

Possible Roles for Products of Polymorphic MHC and Linked Olfactory Receptor Genes during Selection Processes in Reproduction*

ANDREAS ZIEGLER, GOTTFRIED DOHR, AND BARBARA UCHANSKA-ZIEGLER

Ziegler A, Dohr G, Uchanska-Ziegler B. Possible roles for products of polymorphic MHC and linked olfactory receptor genes during selection processes in reproduction. AJRI 2002; 48:34–42 © Blackwell Munksgaard, 2002

PROBLEM: Polymorphic genes of the human major histocompatibility complex [MHC; human leukocyte antigen (HLA)] are probably important in determining resistance to parasites and avoidance of inbreeding. We investigated whether HLA-associated sexual selection could also involve HLA-linked olfactory receptor (OR) genes, which might not only participate in olfaction-guided mate choice, but also in selection processes within the testis.

METHOD OF STUDY: The testicular expression status of HLA class I molecules (by immunohistology) and HLA-linked OR genes (by transcriptional analysis) was determined.

RESULTS: Various HLA class I heavy chains, but not β_2 -microglobulin (β_2m), were expressed, mainly at the spermatocyte I stage. Of 17 HLA-linked OR genes analyzed, eight were found to be transcribed in the testis. They exhibited varying numbers of 5'- or 3'-non-coding exons as well as differential splicing.

CONCLUSIONS: We suggest that testis-expressed polymorphic HLA and OR proteins are functionally connected and serve the selection of spermatozoa, enabling them to distinguish 'self' from 'non-self' [the sperm-receptor-selection (SRS) hypothesis].

Key words:

HLA complex, linkage disequilibrium, major histocompatibility complex, olfactory receptor, oocyte, polymorphism, sperm-receptor-selection (SRS) hypothesis, testis

ANDREAS ZIEGLER
BARBARA UCHANSKA-ZIEGLER

Institut für Immunogenetik,
Universitätsklinikum Charité,
Humboldt-Universität zu Berlin,
Berlin, Germany
GOTTFRIED DOHR
Institut für Histologie und
Embryologie, Karl-Franzens-
Universität Graz, Graz, Austria

*This article is dedicated to the memory of Prof. Dr Wilhelm Burkl, the former Director of the Institut für Histologie und Embryologie of the Karl-Franzens-Universität Graz, who died on 2 August 2001.

Address reprint requests to
Andreas Ziegler, Institut für
Immunogenetik,
Universitätsklinikum Charité,
Humboldt-Universität zu Berlin,
Spandauer Damm 130,
14050 Berlin, Germany.

E-mail: andreas.ziegler@charite.de

Submitted 13 September 2001;
accepted 27 September 2001.

INTRODUCTION

The ability to differentiate between 'self' and 'non-self' is an essential feature of the vertebrate immune system: highly polymorphic molecules encoded by the major histocompatibility complex (MHC) present peptides to thymus-selected T cells. Following thymic selection, these T cells are only able to recognize self MHC molecules complexed with peptides of non-self origin.¹ As specific alleles of MHC class I and II loci determine resistance against various pathogens, including viruses and parasites, the fittest individuals will be those exhibiting maximal heterozygosity at polymorphic MHC loci.^{2,3} Consequently, animals such as mice⁴⁻⁶ or rats,^{7,8} but also humans^{9,10} favor MHC [in humans, human leukocyte antigen (HLA)]-dissimilar partners. The avoidance of inbreeding is a likely added benefit.^{11,12} It has been pointed out that the relative importance of these two mate-choice strategies probably depends on the natural population structure of the species in question.¹³ For example, females of three-spined sticklebacks (*Gasterosteus aculeatus*) do not seem to care whether a potential mate carries other MHC alleles than they themselves, but instead prefer male fish suitable to produce offspring with an optimal (i.e. usually large) number of MHC class II alleles; as a rule, males with fewer alleles are considered less attractive.¹³ On the other hand, in species such as rodents or humans, disassortative mating may primarily serve to avoid inbreeding.

There is a large body of evidence supporting the importance of olfaction in mate choice: in vertebrates, the degree to which individuals are attracted toward each other depends on polymorphic loci within their MHC or closely linked genes.^{4-10,13} These observations on animals have been recently extended also to humans, showing that a human odor was rated the more 'attractive', the fewer HLA antigens were shared by the provider of the odor and its recipient.¹⁴ In support of a role for olfactory stimulants in choosing the 'right' partner, it has even been demonstrated that perfume preferences correlate with the individual's HLA type.¹⁵

In the mammalian species investigated so far, olfactory individuality is apparently due to MHC class I proteins, their breakdown products or other molecules associated with them, which occur in sweat, urine and serum.^{9,14,16-18} Within the olfactory epithelium, these odorants are perceived by olfactory receptors (OR), which belong to the superfamily of G protein-coupled receptors (GPCR). About 1000 typical OR genes (of the 'major olfactory epithelium' type) have been discovered in rodents, and in man more than 900 OR genes exist, possibly as many as 3% of all genes. About 60% of the human OR loci are likely to

be pseudogenes with unclear functional status.^{19,20} OR genes occur in clusters on most human chromosomes, and one of these clusters is HLA-linked (Fig. 1).²¹⁻²⁴ It contains 34 typical OR gene sequences, organized into two subclusters. In addition, five OR loci belonging to a different GPCR family, the type 1 vomeronasal receptor genes (VIR)²⁵ are located telomeric, in the vicinity of the second subcluster (Fig. 1)²⁴ (Horton et al., unpublished data).

