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Characterization and evolution of MHC class II B genes in Galápagos marine iguanas (*Amblyrhynchus cristatus*)

Scott Glaberman [a,*], Maria A. Moreno [b], Adalgisa Caccone [a,c]

[a] Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520-8105, United States
[b] Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8105, United States
[c] Yale Institute for Biospheric Studies, Yale University, New Haven, CT 06520-8105, United States

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ABSTRACT

Major histocompatibility complex (MHC) class II molecules play a key role in the adaptive immune system of vertebrates. Class II B genes appear to evolve in a very different manner in mammals and birds. Orthology is commonly observed among mammal loci, while genes tend to cluster phylogenetically within bird species. Here we present class II B data from a representative of another major group of amniotes, the squamates (i.e. lizards, snakes, amphisbaenians), with the ultimate goal of placing mammalian and avian MHC evolution into a broader context. In this study, eight class II B cDNA sequences were obtained from the Galápagos marine iguana (*Amblyrhynchus cristatus*) which were divided into five locus groups, *Amcr-DAB1* through *-DAB5*, based on similarities along most of the coding and noncoding portions of the transcribed gene. All marine iguana sequences were monophyletic with respect to class II genes from other vertebrates indicating that they originated from a common ancestral locus after squamates split from other reptiles. The β-1 domain, which is involved in antigen binding, exhibited signatures of positive selection as well as interlocus gene conversion in both long and short tracts—a pattern also observed in birds and fish, but not in mammals. On the other hand, the β-2 domain was divergent between gene groups, which is characteristic of mammals. Based on these results, we preliminarily show that squamate class II B genes have been shaped by a unique blend of evolutionary forces that have been observed in differing degrees in other vertebrates.

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1. Introduction

Major histocompatibility complex (MHC) class II genes encode protein receptors that play a central role in the adaptive immune system of jawed vertebrates [1–3]. Class II receptors bind short peptide fragments derived from exogenous pathogens such as bacteria and protozoa, and present them on the surface of specialized antigen presenting cells (e.g. B cells, macrophages, dendritic cells) [4]. This complex of MHC and antigen is then recognized by CD4+ T helper cells, which in turn release cytokines that drive key components of both the humoral and cell-mediated immune responses, including B cell proliferation and the production of cytotoxic T lymphocytes [3,5,6].

Class II receptors are formed by noncovalent association of two polypeptide chains, α and β, which are encoded by separate class II A and B genes respectively [3,7,8]. Each chain contains one membrane distal (α-1 and β-1) and one membrane proximal (α-2 and β-2) extracellular domain, a transmembrane (Tm) region, and a short cytoplasmic (Cyt) anchor. Typically, different exons code for each protein subunit. The antigen binding pocket is generated by folding of the α-1 and β-1 domains; however, contact with the foreign peptide is generally limited to β-1 amino acid residues [9].

In contrast to T cell receptors and antibodies, variation in MHC molecules cannot be generated within the lifetime of an individual in order to recognize a diverse set of pathogens. Consequently, class II B genes are highly polymorphic, and most of this variation is concentrated in the antigen binding β-1 domain [10,11]. Each allele is capable of targeting hundreds or thousands of different foreign peptide fragments [3]. However, there are some class II loci, referred to as nonclassical, that possess little polymorphism and perform functions other than classical antigen presentation [12].

Phylogenetic studies depict a complex history of class II B loci and reveal that the tempo and mode of evolution differ greatly within and among vertebrate groups. Placental mammals possess several orthologous gene clusters (e.g. *DRB*, *DQB*, and *DPB*), some of which are maintained even among distantly related species [8,13]. The patterns of class II B evolution are much less predictable in birds. Passerines possess numerous gene copies that tend to cluster within species in a phylogenetic analysis [14,15]. This is thought to

* Corresponding author. Tel.: +1 203 432 3886; fax: +1 203 432 7394.
E-mail addresses: scott.glaberman@gmail.com, scott.glaberman@yale.edu (S. Glaberman).

