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Genes of the major histocompatibility complex and the evolutionary genetics of lifespan

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Abstract

Mice that presumably differ just in the major histocompatibility complex (MHC) chromosomal region provide the best evidence that MHC genes affect lifespan. Further evidence is that MHC region genes in some cases are known to influence reproduction, growth, and development. Moreover, MHC genetic associations with disease are well documented. This paper summarizes and defines aspects of the molecular biology, cellular function, and evolution of MHC genes (with special emphasis on the polymorphic MHC class I and II genes) which are important in aging, and attempts to integrate these into an evolutionary genetic perspective of senescence. It is suggested that MHC genes provide a mammalian paradigm for the genetics of lifespan because of their intra- and interspecies diversification, evolutionary selection, and age-specific effects.

Introduction

A decrease in the power of natural selection with advancing age is an evolutionary explanation for senescence that is substantiated in part by artificial selection with *Drosophilia* (Rose & Charlesworth, 1980; Rose, 1984; Charlesworth, 1990). Mutation accumulation and antagonistic pleiotropy are related theories which embody the notion of reduced selection for reproductive fitness at later ages (Charlesworth, this volume). Mutation accumulation suggests that heritable mutations may be neutral with respect to reproductive success but may be causative in age-associated pathologies. Antagonistic pleiotropy, on the other hand, asserts that some mutations which have late-age deleterious effects may actually be selected for because they impart increased fitness. The disposable soma theory is complementary to both mutation accumulation and antagonistic pleiotropy in that it describes the evolution of senescence in terms of the physiological costs of maintaining germ line versus somatic cells (Kirkwood, 1977; Kirkwood & Rose, 1991). However, loci which fit predictions of these theories have not been well documented in mammals.

There is direct experimental support for an influence of major histocompatibility complex (MHC) genes on aging in mammals (Smith & Walford, 1977; reviewed in Finch, 1990, and Walford, 1990). The MHC is thus one of only a few chromosomal regions which are known to affect mammalian maximum lifespan disbarring loci involved in genetic diseases that drastically decrease lifespan (and reproductive fitness). In this paper, the biology and evolution of MHC genes, particularly class I genes, will be discussed in relationship to aging. The major contention of this treatise is that many features of MHC class I genes are in accord with evolutionary theories of aging, especially antagonistic pleiotropy. MHC class I genes evolve under the intense selective pressure of pathogens and therefore can significantly increase fitness, yet MHC class I genes are associated with diseases many of which have late-age onsets (i.e. the dis-

eases minimally affect fecundity). Moreover, MHC class I genes influence reproductive schedules and developmental rates. MHC genes therefore may offer a real-life example of antogonistic pleiotropy and provide a model with which to compare other loci that alter the rate of aging in mammals once they are better defined.

A molecular and functional description of the MHC and MHC class I genes will precede a review of the evidence that MHC genes influence rates of development and aging. A case will be made for a role of MHC class I genes in these phenomena. Mechanisms by which MHC class I genes might influence senescence then will be described as quantitative or qualitative and putative examples of each will be given. Various aspects of the phylogeny and evolutionary history of MHC class I genes will then be related to the evolution of differential rates of aging. A summary will point to future directions and experimental approaches.

Molecular and functional description of MHCs

Structure and diversity of MHCs

The MHC of mice is called the *H-2*; in humans the MHC is refered to as HLA. The MHC may be broadly defined as an approximately 2 megabase chromosomal region roughly demarcated by *pgk-2* and *H-2K* in mice and PGK2 and DQA in humans (Klein, 1986). The organization of genes in the *H-2* (Fig. 1) is very similar to that of the HLA with a few notable exceptions. For one, all HLA class I genes are telomeric to class II loci. In fact, *Mus* and *Rattus* appear to be unique in having class I loci centromeric to the class II region (Klein & Figueroa, 1986; Koller *et al.*, 1989). In addition, there is a greater number of class I protein-encoding loci in the *H-2* versus HLA. Importantly, orthologous relationships between *H-2* and HLA class I genes are not discernible in contrast to the majority of other MHC loci in the two species.

Historically and functionally, MHC class I and II loci are the hallmarks of MHCs (Klein, 1986). Class I antigens are composed of the class I gene-encoded heavy chain in noncovalent association with β_2-microglobulin (β_2m) and present peptides derived from cytosolic pools most often to CD8+ (cytotoxic) T cells. Class II antigens are heterodimeric complexes consisting of MHC-encoded α and β chains. Peptides primarily from lysosomal or endosomal compartments are presented to CD4+ (helper) T cells by class II antigens (Yewdell & Bennink, 1990). Class I and II antigens differ in their tissue distribution as well - 'classical' class I antigens (H-2K, D, and L; HLA-A,B and C) are ubiquitously expressed in moderate to high levels in all somatic cells except for those in the brain, where expression is low but detectable (David-Watine, Israel & Kourilsky, 1990). In contrast, class II expression is restricted primary to cells of monocyte and lymphocyte lineages.

