[Frontiers in Bioscience 3, d739-745, July 27, 1998]

EVOLUTION OF THE HUMAN *HLA-DR* REGION

Göran Andersson

Uppsala Genetic Center, Box 7080, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Received 6/10/98 Accepted 6/12/98

TABLE OF CONTENTS

1. ABSTRACT

The genomic organization and number of human *HLA-DRB* class II genes differs between haplotypes in the human population. In contrast, both *HLA-DQ* and *-DP* class II subregions are conserved. Understanding of the evolutionary relationship and age of *HLA-DR* haplotypes has been obtained by nucleotide sequence determination and phylogenetic analyses of intron sequences of all known *DRB* genes from different *DR* haplotypes. These analyses have indicated that the gene organization of some haplotypes has remained conserved for tens of millions of years and that novel haplotypes have originated during hominoid speciation. Thus, in the contemporary human population, both ancestral and species-specific *DRB* gene organizations are found. The plasticity of *DR* gene organization as well as the extensive allelic polymorphism of expressed DR-beta molecules are features which suggest that DR molecules are under distinct selective pressure compared to HLA-DQ and HLA-DP class II molecules. The extensive allelic polymorphism of HLA-DR transplantation antigens is exclusively provided by the beta-chain of these heterodimeric antigen presenting molecules. Most of this polymorphism appears to have been generated recently. Thus, evolutionary mechanisms involved in the preservation of ancestral gene organizations, selection against DR-alpha polymorphism, creation of DR-beta allelic polymorphism and generation of novel gene organizations are acting on the *HLA-DR* region simultaneously.

2. INTRODUCTION

Three distinct isoforms of class II major histocompatibility complex (MHC) molecules are expressed by human antigen presenting cells. They are denoted HLA-DR, -DQ, and -DP. The MHC class II proteins are heterodimers composed of an alpha-chain and a beta-chain. Class II molecules are type I membrane glycoproteins which present exogenous peptides that are recognized by T-cell receptors on helper T cells. Different alpha-chains are encoded by genetically distinct subsets of *A* genes and beta-chains by *B* genes. Human class II genes are located on the short arm of chromosome 6 at position 6p21.3 (1). The *HLA-DR* genes are located in the most telomeric part of the class II region (figure 1). Five distinct human *HLA-DR* haplotypes exist. These differ in the organization and number of *DRB* genes present on each *DR* haplotype (figure 1). Nine *DRB* loci have been described (*DRB1-DRB9*) (2).

The genetic distinction between *HLA-DR*, *-DQ*, and *-DP* genes is manifested by several structural differences in the most extracellular domain (3-4). Among these three different isoforms of class II molecules, which all functions as antigen presenting molecules, the HLA-DR molecules are distinct from DQ and DP molecules by several criteria: *i*) The *DRB1* locus is the most polymorphic class II locus known with approximately 180 alleles described (2). The *DRB1* locus encodes the beta1 chain of the DR molecule and it is expressed in all the five main human *DR* haplotypes. However, as will be discussed below, genetic evidence suggest that the *DRB1* locus may be split into five different subloci. *ii*) In addition to the *DRB1* locus, all *DR* haplotypes, except the *DR8* haplotype, carries a second expressed *DRB* locus (denoted *DRB3*, *DRB4* or *DRB5*). *iii*) In contrast to the polymorphic *HLA-DRB1* locus, the *HLA-DRA* gene, which encodes the alpha chain of the DR molecule is essentially monomorphic. This is true also for DP molecules where the DP-alpha chain only exhibits limited allelic polymorphism. In DQ molecules both the alpha and beta chains exhibit allelic polymorphism. *iv*) Unlike the *DQ* and *DP* regions, the number of *DRB* genes varies between different haplotypes (5). Thus, in the DR molecule, which is the predominantly expressed class II molecule on the cell surface of most antigen presenting cells, the polymorphism is exclusively located in the beta-chain. This might reflect that subtypes of HLA class II molecules are specialised for different purposes and that there are functional differences in peptide presentation and triggering of the immune response. Furthermore, there are two atypical human class II molecules which are encoded by the *DM* and *DO* loci (6-7). These class II molecules do not present peptide antigens but functions as regulators of antigen presentation (8).