All of the potentially functional OR genes within the HLA-linked cluster were found to be polymorphic, resulting in an even higher number of haplotypes for the OR genes than for the closely linked HLA class I loci in the panel of HLA homo- or hemizygous cell lines investigated.²³ Ligands for the products of HLA-linked OR loci are currently unknown. Nevertheless, it is tempting to speculate that a functional connection between MHC and linked OR loci may exist.^{4,21,23,26,27} This idea is supported by the retention of MHC-OR gene linkage in species that are evolutionarily as separate as humans and mice.²⁴

Linkage of genes controlling mating preferences is not uncommon in other phyla. For example, in the mushrooms *Schizophyllum commune* and *Coprinus cinereus*, the large number of mating type specificities are the result of tightly linked arrays of genes for polymorphic pheromone receptors and pheromones^{28,29} which ensure that self-fertilization is prevented, and furthermore allow efficient selection of a genetically different mating partner. Comparable processes operate in several invertebrates as well, where polymorphic interaction proteins on gametes control self-non-self recognition, as e.g. in molluscs³⁰ or sea urchins.³¹ In addition, a cytosolic molecular complex typically associated with the provision of antigenic fragments for presentation by MHC class I molecules, the proteasome, is involved in preventing self-sterility of the ascidian *Ciona intestinalis*.³² A direct role for MHC genes or MHC-linked loci in reproduction is also suggested by the demonstration that mouse oocytes can 'select' sperm bearing a foreign MHC haplotype.^{33,34} Currently, this observation lacks an explanation, because there is growing evidence that spermatozoa do not express MHC molecules,^{35,36} despite some findings indicating the contrary. However, it is conceivable that polymorphic, sperm-expressed products of MHC-linked OR genes might indirectly, by linkage disequilibrium, signal the MHC haplotype carried by a spermatozoon.

Self-non-self discrimination appears therefore as the most important single feature linking the vertebrate immune system and reproduction. Violation of the mechanisms ensuring recognition of self and non-self will lead to autoimmunity or elevated pathogen susceptibility together with an increased risk of