There are over 60 genes in the *H-2*. In addition to the structurally and functionally homologous class I and II genes, the MHCs of humans and mice contain a host of other genes. Those of known function residing between class I and II are commonly referred to as class III and include components of the complement pathway, steroid hydroxylase, tumor necrosis factor α and β, and heat shock proteins. Elsewhere in the MHC reside genes encoding a transcription factor (*Oct-3*; Uehara *et al.*, 1992), some components of the antigen processing pathway (discussed below), and a variety of transcribed loci with unknown function (Abe *et al.*, 1988; Spies *et al.*, 1989; Hanson & Trowsdale,

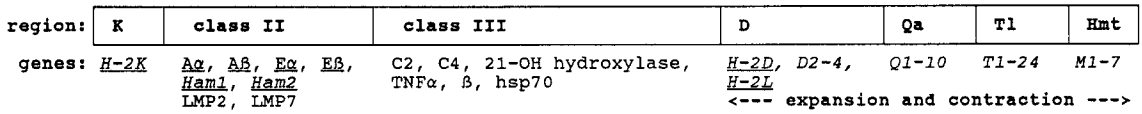

Fig. 1. Gross organization of the *H-2* complex. Commonly referred to regions of the H-2 are boxed and shown with the telomeric end at the right. Some of the genes in each region with known function are listed below the boxed regions and those displaying functionally significant polymorphism are underlined. The arrow below *D – Hmt* is meant to show the highly variable number of class I loci between haplotypes in these regions.

1991). Except for the transporters associated with antigen processing, evidence of functionally significant polymorphism in these nonclass I or II loci, a necessary requisite for loci to be implicated in the observed MHC effects on senescence, is sparse, so these other genes will be excluded from further discussion.

Extensive diversity among MHCs is exhibited both within and between species. Haplotypic (intraspecific) variation is apparent at two levels. First is the astounding variation between alleles of class I and II antigens. For example, to date over 50 serologically defined HLA-B alleles have been detected. The actual number of alleles is much greater, since in several cases more than five alleles differing in antigen binding region sequences share the same serological specificity. Haplotypic variation is rarely limited to a single locus - each haplotype is composed of assortments of alleles at several loci. Second, haplotypic variation entails, especially for class I genes, variation in the number of loci. In mice and to a lesser extent in humans, there is extensive polymorphism in nonclassical class I gene regions such that *H-2* haplotypes may differ by two-fold in the number of nonclassical class I genes (Stroynowski, 1990; O'Neill *et al.*, 1986). Both levels of haplotypic variation may be involved in influencing senescence as discussed below.

Multiformity of MHCs among species is most evident in comparisons of class I genes and the organization of class I gene regions. Sequence variation between *H-2* and HLA class I genes is so great that orthologous loci are difficult, if not impossible, to discern (Kindt & Singer, 1987: Lawlor *et al.*, 1990). This most likely reflects the role of chromosomal expansion and contraction in the diversification of class I genes (discussed below). Orthologous HLA and *H-2* class II loci are more apparent because these genes, unlike class I genes, probably evolved through direct descent (Lundberg & McDevitt, 1992). As with haplotypic variation, there are large differences between species in the number of class I antigen-encoding loci ranging from fifty to sixty in *Peromyscus leucopus* and *Rattus* (Crew *et al.*, 1990; Jameson *et al.*, 1990) to seven in minature swine (Singer *et al.*, 1987). The proposition here is that MHC class I gene diversity between species mirrors the profound interspecific diversity of life history patterns.

Functions of MHC genes

As alluded to throughout the above discussion, MHC class I and II antigens have a primary role in the immune system via their presentation of antigens to T cells. In fact, the whole MHC has been likened to a bacterial operon because of the role of individual, nonhomologous loci in antigen presentation and overall immune function (Robertson, 1991). In this regard, the MHC appears to be unique among eukaryotic chromosomal regions. For instance, several proteins involved in the pathway leading to presentation of virally encoded antigens by MHC class I molecules are encoded within the MHC. The initial step in viral antigen presentation is proteolysis of viral peptides by large multifunctional proteosomes comprised in part of low mobility proteins, LMPs. LMP2 and LMP7 genes have been mapped within the class II region of mouse and human MHCs (Brown, Driscoll & Monaco, 1991; Glynne *et al.*, 1991). Presently, the exact role of LMP2 and LMP7 in the proteosome as well as the degree to which they might influence proteolytic specificity is uncertain. Further down the antigen presenting pathway, two subunits of a transporter protein which putatively translocate proteosome-derived peptides into the endoplasmic reticulum are likewise encoded within the MHC (Monaco, Cho & Attaya, 1990; Trowsdale *et al.*, 1990; Spies *et al.*, 1991; Deverson *et al.*, 1991). In this case, biologically relevant polymorphism with respect to peptide specificity has been recently described (Powis *et al.*, 1992). Finally, the highly polymorphic MHC class I proteins (which originally defined the *H-2*) bind peptides and β_2m in the ER and are transported to the cell-surface where T cells recognize the peptide in association with MHC class I antigen (Yewdell & Bennink, 1990).