In this review article the evolutionary relationship between the *HLA-DR* haplotypes and *DRB* genes will be

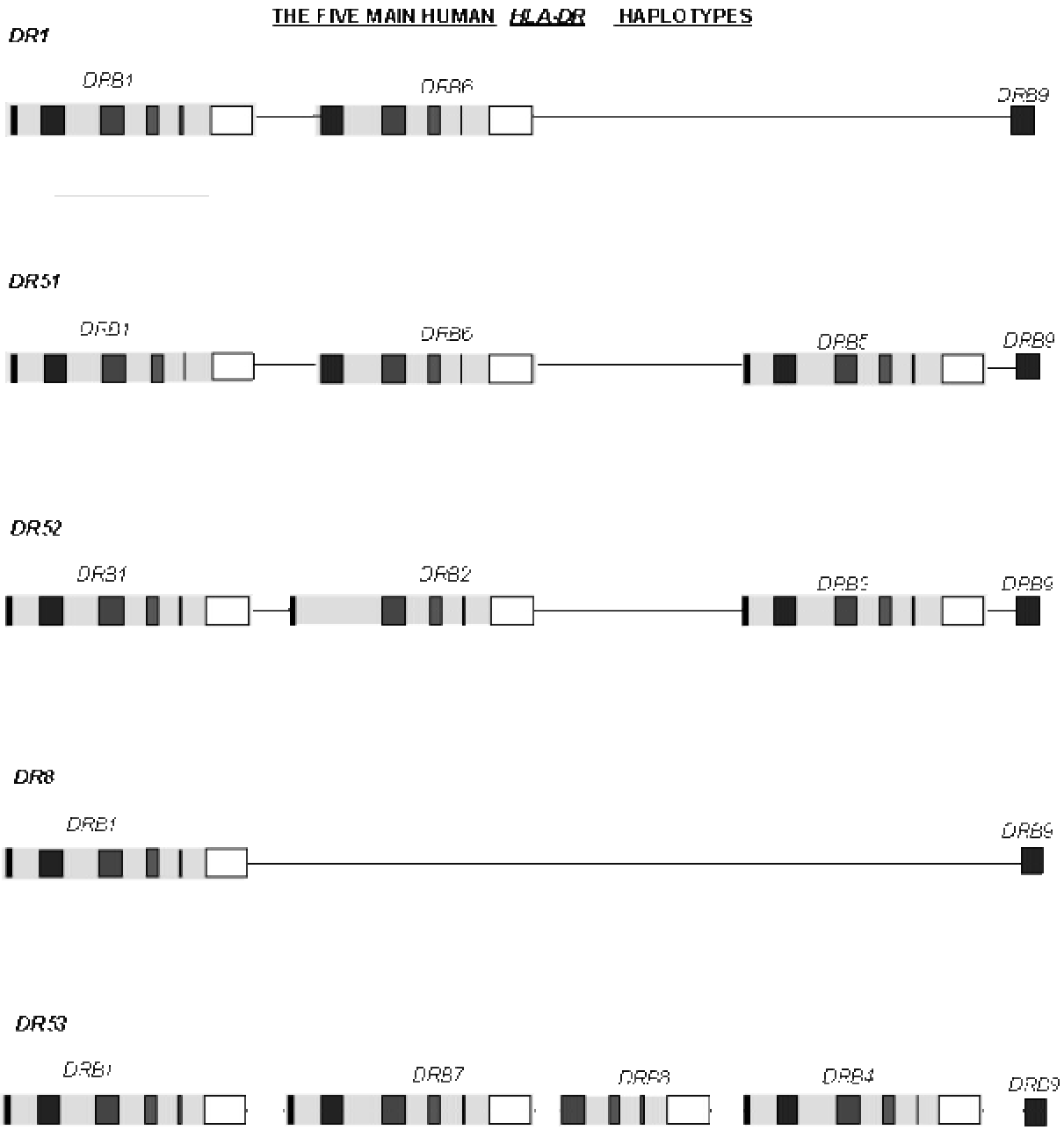

Figure 1. Schematic representation of the five human *HLA-DR* haplotypes. The exons of the *DRB* genes are shown in different shades of grey. Sizes of and distances between the genes are not drawn to scale. The *DRA* locus which is closely linked to the *DRB9* locus is not shown. Adapted in part from reference 45.

discussed based on the available genetic and molecular information.

3. GENETIC COMPLEXITY WITHIN THE HUMAN *HLA-DR* HAPLOTYPES

The non-polymorphic *DRA* gene is linked to a varying number of *DRB* genes in different *DR* haplotypes (5). Based on this variation, five different *DR* haplotypes have been identified (9). They are denoted *DR1, DR51, DR52, DR8* and *DR53*, respectively (figure 1, ref. 9). All *DR* haplotypes carry one expressed *DRB1* gene and except the *DR8* haplotype, another expressed *DRB* gene (*DRB3-5*) is expressed (figure 1). Besides this variation, a number of *DRB* pseudogene loci have been identified. These have been given the designations *DRB2, DRB6-9*) (figure 1).

