

Functional significance of MHC variation in mate choice, reproductive outcome, and disease risk

Dagan A. Loisel, Susan C. Alberts, and Carole Ober

Introduction

The nervous and immune systems both serve essential sensory functions in vertebrates. Whereas the nervous system surveys the sensory landscape of the physical world, the immune system responds to an enormous diversity of self and non-self (i.e., bacterial, viral, fungal) biological stimuli. The two systems are also intimately connected by a common biochemical language (Blalock 1994). A network of shared ligands (e.g., neurotransmitters, hormones, and cytokines) and receptors enables molecular crosstalk between the two systems, facilitating intersystem coordination and intrasystem regulation (Blalock 1994; Boulanger *et al.* 2001). In addition, the activity of one system is crucial to the normal development and function of the other. Immune signaling plays a critical role in normal central nervous system development and function, synaptic remodeling and plasticity, and learning and behavior (Boulanger and Shatz 2004; Ziv *et al.* 2006). Likewise, neural structures and functions contribute to immune homeostasis and host defense (Downing and Miyan 2000). From these three concepts—the overlap of sensory function, bidirectional flow of information, and developmental interdependence—emerges the idea of an integrated neural-immune circuit (Blalock 1994).

The existence of an integrated neural-immune system has profound implications for our understanding of the function and evolution of the human body's most extraordinary genetic system: the immune genes of the major histocompatibility

complex (MHC). MHC genes are highly genetically diverse, among the most diverse in the human genome, and this diversity influences disease susceptibility and resistance. There is little doubt that the ongoing evolutionary battle against pathogens has shaped the evolution of these genes. In contrast, the suggestion that MHC genes are involved in vertebrate mate choice and reproduction, specifically in the context of sexual selection, has been met with much skepticism despite burgeoning evidence in its support. The newly emerging idea of neural-immune integration addresses issues at the heart of this skepticism because it provides a mechanism for the production and detection of MHC-based olfactory cues that would be an essential basis of MHC-based sexual selection. In this chapter, we review the empirical evidence and evolutionary theory underlying current ideas about the importance of natural and sexual selection in the evolution of MHC genes, and we discuss the possible adaptive and non-adaptive consequences of this selective scenario. Only by examining MHC biology in an evolutionary perspective can we truly appreciate the far-ranging non-immune functions of these unique genes, including their underappreciated role in vertebrate olfactory communication, mate choice, and reproduction.

Genes of the major histocompatibility complex

The major histocompatibility complex is a gene-dense region occurring in all jawed vertebrates

that contains several dozen genes involved in adaptive or innate immunity (Fig. 8.1a). These immune genes strongly influence disease resistance, tissue graft acceptance, and fetal tolerance during pregnancy (Ober and van der Ven 1997; Klein and Sato 2000; Lechler and Warrens 2000). In addition to their immune functions, some MHC genes play an important role in nervous system development and function (reviewed in Boulanger *et al.* 2001); MHC class I molecules, for example, are essential for normal structural and synaptic remodeling in the developing and mature central nervous system. Although details about their role in the nervous system are just emerging, it is clear that MHC function extends beyond immunity.

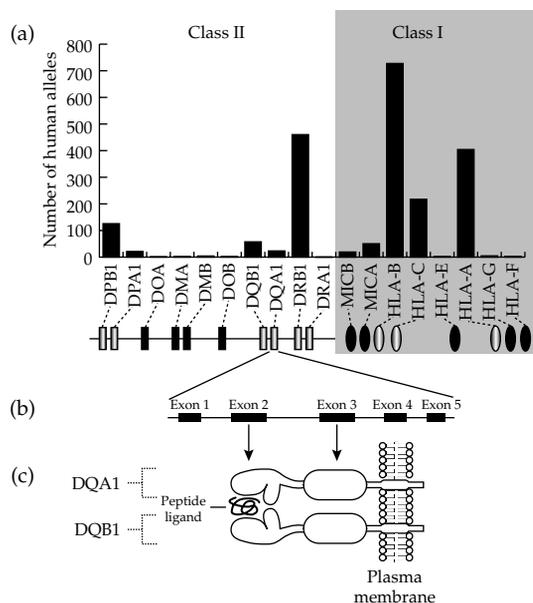


Figure 8.1 Structure and diversity of human MHC genes. (a) The MHC region encompasses roughly 4 million bases on chromosome 6p and contains > 200 genes, approximately 40% of which are involved in immunity. Among these genes, the classical class I genes (shown as open ovals) and class II genes (shown as open rectangles) show extraordinary levels of diversity in humans. Non-classical class I and class II genes (shown as filled ovals and filled rectangles, respectively) are generally less diverse. (b) The exon structure of a class II gene. (c) The structure of a class II molecule. An alpha and beta chain dimerize to form the functional molecule, which together with bound peptide are expressed at the surface of immune cells.

Form and function of MHC molecules

The classical MHC genes encode transmembrane glycoproteins that bind short peptides from degraded self and non-self (i.e., pathogen-derived) proteins and present them on the cell surface to circulating T cells (Figs. 8.1b,c). The range of peptides bound by an MHC molecule is determined by the composition of amino acids comprising its peptide-binding groove. Since all the peptide-presenting MHC genes differ in their peptide-binding groove sequence, each gene binds a different range of peptides. T-cell recognition of a non-self peptide bound by a MHC molecule initiates a complex cascade of immune responses designed to limit the spread or replication of pathogens: cytotoxic T cells proliferate and destroy infected cells, macrophages secrete complement protein to kill phagocytized pathogens, and B cells are activated to produce pathogen-specific antibodies (Lechler and Warrens 2000). Thus, peptide presentation by MHC genes is critical to the development and activation of immune surveillance and response. Disruption of the normal MHC function usually results in profound immunopathology; the consequences range from severe immunodeficiency and death to autoimmune diseases and tumor growth.