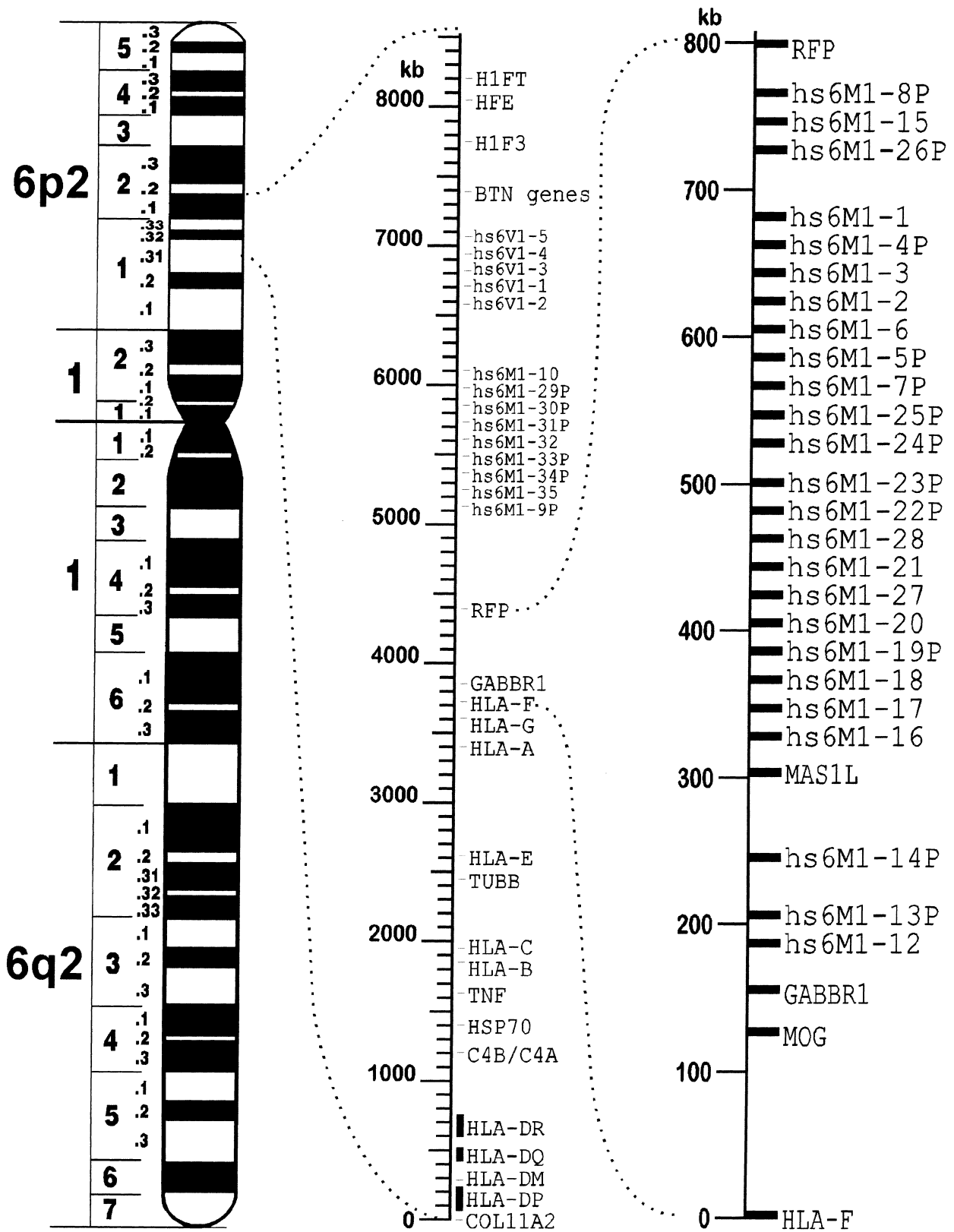


Fig. 1. Human chromosome 6 ideogram. In addition to the HLA complex, all identified OR genes in the vicinity of the HLA complex (hs6M1-1 to -10, and hs6M1-12 to -35P, and the five VIR loci (hs6V1-1 to -5)), and their approximate location on the physical map are indicated. For details, see the article by Younger and colleagues.²⁴

inbreeding, respectively. Presence of OR transcripts has already been found in male germ cells of several mammals, including man, and a role for sperm-expressed OR, e.g. in chemotaxis, been proposed.³⁷⁻⁴³ To support the suggestion of a functional connection between HLA antigens and OR,^{4,26} we analyzed whether HLA-linked OR loci were transcribed in testicular tissue. Furthermore, we investigated the expression of HLA class I molecules and β_2 -microglobulin (β_2m) by male germ cells. The results lead us to suggest that only those OR will be expressed by spermatozoa that have successfully passed a testicular selection step in which self HLA class I heavy chains participate.

METHOD OF STUDY

For immunohistology, tissues from 15 normal human testes were cryopreserved or paraffin-embedded. Sections were immunostained with monoclonal antibodies (mAb) directed either against different epitopes on HLA class I free heavy chains (LA45, L31, HC-10, HC-A2), β_2m (BBM.1 and conventional antisera), or various HLA class I/ β_2m complexes (W6/32.HL, 87G, ME-1), including also appropriate control mAb like W6/32.HK (for details, see Hutter et al., unpublished data).

In the absence of specific antibodies against OR proteins, the transcriptional status of OR genes from the HLA-linked cluster as well as two OR loci from chromosomes 7 and 17 (hs7M1-2 and hs17M1-20, respectively) was assessed using multitissue RNA blots, screening of a cDNA library from testis, polymerase chain reaction (PCR), and rapid amplification of cDNA ends (RACE) procedures on commercial samples of RNA. Sequencing was carried out using a Model 4000 DNA Sequencer (LI-COR,

Lincoln, NE, USA) instrument. Primers were designed based on the genomic sequence previously determined.²⁴ The specificity of the primers was checked by alignment with all known human OR sequences. The genomic locations of the 5'- and 3'-ends of cDNAs from OR genes were assigned by aligning the experimentally determined cDNA sequences with genomic DNA. A list of the primers employed, as well as a detailed description of all experimental procedures can be obtained elsewhere^{23,24} (see also Volz et al., unpublished data).

RESULTS AND DISCUSSION

The presence of HLA molecules within the testis has already been analyzed in several studies, using molecular biological methods and antibodies (reviewed by³⁶), but typically only extratubular expression of HLA proteins was found (see e.g.⁴⁴). However, these results relied on the use of reagents like the mAb W6/32⁴⁵ which is known to detect HLA class I heavy chain (HC) complexed with β_2m . It was therefore possible that free HC, with the possible exception of HLA-E,⁴⁶ had remained undetected. We employed a panel of mAb with differential reactivity towards free and β_2m -complexed HC, as well as against β_2m (Table I).

The results indicate that conventional HLA class I HC/ β_2m molecules, most likely peptide-complexed (see for example Fig. 2),⁴⁷ are only present in cells from the extratubular epithelium, whereas intratubular cells, including spermatozoa and Sertoli cells, were invariably unreactive with the respective mAb, e.g. W6/32.HL. In line with this expression pattern was the reactivity of β_2m -specific reagents which demonstrated the absence of β_2m from seminiferous epithelium, but strong expression by extratubular cells. However, different types of free HLA class I HC were found in

TABLE I. Expression of Free and β_2m -Complexed HLA Class I Heavy Chains in Testicular Tissue

Molecule	Testicular tissue						
	Extratubular epithelium	Tubular epithelium					
		Sgonia	Scytes I	Scytes II	Stids	Sperm	Sertoli cells
HLA class I HC/ β_2m complexes	+++	-	-	-	-	-	-
β_2m	+++	-	-	-	-	-	-
HLA class I HC	+	+/- ^a	+++	+	+/- ^a	-	-

Sgonia: spermatogonia, Scytes I, II: first and second order spermatocytes; Stids: spermatids.

Staining intensity: + + +, very strong; +, moderate; +/-, about 30% of cells weak.