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be the result of either rapid duplication or concerted evolution via genetic exchange among different loci. Few instances of class II orthology have been reported in birds, and only in cases where species are closely related [16–18]. However, a recent study in owls (Strigiformes) showed that two separate loci (*DAB1* and *DAB2*) have been maintained throughout much of the evolution of this order [19], suggesting that a wider breadth of taxa needs to be sampled before achieving any consensus for class II B evolution in birds.

The forces shaping class II B loci differ among regions of the gene. Balancing selection, mediated by host–parasite interactions, is thought to maintain polymorphism in the β-1 domain [20–23]. In mammals, this polymorphism is generated by a mixture of point mutations and intralocus recombination, causing β-1 sequences to cluster within loci in a phylogenetic reconstruction [24]. In birds and bony fish, interlocus recombination plays an additional role in β-1 evolution, often blurring the phylogenetic signal among loci [14,15,25,26]. The β-2 domain, on the other hand, is typically under purifying selection [11]. In mammals, this has resulted in a pattern of divergent evolution, where the history of orthology and duplication of genes can be recovered in a phylogenetic analysis [8,27]. Conversely, in most birds examined, interlocus recombination in long tracts causes loci to cluster within species, effectively extinguishing patterns of orthology among genes [14,16,18].

The dichotomy between mammalian and avian class II B genes raises the question of which mode of evolution is more characteristic of ancestral amniotes. This information can provide a historical context for examining the genetic, physiological, and environmental forces that have led to different MHC patterns in these groups. One way to infer this ancestral pattern is to compare class II B genes of mammals and birds to other amniotes, in order to achieve a consensus. Miller et al. [28] took this approach by examining the MHC in tuatara (order: Sphenodontia). Tuatara along with the order Squamata (i.e. lizards, snakes, amphisbaenians), form the other major lineage of amniotes in addition to archosaurs (birds and crocodilians) and mammals. In the case of squamate reptiles, no sequence-based studies of class II genes have ever been published, even though this group contains approximately seven thousand species [29]—more than all mammals combined. In this study, we present class II B cDNA sequences from the marine iguana (*Amblyrhynchus cristatus*), a squamate species that is endemic to the Galápagos archipelago. We investigate the evolutionary forces that have shaped the different portions of the class II B molecule and compare these results to patterns found in mammals, birds, and other vertebrates.

2. Materials and methods

2.1. 5′ and 3′ RACE of marine iguana cDNA

Blood was taken from a single marine iguana from the island of Santa Cruz, Galápagos. Total RNA was extracted using the Tri Reagent BD kit (Molecular Research Center, Cincinnati, OH, USA) and the protocol provided. Complementary DNA (cDNA) was synthesized as previously described [30,31].

In order to design gene-specific primers (GSPs) for the rapid amplification of cDNA ends (RACE), a pool of short MHC class II B fragments was first generated via PCR of genomic DNA from a single marine iguana. Four degenerate primers were designed using published sequences from other vertebrates (Table 1, numbers 1–4). One of these primers, MHCIIBF22, was located in the β-1 domain and was used in combination with three different primers from the β-2 domain (Supplementary Fig. 1) with the following reactions conditions: 1.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 200 μM of each dNTP, 1 μM each primer, 1.5 mM $MgCl_2$, and 1× PCR Buffer without $MgCl_2$. Cycling conditions were as follows: initial denaturation at 94 °C for 10 min, followed by 30 cycles with 30 s of denaturation at 94 °C, 30 s of annealing at 59 °C, and 90 s extension at 72 °C; and a final extension of 72 °C for 10 min. PCR products were cloned and resultant colonies were purified and sequenced as previously described [30]. PCR yielded numerous products of different sizes, several of which were confirmed to be class II B-like via BLAST search [32].

Table 1
Primers used in amplification of MHC class II B loci from marine iguanas.