According to the current MHC nomenclature,

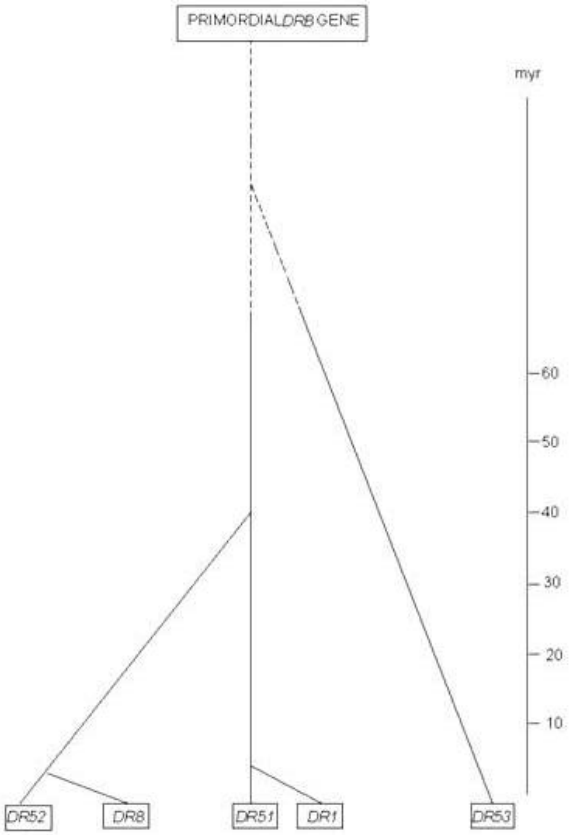

Figure 2. A model for the evolution of *HLA-DR* haplotypes. The estimated evolutionary time scale of the dendrogram is indicated to the right. The two main evolutionary branches were generated early during mammalian speciation. The split between the *DR52* and *DR8* as well as between *DR51* and *DR1* haplotypes occurred after hominoid speciation. Broken lines in the dendrogram indicate that it is not possible to correctly estimate the time point for the split between the two main evolutionary branches. Adapted from reference 13.

DRB1 sequences have been arranged into 13 different allelic lineages (groups of highly related alleles) (2). In phylogenetic analyses, *DRB1* sequences from these allelic lineages cluster within the five haplotypic groups from where they are encoded. The allelic lineages are denoted: *01 and *10 (the *DR1* group), *08 (the *DR8* group), *15 and *16 (the *DR51* group), *03, *11, *12, *13, and *14 (the *DR52* group), and *04, *07, and *09 (the *DR53* group). Thus, *DRB1* allelic lineages can be further grouped into five families which correspond to the five main haplotypic groups. The second expressed *DRB* loci (*DRB3*, *DRB4*, and *DRB5*) exhibit only limited allelic polymorphism in humans (2). This is another quite remarkable feature of *DRB* polymorphism since these loci are expressed largely at similar degree as *DRB1* genes and by the same antigen presenting cells. Moreover, they appear to bind similar types of peptides and similar selective pressures should be acting on these loci.

The *DR8* haplotype, is the least complex *DR* haplotype, which besides a *DRB1*08* gene also carry the *DRB9* pseudogene which is common to all *DR* haplotypes (10-11). Comparison of nucleotide sequences has revealed that the *DRB1* gene of the *DR8* haplotype was generated by a gene contraction event in a *DR52* haplotype (see ref. 34 and references therein). Thus, the promoter, exons 1 and 2 of this gene are highly similar to *DRB1* genes from the *DR52* haplotype. In contrast, the 3'-part of the gene closely resembles the *DRB3* gene from *DR52* haplotypes. This contraction occurred relatively recently on the evolutionary time scale. All other *DR* haplotypes contain either one of the second expressed functional *DRB* gene as well as one or more *DRB* pseudogenes (figure. 1).

In *DR51* haplotypes, the second expressed *DRB* locus is denoted *DRB5* and is located adjacent to a *DRB6* pseudogene. In the human MHC, the *DRB5* locus is unique to the *DR51* haplotype. However, *DRB5* genes are found in several distinct chimpanzee as well as gorilla *DR* haplotypes (26). In some of these haplotypes, the *DRB5* gene is linked to a *DRB6* gene but other combinations of *DRB* genes have been described in these primate species (26). Shared, species-specific as well as composite haplotypes between human, chimpanzee and gorilla appears to be a fundamental feature of the *DR* haplotypes which emphasizes the plasticity of this region during primate evolution.