^a *Subsets of spermatogonia and spermatids were weakly reactive only with the mAb HC-A2 (anti-HLA-A, -G, -E, some -C, -B73).*

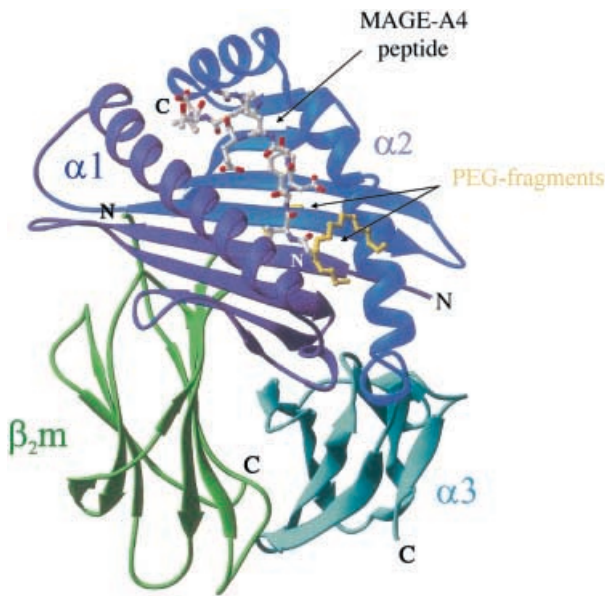


Fig. 2. Structure of the HLA-A*0201 molecule in complex with a decameric MAGE-A4-derived peptide as determined by X-ray crystallography at 1.4 Å resolution.⁴⁷ MAGE-A4 is a protein that is normally expressed only in seminiferous tubules, where it cannot be presented to T cells by HLA molecules. However, certain types of tumors, including melanoma, express MAGE-A4, and a number of HLA class I molecules like HLA-A2 may present MAGE-A4-derived peptides to cytotoxic T lymphocytes. As the reported antigenic complex should be strictly tumor specific, it is considered to be an ideal target for immunotherapy.

considerable amounts in the more immature cell types of spermiogenesis, in particular first order spermatocytes. It appeared that the staining was not restricted to the cell membranes, but was distributed throughout the cytoplasm. This expression pattern was observed with three different mAb, while HC-A2, the reagent with the broadest reactivity, exhibited more extensive staining, which included also some spermatogonia and spermatids. Spermatozoa and Sertoli cells, on the other hand, were always negative. It is likely that HC from the following loci were expressed: HLA-B, -C, -E and probably also -A (Hutter et al., unpublished data). Our results confirm earlier studies with regard to expression of intact, β_2m -associated HLA molecules and β_2m , but in addition demonstrate the presence of various free, polymorphic class I HC in cells from certain stages of male germ cell development.

To our knowledge, this exclusive expression of free HLA class I HC in male germ cells has never been observed in any other human tissue. According to present concepts, HLA class I molecules can only perform an immunologically meaningful function when they are associated with β_2m , and occur on the surface of antigen-presenting cells. Therefore, antigen presentation does not seem to make much sense within seminiferous tubules of the normal testis, where

lymphocytes with T cell receptors (TCR) or natural killer cells with their various receptors (e.g. killer cell immunoglobulin-like receptors, KIR; leukocyte immunoglobulin-like receptors, LIR) are lacking. It has been pointed out that HC contain a number of epitopes that allow the molecules to interact with a variety of different cell surface-expressed ligands:⁴⁸ TCR, CD8, KIR, LIR, and CD94/NKG2A, but all these natural ligands appear to require the intact HLA class I molecule for interaction, and there is no evidence for their expression in seminiferous tissue. We regard it also as unlikely that the pronounced expression of class I HC in male germ cells is an artifact, because the results rely on several different reagents and include appropriate controls. Furthermore, accidental expression of HLA HC in such large quantities, and at such defined cellular sites, appears very improbable. Therefore, it is likely that the expression fulfills another purpose. As the conserved linkage of MHC and OR genes suggests a functional connection,^{4,21,26,27} we explored the possibility that HLA-linked OR genes might be expressed in the testis, allowing a possible interaction with HLA class I HC.

Multi-tissue RNA blots showed clear-cut expression of hs6M1-16 transcripts in testis, kidney and lungs, while the analysis of all other OR tested resulted only in very weak expression in several tissues or no expression at all. Furthermore, cDNA from only a single OR gene, hs6M1-32, was obtained by screening a testis cDNA library. As no other OR transcripts were found in the library, we decided to analyze testicular OR expression by RACE with cDNA prepared from testis RNA. The following OR genes were found to be expressed: hs6M1-3, -6, -16, -18, -20, -21, -27, and hs17M1-20. Like hs6M1-32 isolated from the cDNA library, the transcripts of hs6M1-3, -6, and -20 consisted only of the single coding exon. However, an entirely different situation was seen with hs6M1-21, -27, and -18 (OR genes with identical transcriptional orientation, located on 6p in the order shown,²⁴ see also Fig. 1): transcripts from these loci occurred in several different splice variants, and exhibited extensive 5' exon sharing among each other. Furthermore, the most distant 5' exon of hs6M1-21 was more than 100 kb away from the coding exon, so that the unspliced hs6M1-21 transcript is expected to include five additional OR genes (hs6M1-17, -18, -19P, -20, and -27,²⁴ Fig. 1). The hs6M1-16 gene was located directly centromeric of the hs6M1-17 locus, in opposite transcriptional orientation. hs6M1-16 transcripts contained up to four 5' non-coding exons, and up to two additional 3' non-coding exons. Furthermore, alternative splicing, resembling that identified during the expressed sequence tag (EST)-database searches,²⁴ was found for the hs6M1-16 gene. These variants are

expected to result in an N-terminally truncated OR which may be functional.^{24,49}

