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Limited MHC polymorphism in whales

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Abstract. Little is known about disease and genetic variation in aquatic mammalian species such as whales. In this paper human HLA class I and class II probes were used to study major histocompatibility complex (MHC) genes from two species of whale: Fin (*Balaenoptera physalus*) and Sei (*B. borealis*). Stronger signals were obtained on whale than on equivalent concentrations of mouse DNA. Evidence was obtained for several *DRB*-related genes, a *DNA* gene, one *DQA* gene, and multiple class I genes in whales. Interestingly, the whale genes, from the small panel studied, were less polymorphic than those of humans or mice. The aquatic environment of this mammalian species may be a unique factor in shaping its immune response through the MHC.

Introduction

Extreme polymorphism is a hallmark of the major histocompatibility complex (MHC) class I and class II products in several species, including mice and humans (Klein and Figueroa 1986). These glycoproteins play a crucial role in T-cell recognition of both self and foreign antigens and hence in immune protection of the species (Klein 1986). Polymorphism of MHC products may be a consequence of natural selection, providing protection against an evolving spectrum of new or altered pathogens, although this notion is much debated (Bodmer 1972, Streilein et al. 1984, Klein 1986, Klein and Figueroa 1986, Andersson et al. 1987, Klein 1987). Interestingly, the MHCs of a few mammalian species are relatively non-polymorphic, examples being the cheetah and class I genes of the Syrian hamster (Phillips et al. 1978, Darden and Streilein 1984, Streilein et al. 1984, O'Brien et al. 1985). In the mole rat, the class II *DR* loci, which are of major importance in other species, appear to have been lost altogether, and their functions may have been replaced by the *DP* loci (Nižetić et al. 1987, Schöpfer et al. 1987). Interestingly, this species also contains an expanded complement of class I genes (Vincek et al. 1987).

We decided to examine a marine mammal, the whale, to see if its unusual environment had affected maintenance of these genes and to try to gain some insight into the relevance and generality of MHC polymorphism. Restriction fragment length polymorphism (RFLP) analysis was performed using human probes on whale DNA from blood samples that were collected and stored in Iceland several years ago. This research did not pose any threat to whale stocks (Collins 1985, Swinbanks 1985, Walgate 1985).

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Materials and methods

DNA samples. High relative mass genomic DNA was prepared from 5-ml whale blood samples which had been collected in 10 m*M* ethylenediaminetetraacetate (EDTA) and stored at −20 °C; cells were resuspended in 5 ml of phosphate-buffered isotonic saline. Forty-five milliliters of triton-sucrose was added (containing 1% triton, 10% sucrose, 5 m*M* $MgCl_2$ in 10 m*M* Tris/HCl buffer pH 7.5), and the mixture was centrifuged gently for 20 min. The pellet was resuspended in 5 ml of 75 m*M* NaCl, 25 m*M* EDTA. Proteinase K (200 μg/ml) and RNase /15 μg/ml and sodium dodecyl sulfate (SDS) (0.5%) were added and incubated at 37 °C for 3 h. After phenol extraction and ethanol precipitation the DNA was recovered with a glass hook and resuspended in 10 m*M* Tris 1 m*M* EDTA, pH 8.0. The panel of blood samples was drawn from seven individual Fin whales from the Icelandic coast on two different seasons (1985 and 1986) plus two from Spanish waters. The five Sei whale samples were all from the same season.

Southern blotting. DNA was digested with *Eco* RI to completion, and 10 μg samples were electrophoresed on 0.8% agarose gels. Southern blotting onto Hybond-N membranes (Amersham United Kingdom) was essentially according to standard protocols. Probes were radioactively labeled by hexamer priming (Feinberg and Vogelstein 1983) and were added at a concentration of 5×10^5 dpm/ml. Hybridization was carried

out at 65 °C in 6 × standard sodium citrate (SSC) overnight. Washing was at 65 °C in 6 × SSC, 0.1% SDS. The blots were then exposed to Kodak X-AR5 film for approximately 3 days.

