ABSTRACT

The mammalian immune response is a highly regulated system which treads a delicate balance between preventing disease and producing autoimmunity. The genes involved in this process lie within the major histocompatibility complex (MHC), the most variable portion of the vertebrate genome. Although all class II MHC genes are controlled by a single master switch, the class II transactivator (CIITA), expression across the different loci is not uniform. Class II MHC genes carry regulatory motifs in their proximal promoter region which must be bound by a complex of transcription factors and CIITA to turn on expression. Genetic variation within the promoter region may alter the capacity for this complex to form and thus the ability of the organism to launch an appropriate immune response. The regulatory motifs for three cetacean class II loci, DQA, DQB, and DRB, were identified from the Tursiops truncatus whole genome shotgun sequence. Primers were then designed to PCR amplify those regions in T. truncatus, Delphinapterus leucas, and Orcinus orca. A complete duplication of a 15 bp T-box motif was found in the DQA promoter region of both T. truncatus and O. orca but was absent in D. leucas. This is the first genetic characterization of MHC regulatory motifs in cetaceans and the only known motif duplication event in a mammalian MHC promoter. This regulatory adaptation may divulge unique immunogenetic strategies between the different cetacean species.

BACKGROUND

- CIITA and transcription factors bind SXY in the promoter region
- This complex forms at all MHC Class II genes to turn on expression
- Two genes (DQA, DQB) together create a receptor molecule (DQ)
- Receptors present pathogens to T-cells to initiate the immune cascade
- Activated T-cells have upregulated MHC expression
- Cetacean T-cells are always activated
- Genetic variation in promoter regions affects MHC expression
- Level of MHC expression affects magnitude of immune response
- MHC genetic variability is a useful measure of population immune health

METHODS

1. Locate Genes in WGS. Published mRNA or DNA sequences for three MHC class II loci (DQA, DQB, DRB) in T. truncatus were downloaded from GenBank and queried against four cetacean WGS using NCBI BLASTs.
2. Locate Conserved Regulatory Motifs. Top hit was aligned to the query sequence using BioEdit Sequence Alignment Editor, and GenSCAN was run to determine exon boundaries. Conserved regulatory motifs S, X, and Y were identified at each locus from the proximal promoter regions upstream of the transcription start site, along with locus-specific motifs.
3. PCR & Sequencing. Primers were designed using Primer3 and IDT OligoAnalyzer. PCR reactions were carried out on 10 T. truncatus and 4 O. leucas samples, and amplicons were sequenced on a 3130 Genetic Analyzer.

RESULTS

- Identified DQA, DQB, DRB loci in WGS for five cetacean species
- First characterization of cetacean MHC promoter structure
- Identified DQA, DQB, DRB regulatory motifs
- Found complete duplication of 15 bp regulatory motif
- Present in only two cetaceans: T. truncatus and O. orca
- Variable sites found in T. truncatus DQA, DQB, and DRB (n=10)
- 2 variable sites found within regulatory motifs
- Variable sites found in O. leucas DRB (n=4)

CONCLUSIONS

T-Box Duplication:
- T-box response element, decreases DQA expression
- Potential importance for avoiding autoimmunity
- First known duplication of MHC regulatory motif

Variant Analysis:
- DQ variation in dolphins (n=10), not in belugas (n=4)
- Variation in dolphin DQA, DQB regulatory motifs
- Lack of variation may indicate at-risk populations

Ongoing Work:
The exact role of the cetacean T-box is unclear, pending future expression studies. The species distribution of the duplication is of interest, as it is present in a species with high DQA diversity (T. truncatus) and absent in a species with low DQA diversity (O. leucas).

A next generation sequencing approach has already been completed for high throughput genotyping of multiple MHC loci promoters and peptide binding regions in dolphins, and will also be implemented for belugas. The extent of MHC diversity will be determined to understand the different evolutionary strategies employed by these two species and elucidate the significance of the T-box duplication.

FOR MORE ON BELUGA MHC SEE POSTER#181 (Tatiana Ferrer)

Marine mammals are uniquely exposed to a variety of airborne and waterborne pathogens. As part of the Cetomics Initiative, this work will allow us to relate MHC genetic diversity in a population of whales to the particular pathogens and health threats that they are exposed to.

Works Cited

- flowcharts and images

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