

Short communication

# Characterization of the immunoglobulin A heavy chain gene of the Atlantic bottlenose dolphin (*Tursiops truncatus*)<sup>☆</sup>

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## Abstract

Immunoglobulin constant region heavy chain genes of the dolphin (*Tursiops truncatus*) have been described for IgM and IgG but not for IgA. Here, the heavy chain sequence of dolphin IgA has been cloned and sequenced as cDNA. RT-PCR amplification from blood peripheral lymphocytes was carried out using degenerate primers and a single sequence was detected. The inferred heavy chain structure shows conserved features typical of mammalian IgA heavy chains, including three constant (C) regions, a hinge region between constant region domain 1 (C1) and constant region domain 2 (C2), and conserved residues for interaction with the Fc $\alpha$ R1 and N-glycosylation sites. Comparisons of the deduced amino acid sequences of the IgA heavy chain for the dolphin and the evolutionarily related artiodactyl species showed high similarity. In cattle and sheep, as in dolphins, a single IgA subclass has been identified. Southern blot analysis as well as genomic PCR confirmed the presence of multiple *IGHA* sequences suggesting that *IGHA* pseudogenes may be present in the dolphin genome.

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## 1. Introduction

IgG, with its high plasma concentrations, is often thought of as the major antibody produced by mammals. However, in many species of mammals

the synthesis of IgA predominates. In humans the rate of IgA production is approximately twice that for IgG (Kerr, 1990; Yoo and Morrison, 2005). Thus, while IgA represents only about 15–20% of immunoglobulins in the blood (Kerr, 1990), it is also secreted and often present in high concentration in body fluids such as tears, saliva, gastric fluid, mucosal secretions and milk where it is important in local immune defense against pathogens (Childers et al., 1989). The IgA isotype appeared during evolution before the divergence of birds and mammals (Magor et al., 1998; Mansikka, 1992). In the chicken and duck the IgA heavy chain contains four C-region domains (Lundqvist et al., 2001; Magor et al., 1998; Mansikka, 1992; Zhao et al., 2000),

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while in mammals the IgA heavy chain contains three C-domains with a short hinge region between C1 and C2. The numbers of IgA heavy chain (*IGHA*) genes in the *IGH* locus of mammals varies, from one in mice, swine, sheep and cattle to as high as 13 in the rabbit (Brown and Butler, 1994; Brown et al., 1997; Burnett et al., 1989; Butler and Brown, 1994; Flanagan et al., 1984; Knight et al., 1985; Shimizu et al., 1982; Tucker et al., 1981; White et al., 1998).

The cetaceans (whales, dolphins and their relatives) are a group of marine mammals that has adapted to life in a totally aquatic environment. This has led to distinctive and major morphological, behavioral and physiological adaptations. However, little is known of the adaptations that have occurred in the immune systems of cetacean species in response to the aquatic environment. Given the importance of IgA in immune function at the interface between the organism and its environment, we undertook and report here on the molecular cloning and characterization of the *IGHA* gene in the Atlantic bottlenose dolphin, *Tursiops truncatus*.

## 2. Materials and methods

### 2.1. Sample collection, RNA and DNA preparation

Methods used for the preparation of total RNA from peripheral blood leukocytes and of genomic DNA from spleen samples of dolphins have been reported previously (Mancía et al., 2006). Blood samples were collected from dolphins maintained by the US Navy Marine Mammal Program, San Diego, CA, in accordance with a protocol approved by the Institutional Animal Care and Use Committee under the guidelines of the Association for the Accreditation of Laboratory Animal Care.

### 2.2. Cloning of dolphin *IGHA* sequences

A portion of the dolphin *IGHA* sequence was amplified from peripheral blood leukocyte cDNA from one animal (animal 1) by polymerase chain reaction (PCR, using the Advantage<sup>TM</sup> cDNA PCR kit, Clontech, Palo Alto, CA), with a primer concentration of 10  $\mu$ M and a Mg<sup>2+</sup> concentration of 3.5 mM, an initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, a gradient of 52–63 °C for 30 s, 72 °C for 1 min, followed by soaking for 7 min at 72 °C using degenerate primers (Aveskogh and Hellman, 1998). These were G-1859 (C2 forward, 5'-GAAGAATTCGGCTGCTACAGYGTGTCCAGYRTSCTRCCNGGCTGTGC

and G-1860 (C3 reverse, 5'-GAAGGATCCGGTTTACCCGMCWVVCGGTYGATGGTCTTCTG). Full-length *IGHA* sequence was then completed through the cloning of overlapping sequences using 5' anchored and 3' RACE PCR (Frohman et al., 1988). Primers were G-2183 (VH-FR3 forward, 5'-GACRCRGCRRTR-TATTAYTGTG); G-3001 (C2 forward 5'-CTGCACC-CACCGGCCCTTGA); G-3002 (C2 reverse 5'-CT-TGGGGTCTCTCAGGCCACT); G-3012 (C2 reverse 5'-GGTTTCGTGATGGTGGCGG); G-2185 (C3 forward 5'-GTCCACCTGTGCCGCC); G-1913 (C3 forward 5'-GCGAGAAGTACCTGACCTG); G-2181 (C3 reverse 5'-GTGAAGGCCAGGGGCAGG); G-414 (3'RACE anchor primer, 5'-TCTGAATTCTCGAGTGACATC).

Genomic PCR was performed on DNA from four different animals. Genomic DNA was phenol extracted from tissue necropsy (spleen, animal 2) or peripheral blood leukocytes (PBL, animal 3, 4 and 5). A portion of the dolphin *IGHA* gene from animals 2 and 3 was amplified by genomic PCR using 50–100 ng of genomic DNA with G-2983 (C1 forward 5'-CCACTGAGCC-TAAGGAGCG) and G-1914 (C3 reverse 5'-CACCATG-CAGGAGAAGGAC). PCR (using Advantage<sup>TM</sup> GC genomic PCR kit, Clontech, Palo Alto, CA) used primer concentrations of 10  $\mu$ M, initial denaturation at 95 °C for 1 min, 30 cycles of 95 °C for 30 s, 56 °C for 30 s, 68 °C for 4 min, followed by a soaking for 10 min at 68 °C. Amplicons were agarose gel purified, dA-tailed with Taq DNA polymerase (Fisher Scientific, Pittsburg, PA) and cloned into the pCR<sup>®</sup> 2.1 TOPO<sup>®</sup> vector (Invitrogen, Carlsbad, CA) before sequencing on both strands. Sixteen and eight independent clones were sequenced respectively from animal 2 and 3.

### 2.3. Southern blot analysis

Genomic DNA from animals 1 and 5 was digested to completion with *Bam*HI, *Eco*RI, *Hind*III, *Pst*I and *Mbo*I and separated on 0.6% agarose gels, blot transferred to nylon filters and subjected to hybridization as described previously (Mancía et al., 2006). Probes specific for the dolphin C2 or C3 exon were generated by PCR from genomic white blood cells DNA templates from one animal (animal 3) using primer pairs G-3001/G-3012 and G-2185/G-1914, respectively. After hybridization with <sup>32</sup>P-labeled probes (Mancía et al., 2006) the filters were washed for 2  $\times$  30 min in 1  $\times$  SSPE, 0.1% SDS, at 42 °C and 3  $\times$  30 min in 1  $\times$  SSPE, 0.1% SDS, at 65 °C prior to exposure to X-Omat AR film (Kodak, Rochester, NY) for 48 h at –80 °C.

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