Evolution of MHC genes

Perhaps the most striking property of the MHC is its extraordinary genetic diversity. The classical MHC genes, referred to as human leukocyte antigens or HLA genes, are among the most polymorphic in the human genome, with hundreds of alleles at some loci (Fig. 8.1a) (Garrigan and Hedrick 2003). Nucleotide diversity in the MHC region can reach levels approximately two orders of magnitude greater than the genome average (Garrigan and Hedrick 2003). This variation is not evenly distributed across MHC loci, however. Both polymorphisms and non-synonymous (i.e., amino acid altering) changes occur in excess in the codons involved in peptide binding, suggesting that selection favors variation at these sites (Hughes and Yeager 1998). In addition, several other features of MHC diversity are consistent with the action of selection: too many MHC alleles are observed in most populations,

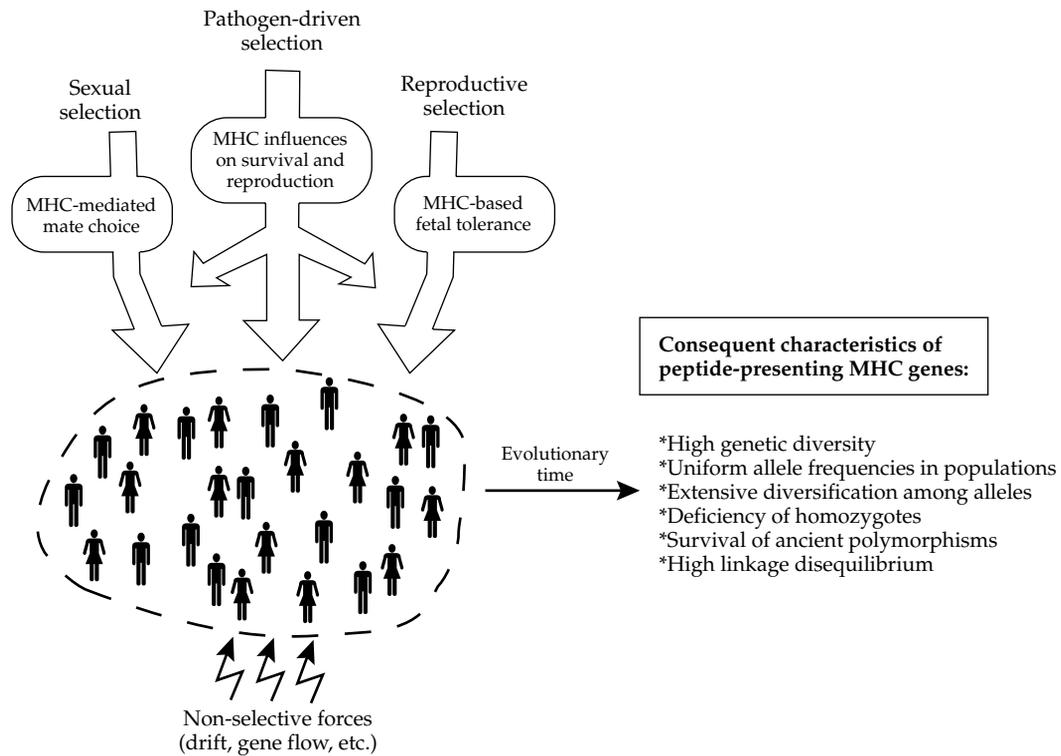


Figure 8.2 Selective forces driving MHC evolution. The standing diversity of MHC genes is subject to a number of selective and non-selective forces. In humans, for example, the remarkable molecular and population genetic features of MHC genes (e.g., high diversity, uniform allele frequencies) are attributed to the action of these evolutionary forces.

allele frequencies are too uniform, alleles differ at too many (often >50) sites, and polymorphisms have persisted for too long to be consistent with neutral evolution (Fig. 8.2) (Apanius *et al.* 1997; Meyer and Thomson 2001; Garrigan and Hedrick 2003). The consensus interpretation of these results is that selection favoring the induction and maintenance of MHC diversity—i.e., balancing selection—has greatly influenced MHC gene evolution.

But, what are the ultimate drivers of selection? Because MHC genes play a fundamental role in immunity and contribute to disease risk, it has been long suspected that pathogen-mediated selection maintains MHC diversity (references in Apanius *et al.* 1997). However, pathogens are but one of several potential drivers of MHC diversity that are consistent with the observed data (Fig. 8.2). There is considerable evidence that non-pathogen-mediated mechanisms, such as sexual selection, autoimmunity, and reproductive selection, also shape the pattern of MHC

evolution (Apanius *et al.* 1997; Meyer and Thomson 2001). In the following sections, we describe the pathogen- and non-pathogen-mediation models of selection, the predictions of these models, and the empirical evidence that supports them.

Pathogen-mediated selection on MHC genes

Pathogen-mediated selection operates when MHC gene variants (i.e., alleles) differ in their ability to protect against infectious organisms. Ample evidence for this phenomenon exists in humans, in that specific human MHC alleles are associated with susceptibility and resistance to a number of infectious diseases, including HIV, malaria, tuberculosis, hepatitis, leishmaniasis, and leprosy (reviewed in Lechler and Warrens 2000; Shiina *et al.* 2004). Similar correlations between MHC alleles and host resistance to bacterial, viral, fungal, and

parasitic infection were observed in experimental infection studies of laboratory (e.g., mice and rats) and captive-raised (e.g., chickens, cows, and fish) animals (reviewed in Apanius *et al.* 1997; Penn 2002; Bernatchez and Landry 2003; Sommer 2005b). Finally, recent field studies have demonstrated that MHC variation influences pathogen resistance in wild populations of fish, sheep, snakes, and mice (reviewed in Sommer 2005b). These results from wild populations are particularly informative, as they illustrate the potential for MHC genes to impact fitness in the natural world.

In addition to the allele-specific effects described above, MHC-heterozygosity effects on disease have been observed in humans, laboratory mice, and captive-raised fish (reviewed in Penn 2002; Sommer 2005b). Specifically, MHC heterozygotes were more resistant to infection or more efficient in recovering from infection than homozygotes; this effect was most pronounced in studies involving serial or multiple-pathogen infections, or infections caused by quickly evolving viruses like HIV (Penn 2002; Sommer 2005b). MHC heterozygotes may be more resistant to infection than homozygotes due to either heterozygote advantage (i.e., heterozygotes are more resistant than the average of the two homozygotes) or heterozygote superiority (i.e., heterozygotes are more resistant than either homozygote) (Penn 2002; McClelland *et al.* 2003).