The *DRB* genes present on the *DR1* haplotype (*DRB1*, *DRB6* and *DRB9*) are highly similar to those found in the *DR51* haplotype. The conspicuous difference between the *DR51* and *DR1* haplotypes is that the latter lacks the *DRB5* locus. The most likely explanation is that *DRB5* was deleted when the *DR1* haplotype emerged (34 and figure 2). *DR1* haplotypes have so far not been found in other primates and might hence be human-specific. In rare cases, human *DR* haplotypes have been described (albeit at low frequencies) which most likely are the result of recombinations between the main *DR* haplotypes (21-25). For example, *DR1*-like haplotypes which contain an expressed *DRB5* gene, which is otherwise unique to *DR51* haplotypes, have been described (figure 1, ref. 25). Furthermore, *DR51*-related haplotypes have been identified which appear to be the result of recombinations between the closely related *DR51* and *DR1* haplotypes (21-24).

The *DR52* haplotype constitutes what appears to be an ancestral haplotype, with the gene organization - *DRB1* - *DRB2* - *DRB3* - *DRB9*, that has remained evolutionary preserved at least some 40 million years (MYR) (13 and references therein). Besides its evolutionary persistence and presence in all great apes, novel *DR* haplotypes, like *DR8* (see above) have been generated recently from the *DR52* haplotype.

DR53 haplotypes have an evolutionary distinct organization of *DRB* genes (*DRB1* - *DRB7* - *DRB8* - *DRB4* - *DRB9*). Moreover, like *DR52* haplotypes, *DR53* haplotypes originated early during mammalian evolution. The *DRB7* and *DRB8* pseudogenes as well as the expressed *DRB4* gene are unique to this evolutionary branch (5, 20). The *DR53* haplotype in itself represents a distinct main evolutionary branch (figure 2). A *DR* haplotype with identical gene organization to the human *DR53* haplotype has been found in the chimpanzee MHC (26).

As indicated above, evidence for the plasticity of the *DR* region has been obtained from studies of *DR* gene organization in chimpanzee, gorilla, and orangutan MHC. These studies revealed that the organization of most human *DR* haplotypes is not conserved within primates (26). Several examples of what appears like composite haplotypes of those found in humans are present in nonhuman primates (26-32). *DRB* genes othologous to all nine human *DRB* loci have been identified in the chimpanzee MHC. However, except for the *DR53* haplotypic group, most of the *DR* haplotypes found in nonhuman primates have distinct organization from those found in the human MHC (26).

As discussed above, *DRB1* alleles cluster together in phylogenetic analysis into five families of allelic lineages (12-15). When these *DRB1* allelic lineages are compared phylogenetically with alleles from other *DRB* loci, it is not genetically evident that all *DRB1* sequences are truly allelic (16). Furthermore, when the polymorphic exon 2 sequences from all available *DRB1* sequences are used as one data set in population genetic studies, alternative conclusions have been obtained concerning the age of MHC polymorphism (17-19). Of utmost importance for drawing conclusions on the evolutionary relationship of *DRB* genes and the age of MHC polymorphism is to include intron sequences in the analyses. This has been used successfully in several recent studies (12-15, 42). For practical reasons (tissue-typing etc) it has been argued that it is more convenient to group all *DRB1* sequences into one locus since they appear functionally equivalent. However, what appears clear from molecular characterization of these genes is that at least *DRB1* alleles from the *DR53* allelic lineage are evolutionary distant from other *DRB1* genes. For example, they lack the *ERV9 LTR* element in intron 5 that is present in other *DRB1* genes (12-13, 20). Furthermore, *DRB1* sequences intermingle with *DRB3*, *DRB4*, and *DRB5* sequences in phylogenetic analysis (16). Clearly, *DRB1* genes from distinct *DR* haplotypes have evolved separately during substantial (>10 MYR) evolutionary times. Therefore, true allelic relationships may have been obscured. To avoid further confusion, locus designations including the haplotype designation may be used, e.g; *DRB1(1)*01*, *DRB1(51)*15*, *DRB1(52)*03*, *DRB1(53)*04*, and *DRB1(8)*08*. This should emphasize which allelic lineage a particular *DRB1* sequence belongs to and allow more stringent conclusions.