The primary function of the MHC class I and II antigens as antigen presenting molecules is manifest in the localization of polymorphic residues to protein domains associated with antigen binding and T cell receptor (TcR) recognition as shown by crystallographic studies (Bjorkman *et al.*, 1987a, 1987b). This is exemplified in Figure 2 - the α1 and α2 domains contain over 75% of the amino acid variation between *H-2D* genes of three haplotypes studied for lifespan (see below). The function of nonclassical class I genes (those in the *Qa*, *Tl*, and *Hmt* regions, Fig. 1) is not clear, though there are indications that some nonclassical class I

```
domain:     [        α1        ][         α2          ][α3][TM,CY]
consensus ATPVAGENT-VREGEINS-TRKIWSVG--CMQRRAR-RTRY--ARG-H-G-MMAA-
    b     ---e-es-kyap---kqwsngalq-l-wlr----shykeh-kn---sry-mkr--r
    k     va-ev-s--d--------d--e-----we--lkh--d----el-hvs-yvv--ttp
    r     --r----d-v--ven--in-s---f-egq-t-----k--lhkne--a-h-m----q
          52233445567778899911111111111111111111111112222223333333
           06352845934907047000112222334666677888999900278812334 56
                              14739137818525894901735781510468356801
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Fig. 2. Comparison of *H-2D* genes from three *H-2* haplotypes which have been studied for lifespan and fecundity. *H-2D* genes from the b, k and r haplotypes (GenBank) were translated and compared to a consensus derived from six *H-2D* genes. Out of 362 positions, 56 were variable between these three haplotypes. Only the variable positions are shown. The position number is at the bottom of the comparison (read vertically). The domains corresponding to the sequences shown are given at the top of the comparison (TM,CY denotes transmembrane and cytoplasmic domains).

proteins may present specific antigens and serve as restriction elements a la the classical transplantation antigens (Stroynowski, 1990; Ito *et al.*, 1990; Wang *et al.*, 1991). As such, polymorphic residues are less concentrated in the α1 and α2 domains.

The sphere of influence of MHC class I genes is not limited to the immune system. Reports that MHC class I proteins are co-immunoprecipitated with receptors for insulin and leutenizing hormone (LH) have led to the consideration that class I antigens act as modulatory subunits with peptide hormone receptors (Verland *et al.*, 1989; Solano *et al.*, 1988). Importantly, MHC class I polymorphism may be manifest in some of these interactions (LaFuse & Edidin, 1980). Judging from the three-dimensional structure of MHC class I molecules (Bjorkman *et al.*, 1987a, 1987b), interaction of MHC class I molecules with other integral membrane proteins on the same cell might occur through the α3 domain. Thus, polymorphism outside of the antigen and TcR binding regions may be relevant to peptide hormone receptor interactions with MHC class I molecules and therefore important in altering reproductive patterns as described below.

The role of MHC-encoded genes in longevity

Evidence from H-2 congenic and recombinant inbred mice

The most direct evidence that the MHC influences the rate of aging is that mice that presumably differ in just the *H-2* have significantly different lifespans (Smith & Walford, 1977). [The qualifier 'presumably' is necessary since it is possible that during the course of breeding other differences between regions outside of the MHC may have arisen]. These studies, originally described in 1977, were repeated ten years later with nearly identical results (R. Walford, pers. comm.), underscoring the robustness of the original observation and the phenotypic stability of the genetic differences. Genetic analyses by E. Yunis and colleagues (Yunis *et al.*, 1984; Watson *et al.*, 1990; Yunis & Salazar, this volume) also have suggested a role of MHC loci in aging. Meyer, Armstrong and Warner (1989) found only a trend toward *H-2* effects on lifespan. However, males of the haplotypes tested in that study also showed only weak differences between lifespan in the studies by Smith & Walford (1977).

Where examined, the $H\text{-}2^r$ haplotype is notable for its superiority with respect to biomarkers of aging (Walford, 1990; Table 1). Lifespan for B10.R111 ($H\text{-}2^r$) males and females is longer than all other haplotypes on the B10 background. B10.RIII females have more delayed reproductive senescence when compared to b and k haplotypes (Lerner *et al.*, 1988; Lerner & Finch, 1991; summarized in Table 1). The concordance between lifespan and reproductive senescence (age at last litter) is striking. Furthermore, age-related declines in unscheduled DNA synthesis, removal of DNA adducts, mitogen-induced lymphocyte proliferation, and mixed function oxidase induction are lessened in B10.RIII relative to B10 and B10.BR mice (Hall, Bergmann & Walford, 1981; Koizumi, Walford & Hasegawa, 1987; Walford, 1990; and R. Walford, pers. comm.).

H-2D genes are the only orthologous loci among the haplotypes examined gerontologically for which DNA sequences are available. Variable residues in the protein sequences of *H-2D* antigens are

Table 1. Comparison of lifespan and fecundity of three *H–2* haplotypes on the B10 background.

Haplotype	Lifespan[1] (weeks)		Fecundity[2]			
	Male	Female	# of litters	Age (weeks) at last litter	# of pups/ female	Pups/litter
$H\text{–}2^k$	149 ± 1.1	161 ± 2.1	7.5 ± 0.5	44.4 ± 2.1	43 ± 3	5.8 ± 0.3
$H\text{–}2^b$	155 ± 0.4	148 ± 1.2	5.4 ± 0.5	37.1 ± 2.4	34 ± 3	6.4 ± 0.3
$H\text{–}2^r$	170 ± 0.8	165 ± 1.0	7.8 ± 0.5	45.3 ± 2.3	50 ± 3	6.7 ± 0.3

[1] Data from Smith & Walford (1977). Lifespan is the mean age of the last 10% surviving in that group which is a valid approximation of maximum lifespan. Differences between lifespans of each haploptype within each sex group are statistically significant ($p < 0.02$).