Three theoretical models of pathogen-driven balancing selection have been developed to explain how MHC diversity is maintained (reviewed in Meyer and Thomson 2001; Hedrick 2002). First, the frequency-dependent selection model posits a cyclical coevolutionary arms race in which the selective value of an allele is inversely proportional to its frequency in the population. New or rare alleles have a selective advantage because few pathogens have adapted to them; this advantage declines as the rare alleles increase in frequency and pathogens evolve resistance. The resulting oscillations in allele frequencies prevent alleles from becoming fixed or eliminated, thereby maintaining MHC variation. Second, in the fluctuating selection model, fitness values change as a function of pathogen frequency or intensity, not as a function of allele frequency as is observed in the

frequency-dependent model (Meyer and Thomson 2001). Fluctuations in the temporal or spatial patterns of pathogens result in selection favoring different MHC alleles at different times, irrespective of their frequency in the population. The changing selective landscape implicit in this model could maintain the high allelic and nucleotide diversity characteristic of peptide-presenting MHC genes (Hedrick 2002). Finally, heterozygote superiority could maintain MHC polymorphism if heterozygotes are more resistant to pathogens, and thus have higher fitness, than both of the homozygotes (Hughes and Yeager 1998; Penn 2002). The three models are not mutually exclusive, and it is likely that their effects combine to maintain the extreme diversity of MHC genes.

Sexual selection on MHC genes

A prediction of the pathogen-mediated mechanisms of MHC evolution is that individuals may increase their fitness by employing MHC-dependent mating preferences that increase the production of offspring with enhanced disease resistance (Penn and Potts 1999). MHC-dependent mating preferences could increase the immunological resistance of offspring by any of the following, non-mutually exclusive processes:

1. producing MHC heterozygotes with enhanced resistance to multiple pathogens;
2. increasing the disparity between the offspring and parental MHC genotypes (in order to protect against pathogens adapted to the parental genotype);
3. facilitating the avoidance of genetic incompatibility at MHC loci or genome-wide inbreeding; and
4. producing offspring with optimal levels of MHC diversity (Penn 2002; Sommer 2005b).

At the level of individual choice, individuals may potentially maximize the fitness of their offspring by choosing mates with particular MHC characteristics. For example, individuals may choose mates based on whether they carry 'good genes,' which confer additive benefits on offspring and may be indicated by condition-dependent indicator traits. If mate choice is based on good genes,

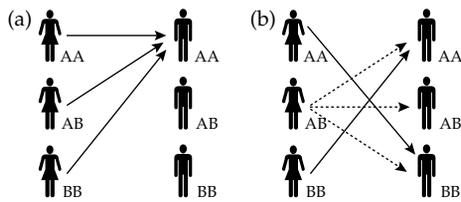


Figure 8.3 Models of mate choice. Both examples show female choice. (a) Mate choice for 'good' genes. In this model, the A allele signals good genes. Mating with the AA males is preferred by all females (solid line), regardless of her genotype, because it maximizes the number of offspring inheriting the good gene. (b) Mate choice for 'compatible' genes. In this model, the A and B alleles represent compatible genes. Females prefer males who are most different from themselves. The heterozygous AB female will not prefer any one male genotype because all matings will result in half her offspring being heterozygous (dotted line). (Adapted from Neff and Pitcher 2005).

then all individuals in a population will have similar preferences (Fig. 8.3a). Alternatively, individuals may choose mates with the most 'compatible genes' to their own, so as to provide offspring with the most adaptive gene combinations (Neff and Pitcher 2005). Mate choice based on compatible genes will result in incongruent mate choice patterns within a population (Fig. 8.3b). MHC genes have been implicated as a target of mate choice for both good genes and compatible genes in non-human animals and in humans; the evidence for both is reviewed below.

MHC-mediated mate choice in non-human vertebrates

The first evidence for MHC-mediated mating preferences indicated that male and female laboratory mice preferred MHC-dissimilar mates (reviewed in Alberts and Ober 1993; Penn and Potts 1999). Subsequent laboratory mouse studies reported conflicting results and this inconsistency was likely due to the methodological shortcomings of those studies. More compelling evidence for MHC-mediated mate choice in mice came from studies of wild-derived house mice living in large, semi-natural enclosures (Potts *et al.* 1991; Potts *et al.* 1992). Here, a significant population-level deficiency of MHC-homozygous offspring was observed and shown to be due to female choice for MHC-dissimilar males

(Potts *et al.* 1991). Subsequent experiments demonstrated that cross-fostering (rearing female mouse pups in MHC-dissimilar families) reversed MHC-disassortative mating preferences, suggesting an important role for post-natal imprinting in the development of MHC-mediated mate choice (Penn and Potts 1998b).

Work on natural populations of non-model vertebrates has detected MHC-dependent mate choice in species of fish, birds, lizards, and rodents (Paterson and Pemberton 1997; reviewed in Bernatchez and Landry 2003; but see also Ekblom *et al.* 2004; Westerdahl 2004; Piertney and Oliver 2006). These studies highlight the complexity and context-dependent nature of MHC-based mating preferences. In some studies, particular MHC alleles appear to be targets of mate choice for good genes, while in others individuals appear to choose mates based on their own similarity (or dissimilarity) to them at MHC loci, suggesting compatible genes that enhance offspring quality via interactions between maternal and paternal MHC genotypes (Fig. 8.3).

Evidence consistent with mate choice for good genes came from a study of ring-necked pheasants. In these birds, MHC genotype was associated with variation in both male spur length, a condition-dependent ornament preferred by females, and adult male annual survival (von Schantz *et al.* 1997). This effect was not due to heterozygosity per se, as spur length did not differ between MHC heterozygotes and homozygotes. Because MHC genotype affected spur length and females generally preferred long-spurred males, these results are generally consistent with the MHC as good genes model (von Schantz *et al.* 1997).

