants from the Scientific and Technological Projects of Heilongjiang Province (GB01B503-02 and GA09B302).

## References

- Reynaud CA, Dahan A and Weill JC. Complete sequence of a chicken lambda light chain immunoglobulin derived from the nucleotide sequence of its mRNA. *Proc Natl Acad Sci USA* 1983, 80: 4099-4103.
- Magor KE, Higgins DA, Middleton DL and Warr GW. cDNA sequence and organization of the immunoglobulin light chain gene of the duck, *Anas platyrhynchos*. *Dev Comp Immunol* 1994, 18: 523-531.
- Grant JA, Sanders B and Hood L. Partial amino acid sequences of chicken and turkey immunoglobulin light chains: homology with mammalian lambda chains. *Biochemistry* 1971, 10: 3123-3132.
- Kubo RT, Rosenblum IY and Benedict AA. The unblocked N-terminal sequence of chicken IgG lambda-like light chains. *J Immunol* 1970, 105: 534-536.
- McCormack WT, Carlson LM, Tjoelker LW and Thompson CB. Evolutionary comparison of the avian IgL locus: combinatorial diversity plays a role in the generation of the antibody repertoire in some avian species. *Int Immunol* 1989, 1: 332-341.
- Tao MH and Morrison SL. Studies of a glycosylated chimeric mouse-human IgG: Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J Immunol* 1989, 143: 2595-2601.
- Mitra N, Sinha S, Ramya TN and Suroli A. N-linked oligosaccharides as outfitters for glycoprotein folding, form and function. *Trends Biochem Sci* 2006, 31: 156-163.
- Petrescu AJ, Milac AL, Petrescu SM, Dwek RA and Wormald MR. Statistical analysis of the protein environment of N-glycosylation sites: implications for occupancy, structure, and folding. *Glycobiology* 2004, 14: 103-114.