The functional advantage of having more than one expressed *DRB* gene on each chromosome remains uncertain. It is possible that the expression of two *DRB* genes from one chromosome might compensate for the lack of polymorphism at the *DRA* locus. The weakness in this argument is highlighted by the DP molecules, where the number of *DPB* genes is constant although the *DPA* locus is relatively non-polymorphic. An alternative explanation might be related to the fact that DQ, DP, and DR molecules have different requirements for peptide interactions (33). DR molecules appear to bind only a more restricted type of peptides (compared to DQ and DP molecules) with a conserved nine-residue motif (33). This requirement might be restricted by the conserved DR-alpha molecule but somewhat compensated by the multitude of possible DR-beta chains.

Based on the available molecular information of different human *DR* regions a model for *HLA-DR* haplotype evolution has been proposed (13, 34). This model is schematically shown in figure 2. Clearly, *DRB* genes arose by several independent gene duplications and recombinations early during primate evolution and predominantly before human speciation. The two main branches of human *DR* haplotypes are both evolutionary old and were generated by independent duplication events early during mammalian evolution (34). The *DR53* haplotype represents one branch and the second branch contains all the other human *DR* haplotypes (figure 2 and ref. 34). A conspicuous difference between the two main branches is that the *DR53* haplotype has remained constant and that closely related haplotypes have not been described in the human population. In contrast, the *DR51/52* haplotypic branch, has been subject to a high degree of diversification. This is manifested by the recent origin of the *DR8* and *DR1* haplotypes (see above).

4. AGE OF THE *HLA-DR* HAPLOTYPES AND *DRB* ALLELES

Within the *DR53* haplotype, *DRB1* genes which belong to the *DRB1*04* allelic lineage have been estimated to be more than 85 myr old (35). Interestingly, this particular allelic lineage has also the largest number (approximately 135) of *DRB1* alleles (2). In contrast, the number of *DRB1*09* alleles is low. Thus, alleles from two lineages expressed from the same haplotypic group have clearly distinct evolutionary histories. The reason for this discrepancy is unknown but the distinction of allelic polymorphism observed for certain subhaplotypes might be the result of previous bottle necks. Furthermore, intron sequences of *DRB* genes from the *DR53* haplotypic group (*DR4*, *DR7*, and *DR9*) are the most phylogenetically divergent. This further strengthens the notion that the *DR53* haplotypic branch was established early during evolution. Evidence that gene conversion-like events have been important during evolution of the *DRB* genes was found since the four *DRB* genes in the *DR4* haplotype display patchwork similarities to each other (20). This has precluded estimations concerning the age and order of the duplication events that generated these *DRB* genes. However, firm evidence for two consecutive duplications was provided by nucleotide sequence analysis of all the four *DRB* genes (*DRB1*0401*, *DRB7*, *DRB8*, and *DRB4*) in this haplotype (20). A common ancestry between all *DR* haplotypes is evident since the *DRB4* gene of the *DR53* haplotypes is phylogenetically most related to the *DRB2* gene of the *DR52* haplotypes (13, 36-37). These *DRB* loci might represent present day copies of a primordial *DRB* gene. Furthermore, the *DRB9* locus is present in all human *DR* haplotypes (see above).

Substantial information regarding the molecular evolution of the *DR* region has been obtained based on the presence of phylogenetically informative retroelements (12-15). The complexity of retroelements in the class II region has recently been reviewed (45). *DRB1* genes of all human *DR* haplotypes within the *DR51/DR52*, evolutionary branch contains a conserved endogenous retrovirus long terminal repeat *(ERV9 LTR)* insertion in intron 5 (12-15). This retroelement was inserted in an ancestral *DRB* gene after the split between the two main evolutionary branches but before the split of *DR51* and *DR52* haplotypes. The inferred insertion

time of the *ERV9 LTR* elements was estimated to approximately 55 myr ago, after the divergence between Old and New World monkeys (12-15).

The initial duplication event that occurred within a primordial *DR51/DR52* haplotypic lineage occurred approximately 60±6 myr ago (figure 2 and ref. 38), which clearly is prior to the insertion of the *ERV9 LTR* element. The second duplication event occurred subsequently to the insertion of the *ERV9 LTR* element approximately 40±5 myr ago (38). The divergence between *DR51* and *DR52* haplotypic lineages occurred at a similar timepoint in evolution (13).