Several other studies found evidence for mate choice based on MHC as compatible genes, and these results suggest that the mating preferences can take at least three forms. First, as in the early laboratory mouse experiments, there is evidence for mate choice for MHC-dissimilar mates in studies of free-living yearling female savannah sparrows, wild-caught Atlantic salmon, and, possibly, free-ranging sand lizards (reviewed in Bernatchez and Landry 2003; Piertney and Oliver 2006). Second, data consistent with mate choice for MHC-similar mates were observed in a wild population of

house sparrows (Bonneaud *et al.* 2006) and in free-ranging Malagasy giant jumping rats (Sommer 2005a). Although these results are contrary to current thinking about MHC mate choice patterns, it has been hypothesized that a preference for MHC-similar mates may emerge when selection favors local adaptations or co-adapted gene complexes (Bonneaud *et al.* 2006). Mate choice for MHC-similar mates would not contribute to the maintenance of MHC diversity. Third, individuals in some species appear to choose mates that are optimally diverse, i.e., neither too similar to nor dissimilar, at MHC loci. For example, in odor preference trials of wild-caught three-spined sticklebacks, females chose males with levels of MHC diversity that would facilitate the production of offspring with an intermediate number of MHC sequence variants (Aeschlimann *et al.* 2003). Subsequent infection experiments showed that sticklebacks possessing an intermediate level of MHC diversity were most resistant to parasite infection (Wegner *et al.* 2003).

Finally, individuals may simultaneously integrate information about both good genes and compatible genes into their mate choice decisions (Colegrave *et al.* 2002; Mays and Hill 2004; Neff and Pitcher 2005). For example, in a laboratory setting, female mice evaluated and utilized both male scent marking rate (as a good genes indicator) and MHC similarity (as a compatible genes indicator) in their mate choice decisions (Roberts and Gosling 2003). Female choice was also shown to be phenotypically plastic. When variation in scent marking was small, MHC similarity was a significant predictor of female preference, but when variation was large, MHC similarity was not a significant predictor (Roberts and Gosling 2003). This suggests that females may be able to optimize fitness by using different choice criteria in different social, environmental, and genomic contexts (Colegrave *et al.* 2002; Mays and Hill 2004; Neff and Pitcher 2005). Mate choice decisions may therefore involve trade-offs between good and compatible genes, potentially complicating the detection of MHC-mediated mate choice in nature. Thus, the failure to detect MHC-mediated mate choice when it is occurring and discrepancies among studies that detect an effect may simply reflect natural

differences in the relative importance of good versus compatible genes.

Role of the MHC in human mate choice

Evidence that MHC genes (i.e., HLA genes) influence human mating preferences comes from both odor preferences experiments and population-based studies. In the former, researchers tested for associations between odor preferences and MHC similarity. In a well-known example, college students were asked to rate the pleasantness of odors from t-shirts worn by other MHC-similar and -dissimilar unrelated individuals (Wedekind *et al.* 1995; Wedekind and Furi 1997). In general, women rated the odors of MHC-dissimilar men as being 'more pleasant' than MHC-similar men, although this preference was reversed for women on oral contraceptives (Wedekind *et al.* 1995; Wedekind and Furi 1997). Men also tended to rate MHC-dissimilar women as more pleasant, although the results were not statistically significant (Wedekind and Furi 1997). In addition, in these same university students, there was evidence of an interaction between MHC genotype and the preference for particular perfume scents (Milinski and Wedekind 2001). These results suggest that humans exhibit MHC-based odor preferences. However, these odor preferences must reflect behavioral decisions with fitness consequences in order to be relevant to the evolution of MHC genes.

Odor preference studies have also provided insight into the mechanisms of preference development. Jacob and colleagues (2002) showed that women can discriminate between men's odor samples as a function of the number of shared MHC alleles. Overall, women most preferred odors from men with whom they shared an intermediate number of MHC alleles (mean of 2.3 matches to the woman out of a range of 0 to 6 matches), while the least preferred odors were from men with significantly fewer matches (mean of 1.5) (Jacob *et al.* 2002). Strikingly, the women's preferences were based solely on matches to the alleles she inherited from her father. This remarkable finding suggests that odor preference development in humans may be sensitive to paternally inherited MHC alleles rather than just behavioral imprinting on familial

MHC-associated odors, as it appears to be in mice (Jacob *et al.* 2002; Potts 2002).

To directly assess the role of MHC genes in mate choice, researchers have also analyzed actual patterns of mate choice, for example by comparing the observed level of MHC allele sharing between couples to that predicted under random mating. Retrospective studies of outbred, ethnically diverse human populations have consistently failed to find evidence for MHC-mediated mate choice (Alberts and Ober 1993; Meyer and Thomson 2001). This is unsurprising, given that most of these studies involved large, outbred, and poorly defined populations, and were confounded by ethnic, racial, or cultural mating preferences.

Two studies examined mating preferences in more isolated, well-defined human populations. Hedrick and Black (1997) looked for MHC effects on mating in isolated South Amerindian tribes that showed population-level deficits of MHC homozygotes (suggestive of selection favoring heterozygotes). They found no evidence for MHC-dependent mating preferences in their comparison of observed and expected sharing of a two-locus MHC haplotype in mating pairs. However, given the technological (i.e., low resolution of only two loci) and methodological (i.e. small sample size; not controlling for inbreeding and stratification in the population) limitations of the study, only extremely strong selection for MHC-mediated mate choice would have been detectable (Penn and Potts 1999).

The second human population study involved a moderately inbred, ethnically homogenous religious isolate living on communal farms in the United States and Canada (Ober *et al.* 1997, 1999). To determine whether mate choice was random with respect to MHC genotype in 411 married couples, Ober and colleagues (1997, 1999) compared the observed number of couples in which the husband and wife matched at a 16-locus MHC haplotype to the number of couples expected to match given the population and mating structure. They found that significantly fewer couples matched at the 16-locus haplotype than expected; this result was confirmed in an independent analysis that was robust to population stratification and inbreeding (Genin *et al.* 2000). In addition, in couples that did share MHC haplotypes, the matched haplotype

was inherited from the mother significantly less often than expected, though this was largely due to a single haplotype (Ober *et al.* 1997, 1999). This deficiency of maternally inherited matched haplotypes suggests a role for imprinting in mate choice.

