Developmental and Comparative Immunology 72 (2017) 57–68

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

The Florida manatee (*Trichechus manatus latirostris*) immunoglobulin heavy chain suggests the importance of clan III variable segments in repertoire diversity

Breanna Breaux [a], Thaddeus C. Deiss [a], Patricia L. Chen [a], Maria Paula Cruz-Schneider [b], Leonardo Sena [c], Margaret E. Hunter [d], Robert K. Bonde [d], Michael F. Criscitiello [a, e, *]

[a] *Comparative Immunogenetics Laboratory, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA*
[b] *Laboratory of DNA Polymorphism, Federal University of Pará, Belém, Pará, Brazil*
[c] *Laboratory of Medical and Human Genetics, Federal University of Pará, Belém, Pará, Brazil*
[d] *Wetland and Aquatic Research Center, U.S. Geological Survey, 7920 NW 71st Street, Gainesville, FL 32653, USA*
[e] *Department of Microbial Pathogenesis and Immunology, College of Medicine, Texas A&M Health Science Center, Texas A&M University, College Station, TX 77843, USA*

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form
23 January 2017
Accepted 23 January 2017
Available online 25 January 2017

Keywords:
Manatee
Immunoglobulin
Clan
Genomic
Mutation

ABSTRACT

Manatees are a vulnerable, charismatic sentinel species from the evolutionarily divergent Afrotheria. Manatee health and resistance to infectious disease is of great concern to conservation groups, but little is known about their immune system. To develop manatee-specific tools for monitoring health, we first must have a general knowledge of how the immunoglobulin heavy (IgH) chain locus is organized and transcriptionally expressed. Using the genomic scaffolds of the Florida manatee (*Trichechus manatus latirostris*), we characterized the potential IgH segmental diversity and constant region isotypic diversity and performed the first Afrotherian repertoire analysis. The Florida manatee has low V(D)J combinatorial diversity (3744 potential combinations) and few constant region isotypes. They also lack clan III V segments, which may have caused reduced VH segment numbers. However, we found productive somatic hypermutation concentrated in the complementarity determining regions. In conclusion, manatees have limited IGHV clan and combinatorial diversity. This suggests that clan III V segments are essential for maintaining IgH locus diversity.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Manatees are largely tropical marine mammals within Superorder Afrotheria, which includes aardvarks, elephants, and tenrecs (Kellogg et al., 2007). The Order Sirenia includes three manatee species, the West Indian (*Trichechus manatus*), the Amazonian (*T. inunguis*), and the African (*T. senegalensis*) manatee that inhabit the Caribbean and Atlantic coasts, the Amazon River drainage, and the West African coasts, respectively (Domning, 1982). The two American species are of particular interest due to a history of overhunting, which caused several bottlenecks and their current vulnerable status (O'Shea, 1988). The West Indian manatee also has significant population fragmentation due to habitat destruction (Vianna et al., 2006). To date, two subspecies have been identified: the Florida manatee (*T. m. latirostris*) and the Antillean manatee (*T. m. manatus*). Because these species maintain a wide distribution across varied aquatic environments, have a history of bottlenecks and founder events, and serve a role as sentinel species within coastal environments (Bonde et al., 2004), characterizing their adaptive immune receptors is essential to supplement conservation efforts. Some earlier studies have quantified the robustness of the manatee immune system (Bossart, 1995; McGee, 2012; Sweat et al., 2005; Walsh et al. 2003, 2005), but the specific antigen binding diversity of the adaptive immune receptors has not been described. Manatees make an interesting non-model species to study because

* Corresponding author. Texas A&M University, Mailstop 4467, College Station, TX 77843, USA.
E-mail addresses: bbreaux@cvm.tamu.edu (B. Breaux), tdeiss@cvm.tamu.edu (T.C. Deiss), pchen@cvm.tamu.edu (P.L. Chen), paula@ufpa.br (M.P. Cruz-Schneider), lsena@ufpa.br (L. Sena), mhunter@usgs.gov (M.E. Hunter), rbonde@usgs.gov (R.K. Bonde), mcriscitiello@cvm.tamu.edu (M.F. Criscitiello).

http://dx.doi.org/10.1016/j.dci.2017.01.022
0145-305X/© 2017 Elsevier Ltd. All rights reserved.

there are little data on the lymphocyte antigen receptors of the Afrotherian branch of mammalian evolution (Guo et al., 2011).

Florida manatees have two significant disease associated agents identified in their populations (*Brevetoxicosis* (red tide) and papilloma virus) (Bossart et al., 1998, 2002; Walsh et al., 2015). The brevetoxins produced by *Karenia brevis* cause disease in two ways. There are acute neurotoxic effects from the brevetoxin when they bind to sodium channels, which activates them and disrupts the normal salt gradient required for neuronal action potentials (Wang, D. Z. 2008). The more fatal aspects of the disease stem from the chronic immunosuppressive effects when the brevetoxins are internalized by leukocytes because it initiates apoptosis, which releases inflammatory mediators that lead to edema and pulmonary hemorrhage (Bossart et al., 1998). Additionally, several parasites have been identified in the Florida populations, including trematodes (*Chiorchis fabaceus, Chiochis groschafti, Pulmonicola cochleotrema, Moniligerum blairi,* and *Nudacotyle undicola*), nematodes (*Heterocheilus tunicatus*) and coccidians (*Eimeria manatus* and *Eimeria nodulosa*), but their impact on population health is not yet understood (Bando et al., 2014). Little is known about the manatee immunoglobulin (Ig) repertoire, with the exception of serological analysis that determined baseline levels of circulating IgG in *T. manatus* (McGee, 2012). Since the *T. m. latirostris* genome is the only assembled manatee genome currently available, we characterized the genomic organization of this manatee species and evaluated the gene expression to assess the potential repertoire diversity.