The *DR1* and *DR8* haplotypes were all generated recently during primate evolution. Their respective evolutionary histories have recently been reviewed (34). The gene organization of the *DR51* haplotype was estimated to be at least 6 myr old and thus precedes hominoid speciation (39). Of particular interest is a human specific *Alu* (*HS-Alu*) element which is located within the *ERV9 LTR* element of the *DRB1* gene of *DR51* haplotypes. This *HS-Alu* element was inserted subsequently to the divergence between the *DR1* and *DR51* haplotypes (13). Analysis for the presence of this *HS-Alu* element should serve a useful tool in human population genetics studies. This particular *HS-Alu* family member shows 98.9% identity to the HS consensus sequence (40-41), and was most likely inserted at this position after human speciation.

Although the two main evolutionary branches of human *DR* haplotypes were established early during the mammalian evolution (34), the emergence of the more recent *DR1* and *DR8* haplotypes provides examples for the ongoing evolution within this multigene family. Concerning the age of class II gene polymorphism, it is evident that a substantial amount of *DRB* allelic polymorphism was generated after human speciation (42-43), and that other *DRB1* alleles are shared between different primate species (44). Based on intron nucleotide sequence comparison it was estimated that more than 90% of the contemporary *HLA-DRB1* alleles were generated after human speciation (42). This should be contrasted to the more ancient emergence of the allelic lineages of which most were generated early during primate evolution.

5.CONCLUSIONS

Several duplication, recombination, and contraction events have occurred during evolution of the *DR* multigene family. The plasticity of the *DR* region with a variation in the number of *DRB* genes compared to the conserved number of *DQ* and *DP* genes is intriguing. These features suggest that genes within the *DR* region is subject to quite distinct selective pressures from other class II genes. This is manifested by the conservation of the *DRA* locus at the one extreme and the allelic and haplotypic polymorphism of the *DRB* loci at the other extreme. The reason for this difference may be related to an evolutionary selection acting on the antigen-presenting function of DR molecules.

Clearly, the events that generated two main haplotypic lineages and most of the present day *DRB* loci, occurred early during mammalian evolution. Subsequently, multiple rearrangements have occurred after the establishment of the two main haplotypic groups. The presence of recombinant *DR* haplotypes in the human population, the recent generation of both the *DR1* and *DR8* haplotypes and of novel *HLA-DRB1* alleles, most of which were generated after the separation of human and chimpanzee, and finally the existence of composite haplotypes observed in non-human primates, are all features which corroborate the plasticity of the *DR* region. Most likely, the large proportion (24%) of retroelements within the class II *HLA-DR* region (45), has been an important contribution in the evolution of this chromosomal region.

6. ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council.

7. REFERENCES

1. Senger, G. Ragoussis, J. Trowsdale, J & D. Sheer: Fine mapping of the MHC class II region within 6p21 and evaluation of probe ordering interphase fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 61, 49-53 (1993)

2. Bodmer, J. G. Marsh, S. G. E. Albert, E. D. Bodmer, W. F. Bontrop, R. E. Charron, D. Dupont, B. Erlich, H. A. Fauchet, R. Mach, B. Mayr, W. R. Parham, P. Sasazuki, T. Schreuder, G. M. T. Strominger, J. L. Svejgaard, A & P. I. Terasaki: Nomenclature for factors of the HLA system, 1996. *Eur J Immunogenetics* 24(2), 105-51 (1997)

3. Brown, J. H. Jardetsky, T. S. Gorga, J. C. Stern, L. J. Urban, R. G. Strominger, J. L & D. C. Wiley: Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364, 33-39 (1993)

4. Paliaksis, K. Routsias, J. Petratos, K. Ouzounis, C. Kokkinidis, M & G. K. Papadopoulos: Novel structural features of the human histocompatibility molecules HLA-DQ as revealed by modeling based on the published structure of the related molecule HLA-DR. *J Struct Biol* 117, 145-163 (1996)

5. Böhme, J. Andersson, M. Andersson, G. Möller, E. Peterson, P. A & L. Rask: HLA-DR beta genes vary in number between different DR specificities, whereas the number of DQ beta genes is constant. *J Immunol* 135, 2149-55 (1985)

6. Kroshofer, H. Hammerling, G. J & A. B. Vogt: How HLA-DM edits the MHC class II peptide repertoire: survival of the fittest? *Immunol Today* 18, 77-82 (1997)

7. Karlsson, L. Surh, C. D. Sprent, J & P. A. Peterson: HLA-DO: An unusual class II molecule. *Immunol Today* 13, 469-471 (1992)