The results of Ober and colleagues' mate choice studies suggest that MHC (or closely linked) genes can have a strong effect on mating preferences, consistent with a pattern of disassortative mating for MHC haplotypes. This preference for MHC-dissimilar mates is also consistent with, and may be partly responsible for, the population-level deficiency of homozygotes for MHC haplotypes observed in this population (Robertson *et al.* 1999). The ability to detect a strong MHC effect on mate choice was likely enhanced by specific features of the population, such as the relatively low number of segregating MHC haplotypes and homogeneity of important social and culture factors (e.g., ethnicity, religion, education, and income) (Ober *et al.* 1997).

Evolutionary implications of MHC-mediated mate choice

Evidence for MHC-mediated mate choice has been observed in several vertebrate taxa (e.g., mammals, fish, birds, and reptiles), suggesting that this phenomenon may be relatively widespread in nature (Slev *et al.* 2006). The evolution of MHC-mediated mating preferences is favored under the models of pathogen-mediated selection proposed to drive MHC gene evolution, and, like pathogen-mediated selection, is capable of contributing to the maintenance of MHC diversity. The occurrence of MHC-mediated mate choice will affect the long-term evolutionary trajectory of the MHC genes, as well as the population genetic characteristics, i.e., the MHC allele frequencies, that influence the relative risk of disease in contemporary populations.

Since MHC-mediated mate choice is essentially a mechanism to preferentially produce offspring with the genetic qualities favored by selection, the evolution of strong MHC-mediated mating preferences in a population should reduce the incidence of infectious, genetic, and/or reproductive diseases. Therefore, poor mate choice decisions may have dire consequences. In the three-spined stickleback fish, where females prefer mates that best complement

their own MHC diversity (Aeschlimann *et al.* 2003), a suboptimal mate choice decision results in offspring more susceptible to parasite infection (Wegner *et al.* 2003). In the human population that showed a deficiency of couples that matched at a 16-locus MHC haplotype (Ober *et al.* 1999), pregnancy loss rate was significantly higher in couples with matching MHC haplotypes (discussed later in this chapter: Ober *et al.* 1998). Although many unanswered questions remain, MHC-mediated mating preferences can potentially influence host immunity and disease risk, and contribute to the extraordinary evolution of the MHC.

MHC-linked olfactory cues

For individuals to make mate choice decisions based on MHC characteristics, they must be able to determine the MHC genotype of their potential mates and, under a compatibility paradigm, of themselves. Following the first reports of MHC-mediated mate choice, it was hypothesized that MHC genes might influence body odors. Research on olfactory communication in rodents has clearly demonstrated the validity of that hypothesis (reviewed in Penn and Potts 1998a; Yamazaki *et al.* 1998). Behavioral experiments have repeatedly shown that mice and rats (both trained and untrained) can distinguish among odors derived from MHC-disparate, but otherwise genetically identical, individuals (Penn and Potts 1998a). Mice, for example, were able to differentiate between odor samples from mice that differed across the entire MHC region, differed at a single MHC gene, or differed by only a few peptide-binding groove residues of a single MHC gene (Penn and Potts 1998a; Yamazaki *et al.* 1998; Penn 2002). This ability to detect MHC-associated odor differences extends across species boundaries, as rats can distinguish among odors from MHC-dissimilar mice and humans, and humans can recognize differences in the odor of MHC-dissimilar mice (references in Penn and Potts 1998a).

Influence of MHC peptide-binding region on odor

Considering that MHC alleles are defined by variation in the peptide-binding region, that this

variation influences pathogen resistance and mate choice, and that animals can discriminate via olfaction among individuals differing only in this region, it follows that the peptide-binding properties of MHC molecules influence the odor profile of an individual. Four hypotheses to explain how the peptide-binding groove of MHC molecules could influence odor have been proposed (reviewed in Penn and Potts 1998a; Yamazaki *et al.* 1998; Penn 2002). First, MHC molecules or fragments thereof may act as odorants; second, the unique array of peptides bound by an individual's MHC molecules may function as odorants; third, partially degraded MHC molecules may assume a new function as carriers for circulating volatile odorants; and fourth, MHC molecules may shape an individual's specific population of commensal microflora, which then produce odorants. Because the proposed mechanisms are not mutually exclusive, they may be operating simultaneously to influence an individual's odor profile (Penn and Potts 1998a; Penn 2002). Recently, however, several independent lines of evidence have converged in support of the second hypothesis: the peptide ligands bound and displayed by MHC molecules also function as chemical signals of individuality (Boehm and Zufall 2006).

MHC peptide ligands as olfactory cues

Although the mechanistic details of how MHC peptide ligands serve as olfactory cues are still emerging, the working hypothesis is that MHC/peptide complexes are proteolytically shed from the cell surface, partially degraded, and then dissolved in bodily fluids, such as urine, serum, saliva, and sweat. The degradation of the MHC/peptide complexes releases the peptide ligands, which are then free to interact with other types of receptors, e.g., those expressed in olfactory sensory neurons (Boehm and Zufall 2006). Because the range of MHC-bound peptide ligands reflects the structural diversity of the peptide-binding groove of the MHC molecules, this hypothesis directly links an individual's MHC genotype with their body odor phenotype.

The ability of MHC peptide ligands to act as olfactory cues of individual identity has recently

been tested in three distinct biological contexts. First, in a behavioral assay of olfactory assessment, male mice spent significantly more time investigating female urine supplemented with MHC class I peptide ligands specific for a different mouse strain than they spent investigating female urine supplemented with peptides specific for their own strain (Spehr *et al.* 2006). Even among the many olfactory cues present in mouse urine, the signal produced by the addition of peptide ligands was sufficient to alter odor preferences. Second, Leinders-Zufall and colleagues (2004) used the bioassay of pregnancy failure to demonstrate that MHC class I peptide ligands act as strain-specific chemosensory signals of MHC genetic identity. Specifically, the probability of pregnancy loss was greatly increased when female mice were exposed to a familiar male urine sample supplemented with unfamiliar peptide ligands.

The third example of MHC peptide ligands influencing behavior was observed in a set of odor preference experiments involving three-spined stickleback fish (Milinski *et al.* 2005). Gravid female sticklebacks prefer the tank water of males possessing levels of MHC diversity that optimally complements their own diversity, i.e., results in offspring with intermediate levels of diversity. The addition of synthetic MHC class I- and II-specific peptide ligands increased the attractiveness of water from males with suboptimal diversity and decreased the attractiveness of water from males with optimal (or supraoptimal) diversity, presumably by signaling that those males were more diverse than they really were (Milinski *et al.* 2005; Boehm and Zufall 2006). Thus, in both mice and sticklebacks, exposure to MHC peptide ligands significantly modifies behaviors associated with social recognition, mate choice, and reproduction.