Results

Detection. The first experiment was to test whether whale genes could be detected with human HLA probes. As shown in Figure 1, the three probes that were used, DRB, DNA and DQA, gave stronger signals on whale DNA in Southern blots than on equivalent concentrations of mouse DNA. This would be consistent with a greater evolutionary distance between mouse and man than between whale and man, or with different rates of sequence divergence between the species. Figure 1 provides additional information concerning organization of the whale MHC. For instance, panel 1 indicates that there are probably several *DRB*-related genes. Panel 2 shows that the DNA gene is conserved in whale, as it is in certain other species such as sheep, although it has been lost from the mouse *H-2* complex (Widera and Flavell 1985, Steinmetz et al. 1986, Scott et al. 1987). In man the *DNA* gene gives rise to a mRNA, although no protein product has been detected and its function is in doubt (Trowsdale and Kelly 1985). The third panel on Figure 1 shows that whales have only one *DQA*-related gene, as does mouse. This is consistent with the notion that duplication of the primordial *DQ* genes, to give rise to *DQA1* and *DQA2*, took place relatively recently, attested by the fact that the two sequences are 94% related in humans (Auffray et al. 1984). Further evidence of this is provided by cattle, which are polymorphic for the number of *DQA* genes and have either one or two per haploid genome (Andersson and Rask 1988).

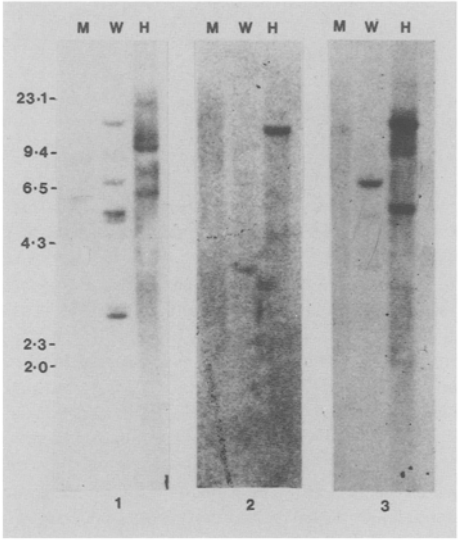

Fig. 1. Southern blots comparing mouse (M), whale (W), and human (H) DNA. Whale DNA was a Fin whale sample and human DNA was from the DR4 lymphoblastoid cell line Preiss (Speilman et al. 1984). Probes were as follows: **panel 1**, DRB (MAβ20) – a full-length DRB cDNA from the DR7-homozygous cell line MANN (Speilman et al. 1984); **panel 2**, DNA cDNA probe PGDZ1, a full-length DNA probe from the DR2 cell line PGF (J. Young and J. Trowsdale, unpublished data); **panel 3**, a DQA genomic fragment 10-8 (Speilman et al. 1984)

Polymorphism. DNA from two species of whale, Fin (*Balaenoptera physalus*) and Sei (*B. borealis*) was used to assess polymorphism of class II genes. Figure 2 shows the results using four α chain probes, DPA, DNA, DQA, and DRA, on blood from several examples of the two species. The DPA probe gave only faint bands, which suggest that *DPA*-related genes may be missing, as they are in mice (Widera and Flavell 1985, Steinmetz et al. 1986). The weak bands may represent cross-hybridization with other α chain sequences. Alternatively, *DPA* may have diverged more than the other α chain genes. The three other probes gave strong nonpolymorphic bands on both species of whale. It is not surprising that *DNA* and *DRA* gave constant bands, since these genes are nonpolymorphic in man. However, *DQA* exhibits considerable variation at the RFLP level in man, as does its equivalent, A_α, in mouse, so it is striking that the gene gives a constant pattern in whales (Auffray et al. 1984, Andersson and Rask 1988). One constant weak band of about 5 kb was observed with all α chain probes and may represent an α chain gene not represented in humans or mice. There is evidence for novel class II α chain genes in both rabbits (*DN*α; T, Kindt, personal communication) and cattle (*DY*α; Andersson et al. 1988).