Therefore, several HLA-linked OR genes are clearly transcribed in testicular tissue, where some of them may be subject to most unusual transcriptional control and splice mechanisms. These results extend those obtained previously by studying testicular OR gene expression, and show that more than 50 different OR genes must be expressed by human testis. *In-situ* hybridizations and analysis of samples enriched for certain tubular cells have shown that OR are primarily expressed by a subset of spermatocytes and early spermatids.^{37-39,41-43} In addition, studies with canine male germ cells have shown that OR genes are not only transcribed, but also translated, and are finally expressed by spermatozoa.³⁸ It appears very likely that OR fulfill a specific function in male germ cell development, or act as chemoreceptors on the surface of spermatozoa.^{37-39,43} Theoretical considerations have even led to the suggestion that testis-expressed OR might become involved in male-male competition and female choice, and that seminal fluid could contain substances to which sperm of other males might be vulnerable.⁵⁰ Tatsura and colleagues⁴³ have expressed similar ideas, and they discuss also mouse VIR genes which they found to be expressed selectively by spermatids, but not by any other cell type within the testis.

The parallel expression of polymorphic HLA class I HC genes and many OR loci within spermatocytes, of which at least the HLA-linked OR loci are also polymorphic, has led us to advance the idea that linkage of HLA and OR genes could have evolved to play a crucial role during sperm maturation and fertilization [the 'sperm-receptor-selection (SRS) hypothesis'], and favor olfaction-driven mate choice, too. Our hypothesis assumes that 'foreign', but not 'self' HLA class I proteins and/or their fragments can interact with the products of sperm-expressed OR genes, a condition which will be met only if olfaction-guided mate choice has brought HLA-different partners (with HLA types 'W' and 'Z' for simplicity) together. It is this type of interplay of the products of closely linked genes which we had in mind when we suggested that the MHC and linked OR genes might constitute an 'Immuno-Olfactory Supercomplex' (IOS).²⁶

The SRS hypothesis is supported by observations made in man and various mammals: (1) in spermatocytes within seminiferous epithelium of the testis, different polymorphic HLA class I HC (of 'W' type), but not β_2m , are expressed (this study); (2) a large number of OR genes, including many polymorphic HLA-linked OR loci, are expressed in these cells as well, although most likely not the whole set available

for expression in olfactory epithelium³⁷⁻⁴³ (this study); (3) the seminal fluid contains soluble intact, β_2m -associated HLA class I molecules (of 'W' type);⁵¹ (4) OR can be expressed on sperm;³⁸ (5) HLA class I molecules (of 'Z' type) are expressed in large amounts on cells surrounding the oocyte, i.e. granulosa cells.⁵²

We postulate that HLA class I HC and OR interact with each other during spermiogenesis, leading to the elimination of self HLA-reactive OR and retention of potentially non-self-reactive OR for expression on mature spermatozoa. The type of testicular selection which we envisage will prevent reaction of spermatozoa with soluble 'W' type HLA molecules within the seminal fluid, but allow interaction with soluble HLA molecules (of 'non-W' type) within the female genital tract. For example, HLA-positive granulosa cells^{52,53} surrounding the HLA-negative oocyte⁵² might shed soluble HLA class I molecules (of 'Z' type) into the fluid of the follicle or the fallopian tube, where they could form a chemoattractive gradient.⁵⁴ This could facilitate fertilization of oocytes by HLA-different sperm, whereas fertilization may be less likely in partners with related HLA types. Furthermore, soluble HLA molecules (of 'W' type) from the seminal fluid have the potential to interact, within the female genital tract, with 'foreign' spermatozoa from a copulation with a second male, because these will most likely carry OR which have undergone testicular immuno-selection on HLA class I HC of 'non-W' type. This mechanism might contribute to successful competition of spermatozoa from the first male with those from the second,⁵⁰ possibly by blocking interaction of the second male's spermatozoa with female HLA molecules (of 'Z' type). Soluble HLA molecules in seminal plasma might therefore be the 'decoy' compounds envisaged by Branscomb and coworkers.⁵⁰

The SRS hypothesis allows to predict the outcome for interactions of sperm-expressed OR selected on self HLA molecules: (1) fertilization of oocytes by spermatozoa from an HLA-different partner will be favored, making the generation of HLA-heterozygous offspring more likely; (2) fertilization of oocytes by spermatozoa from an HLA-similar partner will be discouraged, making the generation of HLA-homozygous offspring less likely; (3) interaction of sperm with soluble self HLA molecules will be prevented (clearly a prerequisite for their successful navigation through seminal plasma filled with self HLA molecules); (4) interaction of foreign sperm with HLA molecules within the seminal plasma provided by the first mating partner is very probable, making fertilization of the oocyte less likely, and in species without development of a vaginal plug after copulation, such as humans, fertilization of the oocyte by sperm from a second male would be discouraged.