Detection of MHC-mediated odors

The small, nonvolatile MHC class I-specific peptide ligands that modify MHC-mediated social behaviors are detected by specialized sensory neurons in both the main olfactory epithelium and the vomeronasal organ in mice (Leinders-Zufall *et al.* 2004; Spehr *et al.* 2006). In both of these olfactory organs, peptide ligands were detected in an allele-specific

manner; i.e., structurally distinct peptide ligands induced different sensory neuron activation patterns. *In vivo* testing showed that class I peptide ligand-specific activation in the main olfactory epithelium affected odor familiarity, while activation in the vomeronasal organ was sufficient to induce pregnancy failure (Leinders-Zufall *et al.* 2004; Spehr *et al.* 2006). The identity of the receptors responsible for recognition of the peptide ligands is unknown. Several candidates for such receptors have been proposed, such as MHC-linked olfactory receptor genes and the V2R pheromone receptors. However, this area requires future study (Boehm and Zufall 2006).

Peptide binding as an integrating principle in MHC evolution

The discovery of a chemosensory function for MHC peptide ligands has important implications for immunity, behavior, and MHC evolution. First, it provides insight into the molecular mechanisms by which an individual can detect olfactory cues of MHC composition in others, can process that information to determine quality or compatibility, and can subsequently bias their behavioral response to maximize their fitness. Second, it serves as a vivid example of neural-immune integration, as the structure of MHC peptide ligands conveys valuable information to both the T-cell receptors monitoring the internal immunological environment and the olfactory sensory neurons surveying the chemosensory world. Finally, it reinforces the idea that MHC genes are subject to a complex mixture of selective forces and constraints. Because the binding of peptide ligands is important to both immune surveillance and chemosensory recognition, the peptide-binding properties of MHC molecules will be influenced by both natural (i.e., pathogen-mediated) and sexual selection (Slev *et al.* 2006). Determining whether synergistic or antagonistic consequences result from this arrangement will be a challenge for future research.

MHC and reproductive outcome

The ability of a female to influence the MHC characteristics of her offspring does not end once

a mate is chosen. In the period between mating and birth, females have an opportunity to exert post-copulatory selection (also called cryptic female choice) to block or abort the production of offspring that would have decreased fitness due to diminished disease resistance, genetic incompatibility, or inbreeding depression (Alberts and Ober 1993; Apanius *et al.* 1997). From an evolutionary perspective, the elimination of these 'less fit' offspring would be adaptive, not pathological (Apanius *et al.* 1997). In non-human animals, there is abundant evidence showing that females use post-copulatory mechanisms to increase the production of offspring with higher genetic quality (reviewed in Tregenza and Wedell 2000; Neff and Pitcher 2005). In humans, the evidence is less convincing, and more controversial (Alberts and Ober 1993; Choudhury and Knapp 2001). However, several studies strongly suggest that the MHC compatibility between mother and fetus influences pregnancy outcome: one study found an excess of heterozygotes in newborn males (Dorak *et al.* 2002) and a number of studies have reported associations between parental MHC sharing and reproductive success (reviewed below).

MHC sharing and reproduction in outbred human populations

Dozens of retrospective studies have examined the relationship between MHC sharing and recurrent spontaneous abortion (RSA) in outbred couples (reviewed in Ober and van der Ven 1997; Choudhury and Knapp 2001; Beydoun and Saftlas 2005). Approximately half of these studies reported increased MHC sharing in couples with RSA compared to control couples. However, among those studies showing a positive association, there was little consistency with respect to the specific MHC genes or regions thought to be responsible. In addition, a meta-analysis of the impact of MHC sharing at particular loci on RSA revealed significant heterogeneity in the odds ratio estimates among studies (Beydoun and Saftlas 2005). Similar discrepancies and inconsistencies were observed in studies of MHC sharing and other reproductive disorders, such as preeclampsia (Saftlas *et al.* 2005) (see Chapter 6 for a different explanation for

preeclampsia). Moreover, parental MHC sharing has been shown to significantly influence pregnancy success after assisted reproductive technology (ART) in couples experiencing unexplained infertility (reviewed in Ober and van der Ven 1997; Choudhury and Knapp 2001). MHC sharing was significantly higher in couples that failed to achieve a successful pregnancy after ART, suggesting that maternal–fetal histoincompatibility improved implantation success and the probability of a successful pregnancy among these couples. Overall, however, the relationship between MHC sharing in outbred couples and reproductive pathology, such as implantation success, recurrent spontaneous abortion, and preeclampsia, remains unresolved.

MHC sharing and reproductive outcome in an unselected population

The most convincing evidence that MHC sharing influences reproductive success comes from a series of population-based studies of the Hutterites (Ober *et al.* 1983, 1992, 1998). The ethnically homogeneous Hutterite population is characterized by a limited number of independent MHC haplotypes, a high natural fertility rate, large family sizes, and a relatively uniform environment (Ober 1999). The communal lifestyle of the Hutterites minimizes non-genetic influences on fertility: for example, birth control use is limited, diet is relatively uniform, smoking is prohibited, and alcohol consumption is moderate (Ober 1999). Because of these features, the Hutterite population is well suited to studies examining genetic influences on fertility and reproduction.

Motivated by the hypothesis that maternal–fetal histocompatibility influenced reproductive outcome, early studies of Hutterite couples revealed a trend towards longer time intervals from marriage to each birth in couples that shared alleles at the MHC genes, *HLA-A*, *HLA-B*, or *HLA-DR* (Ober *et al.* 1983; Ober and van der Ven 1997). The median completed family size of couples that shared *HLA-DR* alleles (6.5 children) was significantly smaller than that of couples that did not share *HLA-DR* alleles (9.0 children) (Ober and van der Ven 1997). To investigate the cause of the longer birth intervals and smaller completed family size, a follow-up

prospective study compared the length of time from the resumption of menses after childbirth to the next positive pregnancy test in *HLA-DR* similar and dissimilar couples (Ober *et al.* 1992). The results showed that couples that shared *HLA-DR* alleles took more than 2.5 times longer to achieve pregnancy. In addition, pregnancy loss rates were significantly increased among couples that shared alleles at another MHC class I locus, *HLA-B*.