[2] Data from Lerner *et al.* (1988). Four measures of fecundity are given. $H\text{–}2^k$ and $H\text{–}2^r$ strains did not statistically differ in any of the measures. $H\text{–}2^b$ mice showed statistically significant differences from $H\text{–}2^r$ and $H\text{–}2^k$ mice in number of litters ($p < 0.05$), age at last litter ($p < 0.01$), and number of pups per female ($p < 0.01$).

shown in Figure 2 to illustrate the magnitude of polymorphism at classical class I loci and to show the complexity in pinpointing differences responsible for influencing the rate of aging (under the assumption the *H-2D* genes are responsible for the altered lifespan between *H-2* congenic lines). Note that polymorphism is more pronounced in the α1 and α2 domains, but there are four variable positions in the relatively well conserved α3 domain.

H-2 congenic mice were developed based upon variation at class I and II antigen encoding loci. Besides differences at these serologically defined loci there may be other tightly linked polymorphic loci. At present, the effects of these loci on MHC/aging relationships can not be excluded. Figure 2 therefore should not be viewed as the only gerontologically or immunologically relevant differences between haplotypes - other differences in the class II, class III, or nonclassical class I gene regions may also be responsible for MHC influence on senescence. The characterization of gross and fine MHC structure among *H-2* haplotypes by gene mapping and DNA sequencing is therefore expected to be enlightening from not only an immunological perspective, but from a gerontological point of view as well.

MHC and disease associations

HLA associations with disease have been known for over twenty years, yet the molecular mechanisms remain elusive (Tiwari & Terasaki, 1985; Bell, Todd & Devitt, 1989). Several aspects, though, are germane to the topic of the evolution of senescence. First, linkage between HLA and disease is never complete. While individuals with the HLA-B27 allele have at least an order of magnitude higher risk than non-HLA-B27 individuals for ankylosing spondylitis (AS), only 2% of HLA-B27 individuals are afflicted with AS (except in familial instances types of AS where the risk is up to 20%; Benjamin & Parham, 1990). Incomplete penetrance may be accounted for in two ways (Bell, Todd & McDevitt, 1989). 1) Serological definition of alleles does not usually resolve differences in the regions which bind peptide antigens and might be the most critical regions with regards to autoimmune disease susceptibility. In fact, some correlations approaching 100% can be identified using hypervariable regions common to several serologically different specificities (Bell, Todd & McDevitt, 1989). 2) More importantly, HLA-associated diseases are polygenic, multifactorial biological phenomena, as is senescence.

Particularly important with regards to the MHC and evolutionary theories of aging, there is often a late-age onset of HLA-associated diseases; individuals in post-reproductive years are predominantly affected (Tiwari & Terasaki, 1985; Klein, 1987). For example, increased mortality is evident in only a small portion of the AS patients (Diethelm & Schuler, 1991). Thus, fitness in an evolutionary sense may not compromised in individuals with disease susceptibility-associated HLA alleles. It therefore seems that MHC genes may be candidate loci for evolutionary theories of aging - namely, mutation accumulation and antagonistic pleitropy.

Most likely reflecting the role of MHC class I and II antigens in presenting virus encoded peptides to T cells, MHC associations are particularly evident in autoimmune diseases. For example, there

are clearly *H-2* haplotypic susceptibilities to experimental allergic encephalomyelitis (a popular paradigm of multiple sclerosis). Insulin dependent diabetes, rheumatoid arthritis, and AS also are thought to have autoimmune components and have strong correlations with certain class II and I (for AS) alleles (Tiwari & Teraskai, 1985). Besides autoimmune diseases, MHC involvement in various stages of neoplasia are also reported (Goodenow, Vogel & Linsk, 1985; Tanaka *et al.*, 1988) and there is some evidence of allelic associations.

MHC involvement in growth and development

The vast majority of literature regarding effects of MHC genes on growth and development implicates class I region genes. However, at the outset it should be made clear that MHC class I genes are not necessary for normal growth and development, as gene 'knockout' experiments indicate (Koller *et al.*, 1990; Zijlstra *et al.*, 1990). Functional, cell-surface expression of MHC class I molecules requires association with β_2m. The gene encoding β_2m was disrupted by homologous recombination in embryonic stem cells and the mice derived from these cells lacked expression of β_2m and consequently surface expression of *H-2* class I antigens (classical and nonclassical alike). They develop normally, though they are extremely vulnerable to some parasites (e.g. *Trypanosoma cruzi*; Tarleton *et al.*, 1992).

However, there is precedence for genes of the MHC to affect various stages of growth and development; in at least three instances, MHC regions encoding nonclassical class I genes have been implicated. *RT1*, the rat MHC, has regions (*RT1.G* and *C*) which probably correspond to *H-2 Tl* and *Qa* regions. Gill and coworkers (Kunz *et al.*, 1980) found that the growth regulatory control locus (*grc*) maps to the RT1.G/C region and is actually composed of two genes which affect body size and testicular development (*dw-3* and *ft*, respectively). Small rats with abnormal testes are observed in the $RT1^l$ haplotype (grc^-), which contains an approximately 70 kb deletion encompassing the *grc*. Probes derived from the *grc* region of grc^+ mice identified a homologous sequence in *Mus musculus* which was mapped to the *Tl* region (Vincek *et al.*, 1990) and at least one *grc* region is related to *H-2 Tla* region genes (Kirisits *et al.*, 1992).