In reality, the situation will be more complicated than outlined above, because the mating partners might share HLA specificities, or even complete HLA haplotypes. In these cases, it could be that the number of OR available for interaction with non-self female HLA molecules is just diminished. The existence of HLA-homozygous individuals proves of course that an interaction between gametes with identical HLA haplotypes can take place. Furthermore, it is very likely that HLA class I molecules are synthesized in codominant fashion by spermatocytes, so that the products of HLA-linked OR genes are most likely not expressed in a haplotype-specific manner by individual spermatozoa. It is therefore improbable that the oocyte can recognize the sperm HLA haplotype via sperm surface-expressed OR. Instead, MHC-dependent sperm choice by oocytes^{33,34} may be a consequence of the interaction of polymorphic female- and male-derived proteins within the fertilized oocyte. The formation and maintenance of the sexually fertile dikaryon of *C. cinereus* provides an example for such processes.²⁹

How plausible is the SRS hypothesis? First of all, the hypothesis offers a logical explanation for the intriguing observation of HLA class I HC expression by spermatocytes. We have pointed out before that there is no 'classical' immunologic reason for the expression of polymorphic HC within an immunologically privileged site such as the seminiferous epithelium, although the expression of HLA-E HC in male germ cells (but not sperm) has been taken as an indication of tolerance induction.⁴⁶ Because there are no cells within the tubuli toward which tolerance might have to be developed, and also no cells which could develop tolerance, we regard our hypothesis as more convincing. A second point in favor of the SRS hypothesis is that it offers a simple way to integrate the universal concept of self–non-self recognition, whose importance in the reproduction of plants and invertebrates can hardly be overstated, also into vertebrate reproductive biology. According to the hypothesis, mammalian germ cells rely for these recognition processes on proteins, namely MHC and OR proteins, with many properties typically observed for such interaction molecules: (1) they are highly polymorphic; (2) some of them are genetically linked and might evolve in tandem; (3) they are obligatory membrane-bound molecules (only OR) with the properties expected of chemoreceptors; (4) they can occur in soluble form (only MHC molecules), facilitating the production of gradients, along which a chemoattracted cell could migrate. Remarkably, both types of molecules perform also seemingly unrelated, complex recognition functions within the immune system and olfaction.

Finally, the SRS hypothesis can be put to the test: (1) immunohistological analysis with OR-specific

antibodies would show which of the OR transcripts give rise to proteins; (2) immunoprecipitations on selected cell populations from the testis could be employed to demonstrate interaction of HLA and OR proteins; (3) the reactivity of spermatozoa with various soluble HLA/ β_2m /peptide complexes could be assessed by immunohistochemical tests; (4) the chemotaxis of purified spermatozoa from males with different HLA types could be investigated with various soluble HLA molecules or by mucus-penetration tests with material from HLA-typed women; (5) follicle fluid and fallopian fluid should be investigated with regard to soluble HLA molecules.

Our hypothesis might also contribute to an understanding of a number of observations which so far lack an explanation. For example, no convincing explanation has been offered for the retention of seemingly disadvantageous, autoimmune disease-associated HLA/OR haplotypes in Caucasians like HLA-A*0101–B*0801–DR3, in which the linkage disequilibrium extends as far as HFE (the hemochromatosis gene),⁵⁵ about four megabase pairs telomeric of HLA-A (Fig. 1). If, however, this haplotype would ensure complete lack of interaction of HLA–A1 and –B8 HC with OR that are part of the extended haplotype, then a selective advantage for OR-bearing sperm might exist, because they will have the chance to carry a higher number of HLA-linked OR species, making them potentially fitter for interactions with non-self HLA molecules. It is also possible that unexplained infertility could be related to the HLA–OR interactions which we postulate, although genes within the HLA class II region are thought to be responsible.⁵⁶

We would also like to point out that the SRS hypothesis shares a number of features with one of the central processes in the immune system, thymic T cell selection. In both selection processes, polymorphic MHC molecules play a crucial role, finally allowing cells (T cells, spermatozoa) bearing the molecules which are the selection targets (TCR, OR) to distinguish between 'self' and 'foreign'. Studies on more primitive vertebrates and their direct evolutionary ancestors will also tell whether MHC molecules performed a function initially within the reproductive or the immune systems. We regard the first possibility as more likely, but there is currently no hard evidence to support this suggestion.

In conclusion, MHC-dependent selection of OR-bearing sperm could support mate choice by introducing a further safeguard to favor the production of MHC-heterozygous offspring. Therefore, we postulate that mammals (and possibly other vertebrates as well) have to overcome two selective barriers to produce offspring: one barrier acts at

the level of the organism, and includes olfaction-driven mate choice,⁴⁻⁸ while the other barrier comprises of receptors on male germ cells (which we assume are OR) and potentially interacting ligands supplied by the female (most likely soluble MHC molecules). We predict that this interaction will only function efficiently when the receptors on spermatozoa have been immuno-selected in the testis to disregard self MHC molecules, and are thus able to respond only when non-self MHC molecules are encountered. This double barrier would fulfill a most useful biological function in favoring both inbreeding avoidance as well as MHC heterozygosity.

Acknowledgments

This work was supported by the European Union and the Volkswagen Foundation (I/75 196). We are grateful to Dr Manfred Milinski, Plön, for encouraging discussions and Mrs Astrid Blaschitz, Graz, for excellent technical assistance.