The association between increased pregnancy loss rate and MHC sharing in the Hutterites was remarkable. To confirm the result, more than 100 subsequent pregnancies were added to the data set and high-resolution genotyping was extended to 16 loci across the greater MHC region (Ober *et al.* 1998). Once again, sharing of *HLA-B* alleles was a significant predictor of pregnancy loss, as was the sharing of alleles at two additional neighboring loci, *HLA-C* and *C4*. However, pregnancy loss rates were highest among couples that shared the entire 16-locus extended MHC haplotype. Although it was not possible to determine whether the primary risk factor for pregnancy loss was associated with the *HLA-B* locus per se, with an *HLA-B*-linked locus, or with the extended haplotype, the results clearly demonstrate strong effects of sharing MHC alleles on reproduction in the highly fertile Hutterite population (Ober *et al.* 1998).

MHC sharing, reproduction, and diversity

In utero selection for MHC-disparate fetuses could contribute to the enormous diversity of MHC alleles observed in human populations (Hedrick and Thomson 1988), particularly in homogeneous populations in which opportunities for MHC sharing among mates regularly arise. Such selection would minimize MHC homozygosity among children of couples that share MHC alleles, thereby increasing the fitness of offspring while at the same time maintaining genetic diversity in the population.

HLA-G in reproduction, immune regulation, and disease

Fetal survival during mammalian reproduction required the evolution of immunological

mechanisms that balanced tolerance of the genetically foreign fetus with maintenance of adequate immune defenses against pathogens. The highly specific patterns of MHC gene expression in fetal cells at the maternal–fetal interface may represent one of those adaptations. In humans, expression of the classical MHC class I (with the exception of *HLA-C*) and class II molecules is absent on fetal trophoblast cells that interface directly with maternal tissues and immune cells (Ober and van der Ven 1997). Conversely, expression of the non-classical class I genes, *HLA-E*, *-F*, and *-G*, is prominent in fetal trophoblast cells (Ishitani *et al.* 2003). It is likely that these non-classical MHC molecules, particularly *HLA-G*, contribute to the establishment and maintenance of maternal tolerance during pregnancy, possible by directing maternal cells toward immunosuppressive phenotypes (Carosella *et al.* 2003; Hunt *et al.* 2005; Hviid 2006).

HLA-G is unlike other MHC genes in several ways. First, although *HLA-G* is capable of classical peptide presentation, it is characterized by relatively low levels of protein polymorphism compared to other class I genes (Fig. 8.1a). Second, the *HLA-G* primary transcript is alternatively spliced into seven transcripts; at least two membrane-bound forms and two soluble forms are translated into protein. Third, *HLA-G* expression is tightly regulated and tissue-restricted in non-pathological circumstances; expression is high on fetal cells at the maternal–fetal interface, but otherwise may only be expressed in the adult thymus and cornea and on certain immune cells (reviewed in Carosella *et al.* 2003; Hunt *et al.* 2005; Hviid 2006).

HLA-G shows remarkable immuno-regulatory abilities. *In vitro* experiments showed that *HLA-G* molecules interact directly with several immune inhibitory receptors and exhibit remarkable immunosuppressive properties (reviewed in Carosella *et al.* 2003; Hunt *et al.* 2005; Hviid 2006). The ability of *HLA-G* to induce cells into immunosuppressive phenotypes is critical to the induction of immune tolerance in pregnancy (Hunt *et al.* 2005), but it may also be significant in other contexts, such as organ transplantation (Carosella *et al.* 2003). For example, the expression of soluble *HLA-G* in heart tissue was associated with a lower risk of rejection after transplantation; a similar pattern

was observed for patients receiving kidney–liver double transplants (references in Carosella *et al.* 2003; Le Maoult *et al.* 2003).

HLA-G in reproductive, autoimmune, and inflammatory pathologies

Because *HLA-G* plays such a critical role in pregnancy, mutations that affect *HLA-G* expression may be deleterious. Indeed, a number of case-control and cohort studies have linked *HLA-G* variation with reproductive complications such as miscarriage, preeclampsia, and pregnancy failure after *in vitro* fertilization (reviewed in Hviid 2006). In the Hutterites, the presence of a single nucleotide polymorphism (-725G) in the 5' *cis*-regulatory region in both partners was associated with sporadic pregnancy loss (Ober *et al.* 2003). In outbred couples, specific *HLA-G* alleles, defined by coding sequence variation, are associated with recurrent spontaneous abortion (reviewed in Hunt *et al.* 2005; Hviid 2006). Finally, risk of recurrent spontaneous abortion was significantly increased in Danish women who were homozygous for a polymorphic 14-base pair insertion in the 3' untranslated region of *HLA-G* (Hviid *et al.* 2004).

Aberrant *HLA-G* expression may also contribute to pathologies such as tumor growth, viral progression, and autoimmune or inflammatory disorders (Carosella *et al.* 2003; Le Maoult *et al.* 2003). For example, the tightly regulated, tissue-restricted patterns of normal *HLA-G* expression may be altered in tumor or virally infected cells so as to facilitate escape from immune surveillance and response (Carosella *et al.* 2003; Le Maoult *et al.* 2003). Moreover, ectopic expression of *HLA-G* has been observed in a number of autoimmune and inflammatory diseases, such as psoriasis, atopic dermatitis, asthma, multiple sclerosis, and inflammatory myopathies. This ectopic expression may represent the cause or consequence of those pathologies. On one hand, the expression of *HLA-G* in adult cells could contribute to the development of pathological inflammatory responses by skewing the host immune cells toward a disease-promoting (Th2-like) phenotype. The expression of *HLA-G* in bronchial epithelial cells from asthma

patients (Nicolae *et al.* 2005) and perhaps in the central nervous system of multiple sclerosis patients (Wiendl *et al.* 2005) supports this possibility. On the other hand, *HLA-G* expression may be induced in response to inflammation or injury. In this case, *HLA-G* molecules may limit the local autoimmune and inflammatory processes in order to protect healthy tissues, such as has been observed in heart transplants (Carosella *et al.* 2003).