Perhaps related to the *grc* locus in the rat, ectopic expression of a *H-2 Tl* region class I gene, *T18*, has remarkable effects superficially resembling delayed immunosenescence (M. Kronenberg, *pers. comm.*). Several independently derived transgenic lines harboring the *T18* gene under control of heterologous promoters exhibit delayed thymus involution compared to nontransgenic littermates. Moreover, cultured splenic T cells from these *T18* transgenic mice have greater persistence and less stringent growth factor requirements than nontransgenic controls. It is not known whether the rat ortholog of *T18* is in the *RT1 grc* region, but it is an attractive hypothesis.

The cleavage rate of of preimplantation embryos is different in B10BR ($H\text{-}2^k$) and B10 ($H\text{-}2^b$) mice, suggesting that a gene designated *Ped* (preimplantation embryo development) is linked to the MHC (Warner, Brownell & Ewoldsen, 1988). Using congenic mice that differ just in the *Qa* region, it was found that *Ped* is closely linked to the *Qa-2* antigen (Warner, Brownell & Rothschild, 1991). These studies also showed that the *Ped* gene (or other genes in the *Qa* region) significantly affect birth weight and litter size, implying that reproductive fitness can be modulated by genes in this region of the *H-2*. Warner and colleagues (Ford *et al.*, 1988; Conley *et al.*, 1988) have shown that the MHC effects on embryo development are demonstrable in pigs as well as as mice. Since the MHC of these animals contains only seven class I genes (Singer *et al.*, 1987) it may offer a more tractable system for understanding MHC influences on early developmental rates.

The differentiation of rat myoblasts to multinucleate myotubes is inhibited by antibodies against rat MHC class I antigens (Honda & Rostami, 1989). These data provide yet another example of MHC class I involvement in growth and extend the range of tissues that may be developmentally influenced by MHC class I antigens.

MHC and reproduction

In addition to the aforementioned effects on testicular development by the *ft* locus in *RT1*, effects of MHC polymorphism are evident in the reproductive physiology of mice, pigs, and chickens (comprehensively reviewed in Lerner & Finch, 1991). Table 1 shows some of the significant features of *H-2* haplotypic variation on fecundity and reproductive senescence in relation to lifespan. Further-

more, the *H-2* (together with other non-*H-2* loci) influences the length of estrous cycles (Lerner *et al.*, 1988). Lerner and Finch (1991) posit that MHC-reproductive relationships reflect interaction of MHC class I antigens with peptide hormone receptors, especially LH receptors. This is a compelling scenario that deserves more experimental attention.

Implication of MHC class I genes in aging

Several features, when taken together, lead to the hypothesis that class I genes may mediate the *H-2* regulation of senescence. MHC class I genes are ubiquitously expressed and are central to immune function, yet are implicated in nonimmume interactions as well. There are disease susceptibility correlations with both class I and II genes and the diseases, like aging, are of a multifactorial and polygenic nature. Finally, the influence of MHC class I genes on developmental rates and reproductive schedules is compelling support for class I involvement. Because class I genes do not seem necessary for normal growth and development in sterile settings, they must be viewed as modulators of aging rates rather than primary determinants.

MHC and lifespan – mechanisms

Differential effects of MHC haplotypes on senescence can be described in mechanistic terms as either due to qualitative or quantitive differences between alleles. Phenotypic variations due to primary sequence differences between alleles are from qualitative mechanisms. Variation in the relative level of expression of each allele is a quantitative mechanism. Clearly, these two possibilities are not mutually exclusive and two alleles may impart phenotypic variation by quantitative as well as qualitative mechanisms.

Quantitative variation

That naturally occurring deletions in the *grc* region of *RT1* lead to altered growth and development implies that quantitative variation in MHC gene expression has phenotypic effects on growth and development. In this case the variation is an all-or-nothing situation. Another example of quantitave variation is the overexpression of *H-2T18* in transgenic mice. The strains used for microinjection express undetectable levels of *T18* in the thymus. Thus, increasing the expression in thymocytes and cell-types that normally do not synthesize appreciable levels of *T18* has effects on a near-universal biomarker of aging in mammals, thymic involution. The effect of *Qa* region genes on pre-implantation embryo development might relate to quantitative variation at the all-or-nothing level as well, because there are large deletions in the $H\text{-}2^{k}$ (Ped^{slow}) versus the $H\text{-}2^{b}$ (Ped^{fast}) *Qa* region (O'Neill *et al.*, 1986).