REFERENCES

- Jameson SC, Bevan MJ: T-cell selection. *Curr Opin Immunol* 1998; 10:214-219.
- Hamilton WD, Axelrod R, Tanese R: Sexual reproduction as an adaptation to resist parasites (a review). *Proc Natl Acad Sci USA* 1990; 87:3566-3573.
- Brown JL: A theory of mate choice based on heterozygosity. *Behav Ecol* 1997; 8:60-66.
- Yamazaki K, Boyse EA, Miké V, Thaler HT, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, Thomas L: Control of mating preference in mice by genes in the major histocompatibility complex. *J Exp Med* 1976; 144:1324-1335.
- Yamazaki K, Yamaguchi M, Baranoski L, Bard J, Boyse EA, Thomas L: Recognition among mice. Evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. *J Exp Med* 1979; 150:755-760.
- Penn D, Potts W: How do major histocompatibility complex genes influence odor and mating preferences? *Adv Immunol* 1998; 69:411-436.
- Singh PB, Brown RE, Roser B: MHC antigens in urine as olfactory recognition cues. *Nature* 1987; 327:161-164.
- Brown RE, Roser B, Singh PB: Class I and class II regions of the major histocompatibility complex both contribute to individual odors in congenic inbred strains of rats. *Behav Genet* 1989; 19:659-674.
- Wedekind C, Seebeck T, Bettens F, Paepke AJ: MHC-dependent mate preferences in humans. *Proc R Soc Lond B* 1995; 260:245-249.
- Ober C, Weitkamp LR, Cox N, Dytch H, Kostyu D, Elias S: HLA and mate choice in humans. *Am J Hum Genet* 1997; 61:497-504.
- Latter BD, Sved JA: A reevaluation of data from competitive tests shows high levels of heterosis in *Drosophila melanogaster*. *Genetics* 1994; 137:509-511.
- Meagher S, Penn DJ, Potts WK: Male-male competition magnifies inbreeding depression in wild house mice. *Proc Natl Acad Sci USA* 2000; 97:3324-3329.
- Reusch TBH, Haeberli A, Aeschlimann PB, Milinski M: Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* 2001; 414:300-302.
- Wedekind C, Furi S: Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proc R Soc Lond B* 1997; 264:1471-1479.
- Milinski M, Wedekind C: Evidence for MHC-correlated perfume preferences in humans. *Behav Ecol* 2001; 12:140-149.
- Singer AG, Beauchamp GK, Yamazaki K: Volatile signals of the major histocompatibility complex in male mouse urine. *Proc Natl Acad Sci USA* 1997; 94:2210-2214.
- Yamazaki K, Beauchamp GK, Singer A, Bard J, Boyse EA: Odortypes: their origin and composition. *Proc Natl Acad Sci USA* 1999; 96:1522-1525.
- Montag S, Frank M, Ulmer H, Wernet D, Göpel W, Rammensee H-G: 'Electronic nose' detects major histocompatibility complex-dependent pre-natal and post-natal odor components. *Proc Natl Acad Sci USA* 2001; 98:9249-9254.
- Glusman G, Yanai I, Rubin I, Lancet D: The complete human olfactory subgenome. *Genome Res* 2001; 11:685-702.
- Zozulya S, Echeverri F, Nguyen T: The human olfactory receptor repertoire. *Genome Biol* 2001; 2:1-12.
- Ziegler A, Ehlers A, Forbes S, Trowsdale J, Uchanska-Ziegler B, Volz A, Younger R, Beck S: Polymorphic olfactory receptor genes and HLA loci constitute extended haplotypes. *In* Major Histocompatibility Complex: Evolution, Structure and Function, M Kasahara (ed.). Tokyo, Springer Verlag, 2000, pp. 110-130.
- Ziegler A, Ehlers A, Forbes S, Trowsdale J, Volz A, Younger R, Beck S: Polymorphism in olfactory receptor genes: a cautionary note. *Hum Immunol* 2000; 61:1281-1284.
- Ehlers A, Beck S, Forbes S, Trowsdale J, Volz A, Younger R, Ziegler A: MHC-linked olfactory receptor loci exhibit polymorphism and contribute to extended HLA/OR haplotypes. *Genome Res* 2000; 10:1968-1978.
- Younger RM, Amadou C, Bethel G, Ehlers A, Fischer Lindahl K, Forbes S, Horton R, Milne S, Mungall AJ, Trowsdale J, Volz A, Ziegler A, Beck S: Characterisation of clustered MHC-linked olfactory receptor genes in human and mouse. *Genome Res* 2001; 11:519-530.
- Dulac C, Axel R: A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 1995; 83:195-206.
- Ziegler A: Biology of chromosome 6. *DNA Sequence* 1997; 8:189-202.
- Isles AR, Baum MJ, Ma D, Keverne EB, Allen ND: Urinary odour preferences in mice. *Nature* 2001; 409:783-784.
- Vaillancourt LJ, Raudaskoski M, Specht CA, Raper CA: Multiple genes encoding pheromones and a pheromone receptor define the B β 1 mating-type specificity in *Schizopyllum commune*. *Genetics* 1997; 146:541-551.
- Halsall JR, Milner MJ, Casselton LA: Three subfamilies of pheromone and receptor genes generate multiple B