Evolution of HLA-G

The unique role of *HLA-G* in human reproduction and immunomodulation suggests that this gene may have experienced a different evolutionary history from the other, classical HLA genes. Whereas the classical MHC genes show strong evidence for diversifying selection on amino acids that interact with T cells or bind peptides (discussed earlier), *HLA-G* shows little diversity in its coding region. However, variation in *HLA-G* that influences its expression appears to have been the target of selection. For example, a null mutation in exon 3 (encoding the $\alpha 2$ domain of the protein) common in populations of African descent shows evidence of positive selection (Aldrich *et al.* 2002), and the highly polymorphic promoter region shows evidence of long-standing balancing selection (Tan *et al.* 2005). It has been suggested that high-expressing *HLA-G* haplotypes may serve to protect the allogeneic fetus during pregnancy, but that lower expressing haplotypes might be adaptive in the presence of *in utero* infection during pregnancy by allowing for a more robust maternal immune response (Aldrich *et al.* 2002; Tan *et al.* 2005). Similarly, the higher expressing haplotypes may enhance the immunomodulatory properties of *HLA-G* in adult cells and predispose toward immune diseases, such as asthma, while protecting healthy tissues in the case of transplants.

The cost of protection: non-adaptive consequences of MHC diversity

In humans, variation in MHC genes is associated with differential susceptibility or resistance to more than 100 diseases belonging to several

disease classes: autoimmune, infectious, reproductive, cardiovascular, neurodegenerative, psychiatric, and metabolic (Klein and Sato 2000; Lechler and Warrens 2000; Shiina *et al.* 2004). MHC theory predicts that natural and sexual selection should favor the production of offspring with enhanced immunocompetence. Indeed, there is substantial empirical evidence that specific MHC alleles (or combinations of alleles) are associated with increased resistance to the pathogenic and parasitic infections that cause many fitness-reducing human diseases, such as HIV, hepatitis B, malaria, and tuberculosis (Lechler and Warrens 2000; Sommer 2005b).

However, it is also true that some MHC allelic variants can increase an individual's risk of disease. For example, MHC genes are among the strongest predisposing genetic factors for autoimmune diseases (Klein and Sato 2000). Genome-wide screens have consistently reported strong linkage to the MHC region and numerous association studies have related specific MHC alleles (or haplotypes) to susceptibility or resistance to diseases like type I diabetes, rheumatoid arthritis, and multiple sclerosis (reviewed in Lechler and Warrens 2000). Autoimmune diseases represent a pathogenic failure of self tolerance characterized by the presence of tissue-damaging self-reactive antibodies and/or T cells. The predisposing effects of MHC genes may result from their involvement in two essential parts of the antigen presentation pathway: the elimination of strongly self-reactive T cells during thymic development and the presentation of antigens to T cells in the course of normal immune surveillance (Marrack *et al.* 2001). Thus, the antigen-binding characteristics of MHC molecules are relevant to the induction of autoimmunity.

Even though autoimmune disease may reduce fitness, many of the MHC alleles linked with increased risk of autoimmune disease are relatively common in contemporary human populations (Apanius *et al.* 1997; Graham *et al.* 2005). Why has selection not eliminated these autoimmune-predisposing alleles from human populations? Several non-mutually exclusive hypotheses to explain why these predisposing alleles continue to persist have been proposed (reviewed in Apanius *et al.* 1997; Graham *et al.* 2005).

First, the benefits conferred by an autoimmune-predisposing allele in the fight against infectious, genetic, and reproductive disease may outweigh even large costs imposed by the associated autoimmune phenotypes (Apanius *et al.* 1997; Graham *et al.* 2005). As long as the disease resistance benefit is greater than the autoimmune cost, selection will maintain the allele in the population. Second, some autoimmune diseases emerge late in life (i.e., when individuals are finished reproducing), and thus impose a very low fitness cost (Apanius *et al.* 1997; Graham *et al.* 2005). In these cases, the deleterious genetic variant would be subject to very weak selection, allowing it to persist in the population for a relatively long period of time. Third, autoimmunity may have emerged relatively recently in human history, perhaps in response to novel or changing pathogens or environments. If so, there might not have been sufficient time for selection to eliminate the autoimmune-predisposing alleles (Graham *et al.* 2005). Finally, autoimmune disease may result from the pathogen-induced manipulation of a normal immune response into a pathogenic response towards self, for example through molecular mimicry (Apanius *et al.* 1997; Marrack *et al.* 2001). Interestingly, the situations involving the infectious-autoimmune balance and molecular mimicry could, in certain circumstances, actually contribute to the maintenance of MHC polymorphism (Apanius *et al.* 1997).

Conclusions

The nervous and immune systems show significant overlap and integration at many levels, e.g., molecular, developmental, and functional. The MHC gene family contributes to this integration, as MHC molecules function in immune and neural signaling processes (and influence mate choice and reproduction) in vertebrates. Given these many roles, the extraordinary diversity of classical MHC genes is probably subject to, and maintained by, multiple selective forces, including pathogen-driven selection, sexual selection, and reproductive selection. With additional research, it may be possible to determine more precisely the relative importance of these selective mechanisms in MHC evolution, as well as the potential non-adaptive

consequences, e.g., autoimmune and inflammatory disease, that may result.

Summary

1. The nervous and immune systems are intimately connected by shared developmental, functional, and biochemical pathways.
2. The extraordinary diversity and remarkable evolution of MHC genes are influenced by several distinct forces, including pathogen-mediated selection, sexual selection, and reproductive selection.
3. MHC diversity influences the risk and progression of infectious, reproductive, autoimmune, and inflammatory diseases.

4. MHC genes play a significant role in olfactory communication, behavior, and mate choice in vertebrates, including humans.

5. The unique evolution of MHC genes contributed to the prevalence of autoimmune and inflammatory diseases in modern human populations.

Acknowledgments

This work was supported by NIH Grants R01 HD21244 and R01 HL72414 to C.O., NSF Grants BCS-0323553 and IBN-0322613 to S.C.A., and funds from the Duke University Biology Department and Program in Genetics and Genomics to D.A.L.