Similarly, as shown in Figure 3, the DRB probe produced a constant pattern within a species of whale, although the patterns differed between the two species. This result is particularly significant since DRB (E_β in mouse) is usually highly polymorphic, and any random sample of six individuals would exhibit widely different patterns with a single enzyme (Trowsdale et al. 1983, 1985). To confirm this finding we have performed the analysis with another enzyme, *Pst I*, which similarly gave a relatively constant pattern (Fig. 3, panel B). A set of bands from 4.6 to 5.1 kb showed some variation in both Fin and Sei whales with *Pst* I, although the strongest bands were constant in all of the samples. Similarly, the DQB probe, which is highly polymorphic in man, produced a simple two-band pattern on the whale samples. One may conclude that in these two species of whale the detectable class II genes are probably less polymorphic than those of humans or mice.

Class I. Finally, we examined class I genes with a probe that detects multiple, related human or mouse class I sequences. As shown in Figure 4, a set of about 15 bands was obtained, indicating that there may be a similar number of class I genes in whales as in humans but less than

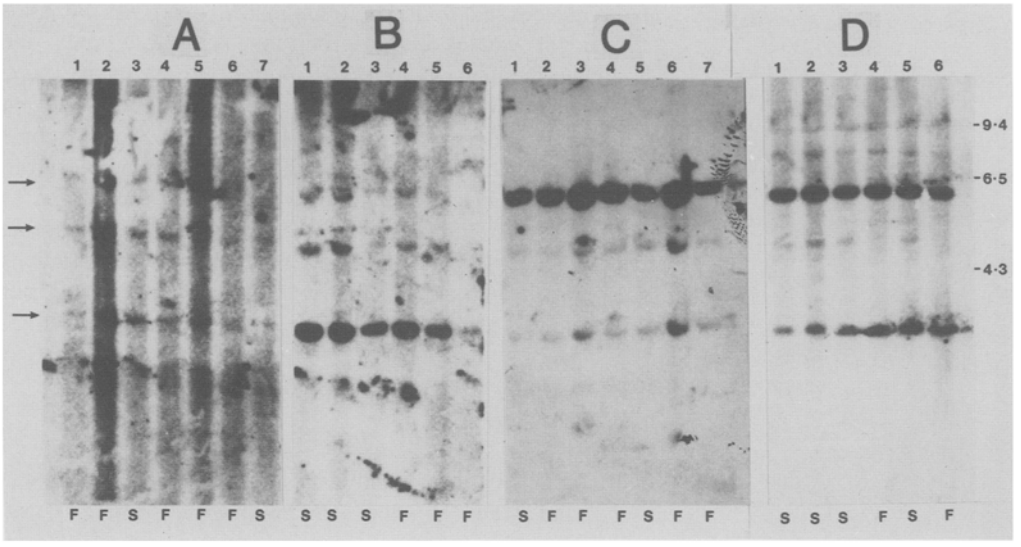

Fig. 2. Examples of Fin (F) and Sei (S) whale DNA cut with *Eco* RI, blotted and probed with DPA (**panel A**: the probe was a full-length DPA cDNA clone) (Trowsdale et al. 1985), DNA (**panel B**: for probe see Fig. 1), DQA (**panel C**: probe from Auffray et al. 1984, and DRA (**panel D**: Lee et al. 1982). Weak DPA bands are indicated by *arrows*