- mating specificities in the mushroom *Coprinus cinereus*. *Genetics* 2000; 154:1115–1123.
30. Swanson WJ, Vacquier VD: Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. *Science* 1998; 281:710–712.
 31. Palumbi SR: All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc Natl Acad Sci USA* 1999; 96:12632–12637.
 32. Marino R, De Santis R, Giuliano P, Pinto MR: Follicle cell proteasome activity and acid extract from the egg vitelline coat prompt the onset of self-sterility in *Ciona intestinalis*. *Proc Natl Acad Sci USA* 1999; 96:9633–9636.
 33. Wedekind C, Chapuisat M, Macas E, Rüllicke T: Non-random fertilization in mice correlates with the MHC and something else. *Heredity* 1996; 77:400–409.
 34. Rüllicke T, Chapuisat M, Homberger FR, Macas E, Wedekind C: MHC-genotype of progeny influenced by parental infection. *Proc R Soc Lond B* 1998; 265:711–716.
 35. Desoye G, Dohr GA, Ziegler A: Expression of human major histocompatibility antigens on germ cells and early preimplantation embryos. *Lab Invest* 1991; 64:306–312.
 36. Hutter H, Dohr G: HLA expression on immature and mature human germ cells. *J Reprod Immunol* 1998; 38:101–122.
 37. Parmentier M, Libert F, Schurmans S, Schiffmann S, Lefort A, Eggerickx D, Ledent C, Mollereau C, Gérard C, Perret J, Grootegoed A, Vassart G: Expression of members of the putative olfactory receptor gene family in mammalian germ cells. *Nature* 1992; 355:453–455.
 38. Vanderhaeghen P, Schurmans S, Vassart G, Parmentier M: Olfactory receptors are displayed on dog mature sperm cells. *J Cell Biol* 1993; 123:1441–1452.
 39. Walensky LD, Roskams AJ, Lefkowitz RJ, Snyder SH, Ronnett GV: Odorant receptors and desensitization proteins colocalize in mammalian sperm. *Mol Med* 1995; 1:130–141.
 40. Vanderhaeghen P, Schurmans S, Vassart G, Parmentier M: Molecular cloning and chromosomal mapping of olfactory receptor genes expressed in the male germ line: evidence for their wide distribution in the human genome. *Biochem Biophys Res Commun* 1997; 237:283–287.
 41. Vanderhaeghen P, Schurmans S, Vassart G, Parmentier M: Specific repertoire of olfactory receptors in the male germ cells of several mammalian species. *Genomics* 1997; 39:239–246.
 42. Walensky LD, Ruat M, Bakin RE, Blackshaw S, Ronnett GV, Snyder SH: Two novel odorant receptor families expressed in spermatids undergo 5'-splicing. *J Biol Chem* 1998; 273:9378–9387.
 43. Tatsura H, Nagao H, Tamada A, Sasaki S, Kohri K, Mori K: Developing germ cells in mouse testis express pheromone receptors. *FEBS Lett* 2001; 488:139–144.
 44. Guillaudeux T, Gomez E, Onno M, Drénou B, Segretain D, Alberti S, Lejeune H, Fauchet R, Jégou B, Le Bouteiller P: Expression of HLA class I genes in meiotic and post-meiotic human spermatogenic cells. *Biol Reprod* 1996; 55:99–110.
 45. Barnstable CJ, Bodmer WF, Brown G, Galfrè G, Milstein C, Williams AF, Ziegler A: Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens – new tools for genetic analysis. *Cell* 1978; 14:9–20.
 46. Fiszer D, Ulbrecht M, Fernandez N, Johnson JP, Weiss EH, Kurpisz M: Analysis of HLA class Ib gene expression in male gametogenic cells. *Eur J Immunol* 1997; 27:1691–1695.
 47. Hillig RC, Coulie PG, Stroobant V, Saenger W, Ziegler A, Hülsmeier M: High resolution structure of HLA-A*0201 in complex with a tumour-specific antigenic peptide encoded by the MAGE-A4 gene. *J Mol Biol* 2001; 310:1167–1176.
 48. Sawicki MW, Dimasi N, Natarjan K, Wang J, Margulies DH, Mariuzza RA: Structural basis of MHC class I recognition by natural killer cell receptors. *Immunol Rev* 2001; 181:52–65.
 49. Ling K, Wang P, Zhao J, Wu Y-L, Cheng Z-J, Wu G-X, Hu W, Ma L, Pei G: Five transmembrane domains appear sufficient for a G protein-coupled receptor: functional five-transmembrane domain chemokine receptors. *Proc Natl Acad Sci USA* 1999; 96:7922–7927.
 50. Branscomb A, Seger J, White RL: Evolution of odorant receptors expressed in mammalian testes. *Genetics* 2000; 156:785–797.
 51. Koelman CA, Coumans ABC, Nijman HW, Doxiadis IIN, Dekker GA, Claas FHJ: Correlation between oral sex and a low incidence of preeclampsia: a role for soluble HLA in seminal fluid? *J Reprod Immunol* 2000; 46:155–166.
 52. Dohr GA, Motter W, Leitinger S, Desoye G, Urdl W, Winter R, Wilders-Truschnig MM, Uchanska-Ziegler B, Ziegler A: Lack of expression of HLA class I and class II molecules on the human oocyte. *J Immunol* 1987; 138:3766–3770.
 53. Desoye G, Dohr GA, Motter W, Winter R, Urdl W, Pusch H, Uchanska-Ziegler B, Ziegler A: Lack of HLA class I and II antigens on human preimplantation embryos. *J Immunol* 1988; 140:4157–4159.
 54. Eisenbach M, Tur-Kaspa I: Do human eggs attract spermatozoa? *BioEssays* 1999; 21:203–210.
 55. Cattley SK, Williamson JF, Tay GK, Martinez OP, Gaudieri S, Dawkins RL: Further characterization of MHC haplotypes demonstrates conservation telomeric of HLA-A: update of the 4AOH and 101HW cell panels. *Eur J Immunogenet* 2000; 27:397–426.
 56. Gill TJ III: Mechanisms of action of major-histocompatibility-complex-linked genes affecting reproduction. *Am J Reprod Immunol* 1999; 41:23–33.