Relevant to the issue of quantitative variation is the trend that innappropriate expression of MHC class I and II genes has dramatic physiological effects. For example, ectopic expression of $H\text{-}2K^{b}$ under control of the mylein basic protein gene promoter causes severe hypomyelination (Turnley *et al.*, 1991). Likewise, destruction of pancreatic β cells occurs when MHC class I antigens are overexpressed in these cells (Allison *et al.*, 1988). Regardless of the pathological mechanisms in these experimentally induced conditions, it is evident that such drastic changes in expression would decrease fecundity. However, a less severe increase in expression might be positively selected owing to better immune protection. Neurons express MHC class I antigens at very low levels and as an outcome viral infection is persistent (Joly, Mucke & Oldstone, 1991). It is reasonable to hypothesize that in some instances increased expression in neurons would be advantageous. Mutations leading to this situation might increase fitness but with detrimental consequences at later ages.

Also under the rubrik of quantitative variation should be mentioned the observed changes in MHC expression with age. Age-related increases in cell-surface *H-2* class I antigens have been reported (Sidman *et al.*, 1987; Janick-Buckner & Warner, 1990; Janick-Buckner *et al.*, 1991). Where tested, the increase in cell-surface expression parallels an increase in MHC class I encoding mRNA (Janick-Buckner *et al.*, 1991; M. Crew, unpublished data). It is not yet known whether there are differences between *H-2* haplotypes in the magnitude of the increased class I expression with age, but this aspect certainly merits consideration. In addition, studies at the mRNA level have utilized probes that cross-hybridize with all class I mRNAs. There may be drastic age-associated changes in the expression of nonclassical class I antigens that are masked by this method.

Qualitative (allelic) variation

Relationships of the MHC to various diseases are probably by and large due to qualitative (i.e. amino acid) differences between alleles rather than altered expression of an allele. For example, in HLA-B27 AS individuals versus healthy individuals there are no clear differences in HLA-B cell-surface levels (Benjamin & Parham, 1990). The same holds true for other HLA alleles associated with disease, probably without exception (Bell, Todd & McDevitt, 1989; Tiwari & Terasaki, 1985).

Likewise, if classical MHC class I antigens are involved in reproduction and developmental rates, then qualitative mechanisms may be invoked since the expression of the classical class I genes probably does not differ appreciably between haplotypes (O'Neill & McKenzie, 1980). However, as mentioned, haplotypic variation in age-related changes in MHC class I expression has yet to be thoroughly explored.

Phylogeny and evolutionary history of the MHC

Darwinian evolution entails natural selection of existing genetic variation. MHC evolution has inspired much debate, in part due to the difficulty in distinguishing the mechanisms giving rise to genetic diversity from the selection for and against variants. Pease *et al.* (1991) emphasized the importance in realizing that the mechanisms of creating genetic variation within the MHC are different from the mechanisms by which new alleles are selected and that the full range of selective forces acting on MHC evolution are not fully understood. However, the principle that unconventional mutations coupled with intense selection by pathogens are intrinsic to the evolution of MHC class I and II genes is widely supported.

Mutational mechanisms

DNA sequence comparisons have revealed that both conventional (single point) mutations and unconventional mutations such as gene conversion events (also referred to as template-directed exchange or nonreciprocal recombination) have contributed to the diversification of MHC class I and II genes (Parham *et al.*, 1989; Lawlor *et al.*, 1990; Pease *et al.*, 1991). Two types of gene conversion events are observed which are defined by the 'donor' sequence (i.e. the sequences contributing to the observed mutation): 1) intraloci (between alleles) and 2) interloci. The former is a leitmotif in HLA evolutionary histories (Kuhner *et al.*, 1991), while both are observed in the phylogeny of *H-2* class I gene (Pease *et al.*, 1991). Putative examples of interloci conversion are not limited to MHC class I genes but are also discerned in other multigene families (*c.f.* Becker & Knight, 1990; Wines *et al.*, 1991).

Variability in class I genes is predominantly localized to the regions encoding antigen-binding and TcR interacting residues. Intra- and interloci sequence exchange clearly takes part in generating this diversity. Yet gene conversion-like events may also homogenize class I genes in regions outside of the hypervariable extracellular domains (Rada *et al.*, 1990). Homogenization is thought to engender species-specific residues which are evident in comparing $\alpha 3$ to cytoplasmic domain-encoding gene sequences of rat and mouse MHC class I genes (Rada *et al.*, 1990) and in comparisons of *Peromyscus leucopus, Rattus*, and *Mus* MHC class I transmembrane domain-encoding exon sequences (Crew *et al.*, 1991). Diversification of hypervariable regions versus homogenization of more conserved domains may have consequences pertinent to nonimmune functions of MHC class I genes. Mutations outside of the antigen-binding regions may be selectively neutral or even negative, but may be outweighed by the benefits of diversity in the antigen-binding region.

For MHC class I genes, an additional level of diversification is apparent which relates to the expansion and contraction via unequal crossover of class I loci. The classical class I antigens between species are not necessarily related by direct descent. Instead, over evolutionary time, class I gene loci vary in their capacity as antigen presenters (Hughes, 1991). Nonclassical class I loci may later be called upon to serve as a classical class I antigens (Wang *et al.*, 1991). Such is the inference from examination of class I genes in New World primates. The classical class I loci in cotton top tamarins appear to have descended from loci homologous to certain HLA nonclassical class I genes (Watkins *et al.*, 1990). Thus, keeping a battery of unused class I genes might be advantageous in future environmental settings though, as described above, the variability in nonclassical class I gene