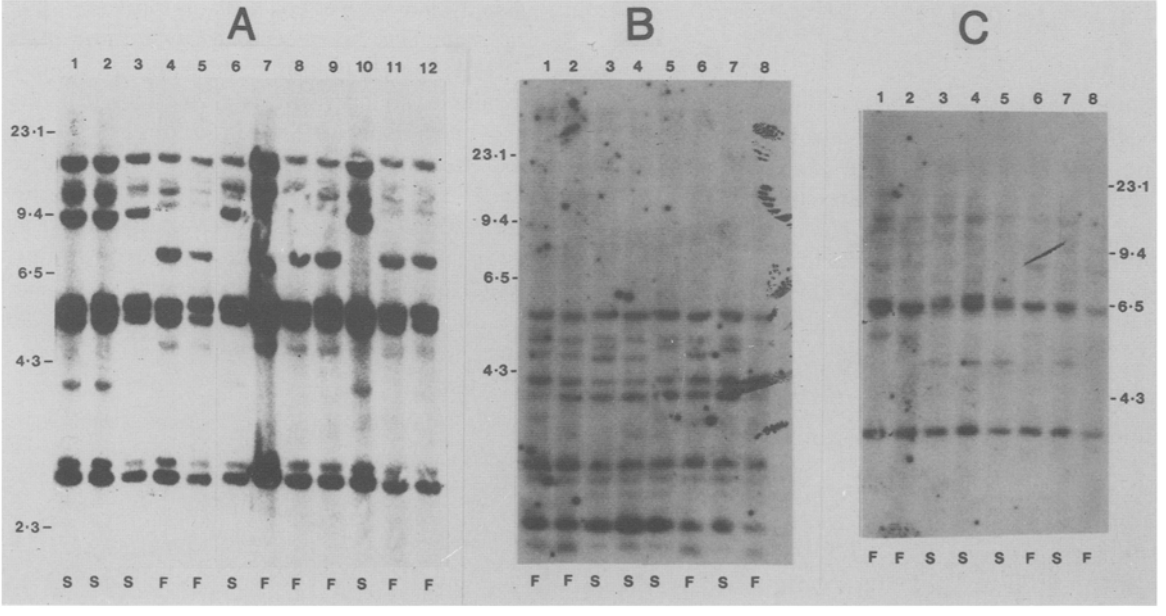

Fig. 3. Southern blots of *Eco* RI-cut whale DNA samples probed with a DRB cDNA probe (**panel A**, *Eco* RI; **panel B**, *Pst* I) and a DQB probe (**panel C**, *Eco* RI). For details see previous figures

in mice (Orr 1983). Extreme variation in the number of these genes has been noted before, some species having about 50 members and others, such as the pig, possessing only six per haploid set (Klein 1987). Patterns for the two whale species differed, although they were relatively constant within a species. Variation was noted in bands at 8.8 and 9.4 kb (Fig. 4). This indicates that one or more whale class I genes may be polymorphic, although the patterns observed were less variable than those obtained from any random samples of human DNA (Orr 1983). Protein analysis, preferably by isoelectrofocusing, would be useful to determine whether this variation is a property of the expressed products.

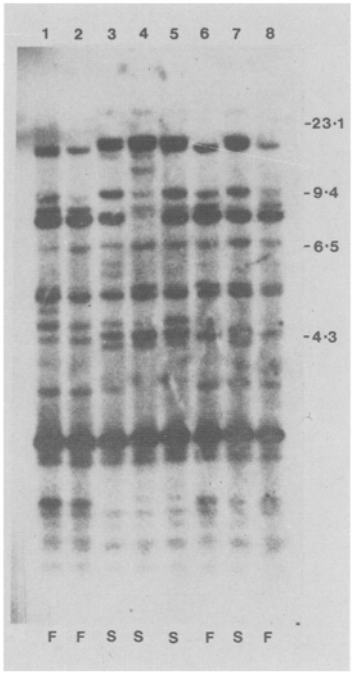

Fig. 4. Southern blots of *Pst* I-cut whale DNA probed with an HLA class I probe (Sood et al. 1981). For details, see previous figures

Sampling. Seasonal migration of whales is generally accepted, although little direct evidence is available. In the North Atlantic, they migrate north in the summertime to the feeding ground and south in the wintertime to the alleged breeding ground. They probably come in herds to the feeding grounds, but not necessarily to the same feeding ground each year (Sigurjonsson 1985, Donovan 1986, Arnason and Spillaert, unpublished data). As far as is known, whales tend to group into very large herds, and even though we took the precaution of using blood obtained on different years, it is difficult to rule out sampling from the same herd each time. To overcome these objections we repeated the analysis on samples from the same species obtained in waters off Spain, and as shown on Figure 5, similar results were obtained. The fact that the class I and DRB probes exhibited minor variations between individuals supports the contention that our samples are not from a single totally inbred population.

