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BRIEF COMMUNICATION

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An immunogenetic basis for the high prevalence of urogenital cancer in a free-ranging population of California sea lions (*Zalophus californianus*)

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Abstract In response to an unprecedented prevalence of cancer recently identified in free-ranging populations of California sea lions [(CSL) (*Zalophus californianus*], we examined the role of the immunologically important major histocompatibility (MHC) genes in this disease epidemic. Associations between MHC genes and cancer have been well established in humans, but have never before been investigated in wildlife. Using a previously developed technique employing sequence-specific primer-based PCR with intercalating dye technology, MHC genotypes were examined from 27 cancer-positive and 22 cancer-negative CSL stranded along the California coastline. Analyses elucidated an underlying immunogenetic component to the high prevalence of urogenital cancer in sea lions. Furthermore, these results demonstrate the functional relevance of CSL class II MHC by revealing a non-random nature of cancer susceptibility associated with the presence of specific genes.

Keywords Major histocompatibility complex · California sea lion · Urogenital carcinoma

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An unprecedented prevalence of urogenital cancer has recently been identified in free-ranging populations of California sea lions [(CSL) *Zalophus californianus*] (King et al. 2002). An interest in the role of the immune system in the etiology of this disease stimulated the present study to examine genetic diversity within the major histocompatibility complex (MHC), the products of which encode trans-membrane glycoproteins that bind and present foreign peptides to T lymphocytes (Paul 1999; Klein and Sato 2000a, b). The high level of MHC genetic variation found in most natural populations essentially determines the repertoire of antigenic determinants to which an individual is capable of recognizing and responding (Zinkernagel 1979; Reizis et al. 1998). In previous studies, an unusual mechanism for generating immunogenetic diversity was identified in sea lions, and techniques were developed to evaluate this at a population level (Bowen et al. 2002, 2004). Briefly, *Zaca-DRB* appears to constitute a gene family composed of at least eight loci, each of which exhibits limited variability, and which are present in variable configurations among individuals. This unusual mechanism for generating MHC *DRB* diversity is similar to that observed in the rhesus macaque, but has not been reported in other species. MHC variation and disease associations were identified in stranded CSL, with and without urogenital carcinoma; genomic DNA was amplified with eight sequence-specific primer (SSP) pairs flanking the putative peptide-binding site, using an intercalating fluorescent dye PCR (Bowen et al. 2004).

The sample population for this study was selected from adult CSL stranding along the Pacific coast of the United States. Extensive necropsies were performed, and the

cancer status of each individual was defined based on detailed histopathological examination of multiple tissues (King et al. 2002). The class II MHC genotype was determined for both cancer positive (n=27) and cancer negative (n=22) animals, using a combination of eight genes (*Zaca-DRB.A–Zaca-DRB.H*) (Bowen et al. 2002, 2004).

The *Zaca-DRB* genotype of 49 California sea lions was examined using previously developed SSP methodology (Bowen et al. 2004). DNA was extracted from hind-flipper skin punches, using standard protocols (DNeasy, Qiagen, Valencia, Calif.), and the presence or absence of each SSP-derived sequence type determined using PCR with an intercalating fluorescent dye. A series of eight SSP-based PCR reactions was performed on each individual. Each reaction contained 500 ng DNA in 25-µl volumes with 20 pmol SSP, Tris-Cl, KCl, $(NH_4)_2SO_4$, 2.5mM $MgCl_2$ (pH 8.7), dNTPs, HotStar *Taq* DNA Polymerase (Quantitect SYBR Green PCR Master Mix, Qiagen), and 0.5 U uracil-*N*-glycosylase (Roche, Indianapolis, Ind.). Amplifications were performed in an iCycler (Bio-Rad, Hercules Calif.), under the following conditions; 2 min at 50°C, followed by 15 min at 95°C; and 35 cycles at 94°C for 30 s, 58°C for 30 s; and 72°C for 30 s; with a final extension step of 72°C for 10 min. Reaction specificity was monitored by melting curve analysis, using a final data acquisition phase of 60 cycles at 65°C for 30 s, and verified by direct sequencing of randomly selected amplicons.

Associations among MHC genotype, gene multiplicity, and presence of cancer were examined by logistic regression. In comparing the genotype of stranded animals with and without cancer, the presence of the MHC class II locus, *Zaca-DRB.A*, strongly associated with an increased risk of cancer in the stranded sea lions (odds ratio = 3.64, 95% confidence interval = 1.11–11.97, Table 1). While associations between cancer and the other *Zaca-DRB* loci were not evident, increased sample size would strengthen confidence in these observations. No relationship could be identified between the total number of unique *DRB* genes and the presence of cancer in an individual. In view of the fact that CSL class II MHC diversity is generated by selective combinations of genes drawn from a large pool of available loci (Paul 1999), these results indicate that class II MHC gene multiplicity is not a factor in conferring susceptibility to this disease (odds ratio = 1.67, 95% confidence interval = 0.91–3.088).

While additional genetic, environmental, and/or pathogenic factors may be involved in this disease, a pattern of association between the presence of specific class II MHC genes and cancer risk emerges from this study. This is the first established relationship between MHC and cancer in wildlife, although similar links between MHC and infectious disease have been reported in Soay sheep (Paterson et al. 1998).

The phenomenon of class II MHC genes conferring susceptibility to cancer is well established in humans (Daniilidis et al. 1997; Dorak et al. 1999; Ferrera et al. 1999; Lin et al. 2001), yet the potential mechanisms underlying this process are unknown. It is also unclear what keeps these demonstrably harmful genes from being eliminated by natural selection (McClelland et al. 2003). As contaminants and herpesviruses have both been linked with cancer in marine mammals (Lipscomb et al. 2000; King et al. 2002; Martineau et al. 2002), further studies are needed to determine whether the MHC–cancer association observed in this study is influenced by additional environmental or pathogenic factors.

Table 1 Frequency of gene locus (*Zaca-DRB-A–Zaca-DRB-H*) and genotype (n=19) in stranded cancer-positive and -negative California sea lions

Zaca-DRB locus or *Zaca-DRB* genotype	Stranded cancer positive (n=27)	Stranded cancer negative (n=22)
Locus		
-"*Zaca-DRB.A*[a]	17	7
-"*Zaca-DRB.B*	20	13
-"*Zaca-DRB.C*	19	15
-"*Zaca-DRB.D*	19	16
-"*Zaca-DRB.E*	27	22
-"*Zaca-DRB.F*	27	22
-"*Zaca-DRB.G*	18	14
-"*Zaca-DRB.H*	27	22
Genotype		
-"*ABCDEFGH*	5	1
-"*ABCDEFH*	2	0
-"*ABCEFGH*	2	0
-"*ABCEFH*	1	0
-"*ABEFGH*	1	0
-"*ABEFH*	1	1
-"*ACDEFGH*	2	0
-"*ACDEFH*	1	2
-"*ACEFGH*	1	2
-"*ACEFH*	1	1
-"*BCDEFGH*	2	4
-"*BCDEFH*	0	1
-"*BCEFGH*	0	1
-"*BDEFGH*	3	2
-"*BDEFH*	3	2
-"*BEFGH*	0	1
-"*CDEFGH*	1	3
-"*CEFGH*	1	0
-"*DEFH*	0	1

[a]The presence of the MHC class II locus, *Zaca-DRB-A*, was associated with an increased risk of cancer in the stranded sea lions (odds ratio = 3.64, 95% confidence interval = 1.11–11.97, P=0.03)

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