regions may affect developmental growth rates and reproductive schedules.

Lastly, there are conflicting views concerning the rate of mutation within the MHC. On the one hand, at least one allele, *H-2K*^{b}, apparently has a higher mutation rate than the rest of the genome (Nathenson *et al.*, 1986). However, there is little evidence that this is the case in other MHC genes (Flaherty, 1988). The high degree of polymorphism shown by MHC class I and II genes may be accounted for by intense selection for variation rather than increased mutation rates. Further, intra- and interlocus exchange have similar advantages over single base substitutions. Purifying selection should remove single base substitutions more than gene conversion generated mutations, since sequences within coding regions are exchanged to equivalent positions in homologous genes. As Howard (1992) points out, because of this the frequency of gene conversion need not be higher than conventional single base substitutions.

Selective pressures

Since MHC class I and II antigens bind foreign peptides for presentation to T cells, resistance to pathogens has long been proposed as a selective force in maintaining diversity in MHC genes (Doherty & Zinkernagel, 1975). Hughes and Nei (1988) showed that in allelic comparisons of MHC class I genes, nonsynonymous substitutions prevailed in the antigen-binding regions of class I antigens but not elsewhere in the extra-cellular domain-encoding portions of the genes. This suggests that polymorphism is positively selected for and that MHC genes probably evolve by overdominant selection of new alleles.

Recently, the first example of increased resistance to an infectious agent conferred by HLA alleles was discovered (Hill *et al.*, 1991), thereby substantiating previous intuitions. In a survey of HLA alleles in areas hyperendemic with malaria, usually rare class I (Bw53) and II (DRB*1302-DQB*0501) alleles were found to occur more frequently in these areas. Moreover, the rare alleles were highly associated with malaria resistance - the frequency of HLA-Bw53 was eight times higher in healthy individuals than in those with severe cases of malaria. These findings strongly support the role of pathogens in the evolution of MHC polymorphism.

Another side to positive selection for heterozygosity at MHC loci is found in the studies of Potts *et al.* (1991) where genotype frequencies were followed in an enclosed population of mice with known *H-2* types. That female mice preferentially mated with MHC-disparate males was obvious in the observed versus expected frequency of *H-2* heterozygotes. The *H-2* influences urinary odor and presumably this is the basis for mating preferences (Boyse, Beauchamp & Yamazaki, 1987; Yamazaki *et al.*, 1990). It seems plausible that avoidance of MHC homozygosity by odor type-determined mate selection evolved for enhanced pathogen resistance (Howard, 1991).

MHC in nonmammals

Functionally and structurally homologous molecules to mammalian MHC class I and II antigens can be discerned in all vertebrate classes. Molecular cloning and DNA sequence data have been reported for MHC class I genes of fish, reptiles, amphibians, and birds (Kaufman, Skjoedt & Salomonnsen, 1990; Hashimoto, Nakanishi & Kurosawa, 1990; Grossberger & Parham, 1992). The best studied nonmammalian MHCs (in terms of relative functional equivalence to mammalian MHCs and molecular characterization) are those from chickens and *Xenopus* (reviewed in Kaufman, Skjoedt & Salomonnsen, 1990).

The *Xenopus laevis* MHC (*Xela*) exemplifies features of nonmammalian MHCs relevant to this discussion (Flajnik & Pasquier, 1990). *Xela* encodes class I and II antigens (with each haplotype having only one detectable class I locus) and complement components as well, suggesting a genetic linkage that has persisted for over 300 million years. During amphibian development there are dramatic changes in the expression of MHC genes. Tadpoles express class II genes - adults express class I genes. Thus, while not causative of metamorphosis, developmental influences of MHC class I molecules may not be limited to mammals.

Perhaps the most relevant feature of interclass comparative analyses is that polymorphism is the rule rather than exception. This fact argues that the overall function of class I molecules is nearly identical across vertebrate classes and therefore the evolutionary selection is similar. The implication is that the evolution of aging and the MHC paradigm may be universally applied to vertebrates.

Evolution of the MHC and evolution of lifespan - comparison of rates and mechanisms

Evolutionary rates

Whether or not the mutation rate in the MHC is higher than in the rest of the genome, it seems clear that MHC class I genes evolve rapidly owing to the intense selection for variation. The recent identification of new HLA-B alleles (resulting from sequence exchange between existing alleles) in South American Indian tribes underscores this and suggests that new alleles can arise to appreciable frequencies in less than 40,000 years (Belich *et al.*, 1992; Watkins *et al.*, 1992). There are few clues as to the evolutionary rate at which mammalian senescence is altered. Extrapolating from studies in invertebrates, one can expect it also to be rapid - in *Drosophilia*, significant alteration in lifespan is observed after 15 generations with judicious selection for delayed reproduction (Rose, 1984; Rose, 1990).