Discussion

In conclusion, this study has shown that characterization of the MHC in whales is feasible with human DNA probes; that, with the exception of DP and the low numbers of class I genes, whale MHC is not radically different from man; and that there is overall rather limited variation, particularly at class II loci. The number of whales in this study was of necessity quite small, and the issue of the low level of polymorphism is strongly suggested from this study but not yet conclusively proven.

Several arguments have been put forward to explain the lack of MHC polymorphism in other species, including, for example, founder effects, evolutionary bottlenecks, or an uncommunal life style (Phillips et al. 1978, Darden and Streilein 1984, Streilein et al. 1984, O'Brien et al. 1985, Klein and Figueroa 1986, Klein 1987). One idea put forward for the lack of class I variation in Syrian hamsters was its solitary existence, but the finding of extensive MHC class I polymorphism in the mole rat,

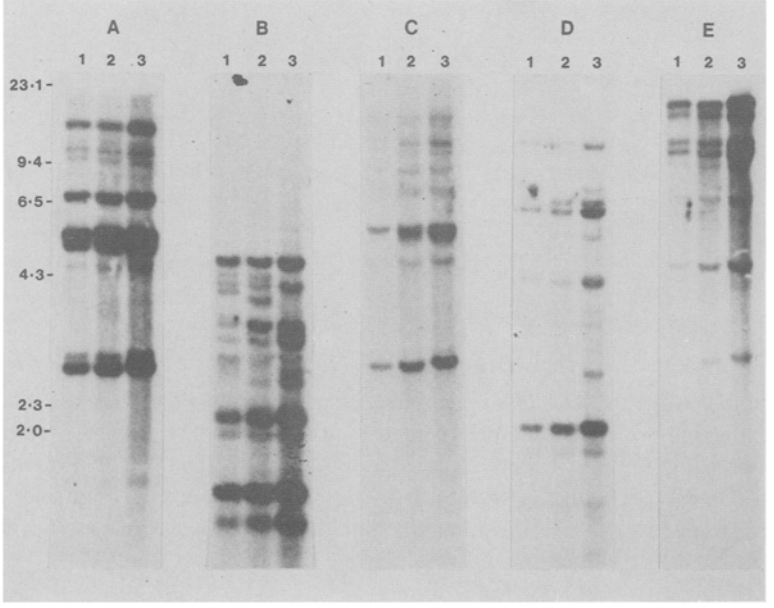

Fig. 5. Comparison of Fin whale samples from the coast of Spain with Icelandic whales. Each panel contains two samples from different whales caught off the Spanish coast (*tracks 1* and *2*) and one from Iceland for comparison (*track 3*). **Panels A** and **B**, DRB probe with *Eco* RI and *Pst* I, respectively; **panel C**, DQB probe, *Eco* RI; **panel D**, class I probe, Pst I; **panel E**, class I probe, *Eco* RI. For details, see previous figures

another solitary species, did not support this argument (Nižetić et al. 1987). However, it was reported recently that Balkan mole rats apparently do show limited polymorphism of both classes of MHC genes (Nižetić et al. 1988). The aquatic environment obviously imposes much less contact in whales than in most other mammalian species, and close contact may be responsible for the spread of most mammalian diseases. Since disease resistance is most likely the force behind MHC polymorphism, the selection pressure for polymorphism may not be so pronounced in marine mammals compared to their land-dwelling relatives.

The genetic diversity within whale species will be of considerable interest in the future, particularly in discussions over threats to some whale species by commercial whaling (Collins 1985, Swinbanks 1985, Walgate 1985). Little is known about cetaceans at the DNA level although their karyotypes are remarkably similar ($2n=44$) (Arnason 1981). The use of highly repetitive DNA probes also points to homogeneity between the species, even between odontocetes (toothed whales) and mysticetes (whalebone whales) (Arnason et al. 1984). We hope that the experiments presented in this paper may eventually be of benefit to cetaceans as well as to certain other mammals.

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