Immunoglobulins are both the secreted and membrane bound receptors of B cells that can opsonize and neutralize extracellular antigens and activate the complement cascade. The adaptive immune receptor loci are exceptional in their organization and rearrangement mechanism which creates the diversity needed to recognize a wide variety of antigens. The Immunoglobulin heavy chain (IgH) locus contains a unique set of several variable (V), diversity (D), and joining (J) segments (Tonegawa, 1983). During development, each B cell recombines one of each segment at the DNA level so that each cell produces one receptor, allowing for the specific proliferation of cells and receptors that recognize the invading antigen (Nossal and Lederberg, 1958).

To increase the diversity of these receptors beyond the combination of different segments, there are two mechanisms driven by the enzyme activation-induced cytosine deaminase (AID) in most vertebrates: somatic hypermutation (SHM) and class-switch recombination (CSR). These mechanisms usually proceed after antigen recognition, except in sheep where mutation occurs independent of external antigen exposure (Reynaud et al., 1995). AID also mediates another uncommon mechanism of diversification called gene conversion, seen in rabbits and chickens. In SHM, deaminating a cytosine results in repair mechanisms that can cause a point mutation. CSR occurs when there are two double stranded breaks in the switch regions of two constant regions, which are resolved by bringing the two breaks together and deleting the intervening sequence. This allows for isotype switching which gives the receptors different functions.

Mammalian V segments can be separated into three clans based on sequence similarity primarily in the framework 1 and 3 regions (Kirkham et al., 1992). In most mammals, there are germline V segment representatives for each clan, but some species lack V segments from one or two clans. However, clan III is usually retained, such as the platypus that only has clan III V segments (Zhao et al., 2009). Clan III is also the most homologous to ancestral V segments in fish (Andersson, 1995; Zhao et al., 2009). However, there are three mammalian species that do not have clan III (cow, sheep, and horse), where it appears to be coupled with low numbers of V segments (Dufour et al., 1996; Niku et al., 2012; Walther et al., 2015). The mechanism and selection pressure behind the maintenance of these clans is unclear, but there do seem to be functional differences between the three clans (Schroeder and Wang, 1990; Tutter and Riblet, 1989).

While the immunoglobulin heavy chain locus structure is similar across jawed vertebrates, there are differences in the potential repertoire diversity and the range of functions of the available constant regions. By comparing the manatee segmental diversity and expression with other species, we can see which segments are important both within and between species and determine how well equipped manatees are for defense against infectious disease in their environment.

2. Materials and methods

2.1. Sample collection

Blood was collected into EDTA-containing vacutainer tubes from the flipper of *T. manatus latirostris* during wild capture health assessments in Crystal River, Florida in November of 2014. The blood was processed at the site of collection using the LeukoLock Total RNA Isolation System (Life Technologies, Carlsbad CA) to preserve RNA from peripheral blood leukocytes. The filters were transported to Texas A&M University at room temperature and then stored at −20 °C.

2.2. Total RNA isolation and cDNA synthesis

RNA was isolated from the filter-bound leukocytes using the LeukoLock Total RNA Isolation System, following the manufacturer's instructions. The quantity and quality of the RNA samples were assessed using a NanoDrop 2000c spectrophotometer (Thermofisher, Waltham, MA) and stored at −80 °C. The 5′ RACE cDNA libraries were prepared from the leukocyte RNA using the GeneRacer kit (Life Technologies) with GeneRacer oligo dT and random primers in equal ratios.

2.3. Identification of IgH genes in the genome

The Igμ constant region for the manatee was annotated by aligning the Asian elephant (*Elephas maximus*) Igμ transcript against the *T. m. latirostris* genome (Broad v1.0/triMan1). Once the scaffold containing the Igμ constant region was identified, BLASTx was used to search for potential upstream V segments and downstream constant regions. The Recombination Signal Sequence Site (http://www.itb.cnr.it/rss/index.html) was used to predict recombination signal sequences (RSSs) to identify D and J segments. D segments were predicted based on two opposite facing 12-spacer RSSs located between the most 3′ V segment and most 5′ J segment. J segments were predicted based on a reverse orientation 23 RSS that was followed by either a FGXG or a WGXG motif. Genomic V and D segments were separated into families based on 70% nucleotide identity (Brodeur and Riblet, 1984). Both V and D segment families were ordered by the number of segments within the family, starting with the largest.

2.4. IgH RACE PCR, cloning, and sanger sequencing

The 5′ RACE products were amplified by PCR using the 5′ Generacer adaptor forward primer and a *T. manatus* Igμ constant region reverse primer (Table S1). The 3′ RACE products were amplified by PCR using several forward primers specific for the framework regions of different V and the 3′ Generacer reverse primer. The amplicons were purified from a 0.8% agarose gel after electrophoresis in tris/acetic acid/EDTA (TAE) buffer, cloned into pCR II vector

Download English Version:

https://daneshyari.com/en/article/5540168

Download Persian Version:

https://daneshyari.com/article/5540168

Daneshyari.com