Evolutionary mechanisms

Evolutionary mechanisms for altering aging rates are well developed at a theoretical level. Antagonistic pleiotropy predicts that some loci attain mutations which impart increased fitness but may be progressively more detrimental with advancing age. There is a striking similarity here to the evolution of MHC class I and II genes in that mutations may be strongly selected for by adding to protectiveness against infectious agents. However, along with this comes increased susceptibility to diseases which mainly occur in post-childbearing years. The trade-off between enhanced fitness early in life versus detrimental effects later may relate to the function of MHC class I and II genes, representing a balance between presentation of foreign peptides (imparting increased fitness) and self-peptides (resulting in old-age deleterious effects).

A requirement for evolutionary theories of aging is that there is sufficient variation for natural selection to act upon. Taking into account the large number of MHC class I and II alleles even in small populations, the MHC clearly satisfies this requirement. Furthermore, considering MHC-disparate mating preferences (Potts *et al.*, 1991), the MHC might be critical in maintaining diversity throughout the genome and therefore may indirectly influence the capacity for evolutionary changes in aging rates by maintaining variation at non-MHC loci.

In populations with high mortality rates, mutations with late-age-specific effects may equilibrate to a higher frequency than that observed in low mortality rate populations (Charlesworth, 1990). Perhaps the observed differential frequency of alleles among racial and ethnic populations reflects this theoretical prediction.

Molecular mechanisms

Molecular mechanisms that lead to alteration in life history patterns, including lifespan, are not well described. Here, molecular mechanisms by which the MHC may affect senescence have been classified as quantitative or qualitative. The latter is typified by gross deletions of MHC nonclassical class I genes. An allele at the *age-1* locus in *C. elegans* which increases lifespan by over 60% is a null allele (Johnson *et al.*, 1990), as would be genes that are deleted in some *H-2* haplotypes. Thus, quantitative mechanisms as described here for MHC genes may be broadly operative in senescence.

At the nucleotide level, the basis for qualitative variation among MHC class I genes is largely due to genetic exchange rather than substitution. Besides the aforementioned trade-off between presentation of self versus nonself peptide antigens, there may be additional physiological consequences which relate to this type of mutational mechanism. That is, while mutations in the antigen-binding regions may be positively selected for, concomitant mutations outside of the antigen-binding domains may also occur, for example, in the $\alpha 3$ domain. The $\alpha 3$ domain may interact with peptide hormone receptors, and thus if selection for antigen binding region mutations were strong enough, the frequency of a new allele which differentially associates with hormone receptors would increase significantly.

Summary and future directions

Evolutionary theories of aging are instructive, but loci which fit predictions of the theories are lacking. Accumulating evidence suggests that MHC region genes modulate developmental, reproductive, and aging rates. Especially implicated are the MHC class I genes, and much is known about their evolutionary history in terms of selection and mechanisms generating diversity. The premise put

forward here is that MHC class I genes are an appropriate model to experimentally address evolutionary theories of senescence in vertebrates.

There are several ways to further substantiate and develop this hypothesis. Corroborative evidence might be found in further analyses of MHC genes in species of wide-ranging lifespans. Along these lines, examination of insular (low mortality rate) populations might be informative, especially if laboratory lifespan data are available. What kind and frequency of MHC alleles are observed relative to noninsular populations (e.g. island versus mainland populations), and how does this compare to the divergence at other loci?

Selection for delayed reproduction in mammals as has been done in *Drosophilia* (Rose, 1984) may be feasible (Johnson, 1988; Rose, 1988). If such studies are performed successfully, a comparison of the type and frequency of MHC alleles that exist in the young versus old strains would be highly recommended.

Causal effects of MHC genes on aging rates perhaps may be unequivocally proven by transgenic mice experiments. Two types of transgenic experiments could seperately address the issue of qualitative versus quantitative mechanisms defined above. Qualitative effects of MHC alleles may be addressed by site-specific recombination techniques. For example, replacing *H-2D*^{b} in C57B10 mice with the *H-2D*^{r} gene would directly test the hypothesis that *H-2D* genes are responsible for the observed differences in lifespan and biomarkers of aging between B10.RIII (*H-2*^{r}) and C57B110 (*H-2*^{b}) mice. The more routine transgenic techniques (random integration sites) are more applicable to quantitative mechanisms.

It is worth emphasizing that since MHC genes are distributed among all vertebrate classes, consideration of the effects of MHC genes on life history patterns should not be limited to mammals. The diversity of MHC genes among vertebrates mirrors the diversity of life history patterns, including aging rates.

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