8. Liljedahl, M. Winqvist, O. Surh, C. D. Wong, P. Ngo, K. Teyton, L. Peterson, P. A. Brunmark, A. Rudensky, A. Y.

Fung-Leung, W. -P & L. Karlsson: Altered antigen presentation in mice lacking H2-O. *Immunity* 8, 233-243 (1998)

9. Trowsdale, J: "Both man, bird beast" Comparative organisation of MHC genes. *Immunogenetics* 41, 1-17 (1995)

10. Gongora, R. Figueroa, F & J. Klein: The HLA-DRB9 gene and the origin of HLA-DR haplotypes. *Hum Immunol* 51, 23-31 (1996)

11. Gongora, R. Figueroa, F. O'hUigin, C & J. Klein: HLA-DRB9 - Possible remnant of an ancient functional DRB subregion. *Scand J Immunol* 45, 504-10 (1997)

12. Svensson, A. C. Setterblad, N. Sigurdardottir, S. Rask, L & G. Andersson: Primate DRB genes from the DR3 and DR8 haplotypes contain ERV9 LTR elements at identical positions. *Immunogenetics* 41, 74-82 (1995)

13. Svensson, A. C. Setterblad, N. Pihlgren, U. Rask, L & G. Andersson: Evolutionary relationship between human major histocompatibility complex HLA-DR haplotypes. *Immunogenetics* 43, 304-14 (1996)

14. Satta, Y. Mayer, W. E & J. Klein: Evolutionary relationship of HLA-DRB genes inferred from intron sequences. *J Mol Evol* 42, 648-57 (1996)

15. Satta, Y. Mayer, W. E & J. Klein: HLA-DRB intron 1 sequences: Implications for the evolution of HLA-DRB genes and haplotypes. *Hum Immunol* 51, 1-12 (1996)

16. Andersson, G. Andersson, L. Larhammar, D. Rask, L & S. Sigurdardóttir: Simplifying genetic locus assignement of HLA-DRB genes. *Immunol Today* 59, 58-62 (1994)

17. Ayala, F. J: The myth of Eve: molecular biology and human origins. *Science* 270, 1930-36 (1995)

18. Erlich, H. A. Bergström, T. F. Stoneking, M & U. Gyllensten: HLA sequence polymorphism and the origins of humans. *Science* 274, 1552-54 (1996)

19. Hickson, R. E & R. L. Cann: MHC allelic diversity and modern human origins. *J Mol Evol* 45, 589-98 (1997)

20. Andersson, G. Larhammar, D. Widmark, E. Servenius, B. Petersson, P. A & L. Rask: Class II genes of the human histocompatibility complex: organization and evolutionary relationship of the DRbeta genes. *J Biol Chem* 262, 8748-58 (1987)

21. Bidwell, J. L. Bidwell, E. A. Dupont, E. Andrien, M. Bouillenne, C. Klouda, P. T & B. A. Bradley: Molecular characterization of a recombinant HLA-DR1/DR2 haplotype. *Hum Immunol* 33, 289-93 (1992)

22. Corell, A. Varela, P. Morales, P. Paz-Artal, E. Martinez-Laso, J. Martin-Villa, J. M. DuToit, E & A. Arnaiz-Villena: A study of DR2-LUM haplotype generation and the DRB6*0202 linkage to DRB1*1601. *Immunogenetics* 38, 460-1 (1993)

23. Gebuhrer, L. Tiercy, J. M. Freidel, A. C. Pascale-Labonne, M. Lambert, J. Jeannet, M & H. Betuel: Apparent HLA DR triplet due to the coexpression of a DRB5 encoded molecule on a DR1 haplotype. *Hum Immunol* 34, 91-8 (1992)

24. Wade, J. A. Hurley, C. K. Hastings, A. Ehrenberg, P. Johnson, A. H. Martell, R. W & E. D. DuToit: Combinatorial diversity in DR2 haplotypes. *Tissue Antigens* 41, 113-8 (1993)

25. Robbins, F. Hurley, C. K. Tang, T. Yao, H. Lin, Y. S. Wade, J. Goeken, N, & R. J. Hartzman: Diversity associated with the second expressed HLA-DRB locus in the human population. *Immunogenetics* 46, 104-10 (1997)

26. Bontrop, R. E: MHC class II genes of nonhuman primates. In: Molecular biology and evolution of blood group and MHC antigens in primates. Eds Blancher, A. Klein, J & W. W. Socha, Springer-Verlag, Berlin, Heidelberg, pp. 358-371 (1997)