	Gene	Primer name	Primer sequence (5′ to 3′)	Primer usage	Annealing temp.
1	All class II	MHCIIBF22	AGHWCGCSCRCTTCGACAGCG	Genomic PCR	58
2	All class II	MHCIIBR26	AAGCTGGSRTGCTCCACCCRGCA	Genomic PCR	58
3	All class II	MHCIIBR27	ACGTCTCCCSGCTCCRSCTTYGT	Genomic PCR	58
4	All class II	MHCIIBR29	AGCATCACCTGGATCTGGAAGGTC	Genomic PCR	58
5	All class II	MHCIIB-Ex4-R2	ACATCTTGCTCCKGGSARAGTC	Southern probes	59
6	*Amcr-DAB1*	MHCIIB-GR1_F3	TTCCTCCTCCAGTTTCTGAGAC	Southern probes	59
7	*Amcr-DAB3*	MHCIIB-GR3_F2	TCCCTACTTTTCCTCTTCCAGTTTCTG	Southern probes	59
8	*Amcr-DAB4*	MHCIIB-GR4_F2	ACTTAACCACATCTCCCTTGAAGAAATC	Southern probes	59
9	*Amcr-DAB5*	MHCIIB-GR2_F1	AGGAAGTCTTCAGGGCCACTC	Southern probes	59
10	*Amcr-DAB1*	R-MHC-GR1-F1	ATGAGCATCACCCCCGCAGAGCAC	3′ RACE	63
11	*Amcr-DAB3*	R-MHC-GR1234-F1	TGATCTGCRAYGTGGCCRGRTTCTG	3′ RACE nested	63
12	*Amcr-DAB3*	MHC2-GR1-5R-R2	AGACTTTGCATCCTCTCAGCAGC	5′ RACE	60
13	*Amcr-DAB3*	MHC2-GR1-5R-R3	TCTTTGCAGTGCTGAAGTCTAGCTC	5′ RACE nested	60
14	*Amcr-DAB2*	Cl2GR1vA-F1	AGCGGAGGGCACAGGAAGGGAC	3′ RACE	65
15	*Amcr-DAB2*	Cl2GR1vA-F2	TCTCTCTGTGGCAGCGGTCAGC	3′ RACE nested	65
16	*Amcr-DAB2*	MHC2-GR1-5R-R1	AGTCCTTCTTTGTCCTCTCCAGAC	5′ RACE	60
17	*Amcr-DAB2*	GR1vA-5R-R1	ACCTCCTTCTGGCCGTTCAAGTGC	5′ RACE nested	61
18	*Amcr-DAB3*	R-MHC-GR3-F1	TTGAAGATCTCAGCCACAGAGCAG	3′ RACE	60
19	*Amcr-DAB3*	R-MHC-GR3-F2	AGGACTCCTCTTCTCAAAACACTTTG	3′ RACE nested	60
20	*Amcr-DAB3*	MHC2-GR3b-5R-R1	AAGCACCAAAGCCCCTCTCCAAG	5′ RACE	60
21	*Amcr-DAB3*	MHC2-GR3b-5R-R2	AAGTAATTCCTGTCCCCACTCACC	5′ RACE nested	60
22	*Amcr-DAB4*	Cl2-DDB-3R-F2	TGCCGCCTGGGAAAGAAACAGC	3′ RACE[a]	65
23	*Amcr-DAB4*	MHC2-GR2a-5R-R3	TCAGGGCCGTGGTCATCACTCTG	5′ RACE	61
24	*Amcr-DAB4*	MHC22-A7-R	TGATGCTCATCTTGGGCTGAACCAG	5′ RACE nested	63
25	*Amcr-DAB5*	R-MHC-GR2&4-F1	ATGAGCATCACCCCCGCAGAGCAT	3′ RACE	63
26	*Amcr-DAB5*	R-MHC-GR1234-F1	See above	3′ RACE nested	63
27	*Amcr-DAB5*	MHC2-GR2a-5R-R1	ATCCTGCAAAGAAATCACCCAGAAGC	5′ RACE	59
28	*Amcr-DAB5*	MHC2-GR2a-5R-R2	ACCGGAGTCACCAGTGAAAAGACG	5′ RACE nested	61

[a] No nested step was required for 3′ RACE.

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