27. Brändle, U. Ono, H. Vincek, V. Klein, D. Golubic, M. Grahovac, B & J. Klein: Trans-species evolution of Mhc haplotype polymorphism in primates: organization of DRB genes in the chimpanzee. *Immunogenetics* 36, 39-48 (1992)

28. Figueroa, F. O'hUigin, C. Inoko, H & J. Klein: Primate DRB6 pseudogenes: Clue to the evolutionary origin of the HLA-DR2 haplotype. *Immunogenetics* 34, 324-37 (1991)

29. Kasahara, M. Klein, D. Weimin, F & J. Gutknecht: Evolution of the class II major histocompatibility complex alleles in higher primates. *Immunol Rev* 113, 65-82 (1990)

30. Kasahara, M. Klein, D. Vincek, V. Sarapata, D. E & J. Klein: Comparative anatomy of the primate major histocompatibility complex DR subregion: evidence for combinations of DRB genes conserved across species. *Genomics* 14, 340-49 (1992)

31. Klein, D. Vincek, V. Kasahara, M. Schönbach, C. O'hUigin, C & J. Klein: Gorilla major histocompatibility complex DRB pseudogene orthologous to HLA-DRBVIII. *Hum Immunol* 32, 211-20 (1991)

32. Schönbach, C. Vincek, V. Mayer, W. E. Golubic, M. O'hUigin, C & J. Klein: Multiplication of Mhc-DRB5 loci in the orangutang: implications for the evolution of the DRB haplotypes. *Mammalian Genome* 4, 159-70 (1993)

33. Rammensee, H. G. Friede, T & S. Stevanoviic: MHC ligands and peptide motifs: first listing. *Immunogenetics* 41, 178-228 (1995)

34. Svensson, A. C & G. Andersson: Presence of retroelements reveal the evolutionary history of the human DR haplotypes. *Hereditas* 127, 113-24 (1997)

35. Figueroa, F. O'hUigin, C. Tichy, H & J. Klein: The origin of the primate MHC-DRB genes and allelic lineages as

deduced from the study of prosimians. *J Immunol* 152, 4455-65 (1994)

36. Gorski, J. Rollini, P & B. Mach: Structural comparison of the genes of two HLA-DR supertypic groups: the loci encoding DRw52 and DRw53 are not truly allelic. *Immunogenetics* 25, 397-402 (1987)

37. Vincek, V. Klein, D. Figueroa, F. Hauptfeld, V. Kasahara, M. O'hUigin, C. Mach, B. & J. Klein: The evolutionary origin of the HLA-DR3 haplotype. *Immunogenetics* 35, 263-71 (1992)

38. Mñuková-Fadjelová, M. Satta, Y. O'hUigin, C. Mayer, W. E. Figueroa, F & J. Klein: Alu elements of the primate major histocompatibility complex. *Mammalian Genome* 5, 405-15 (1994)

39. Klein, J. O'hUigin, C. Kasahara, M. Vincek, V. Klein, D & F. Figueroa: Frozen haplotypes in Mhc evolution. In: Molecular Evolution of the Major Histocompatibility Complex. Eds: Klein J & Klein D, Springer Verlag, Heidelberg, pp. 261-86 (1991)

40. Batzer, M A. Kilroy, G E. Richard, P E. Shaikh, T H. Desselle, T D. Hoppens, C L & P. L. Deininger: Structure and variability of recently inserted Alu family members. *Nucleic Acids Res* 18, 6793-98 (1990)

41. Batzer, M A. Stoneking, M. Alegria-Hartman, M. Bazan, H. Kass, D H. Shaikh, T H. Novick, G E. Ioannou, P A. Scheer, W D. Herrera, R J & P. L. Deininger: African origin of human-specific polymorphic Alu insertions. *Proc Natl Acad Sci USA* 91, 12288-92 (1994)

42. Bergström, T. F. Josefsson, A. Erlich, H. A & U. Gyllensten: Recent origin of HLA-DRB1 alleles and implications for human evolution. *Nature Genetics* 18, 237-42 (1998)

43. Titus-Trachtenberg, E. A. Rickards, O. DeSteano, G. F & H. A. Erlich: Analysis of HLA class II haplotypes in the Cayapa indians of Ecuador: a novel DRB1 allele reveals evidence for convergent evolution and balancing selection. *Am J Hum Genet* 55, 160-7 (1994)

44. Fan, W. Kasahara, M. Gutknecht, J. Klein, D. Mayer, W. E. Jonker, M & J. Klein: Shared class II MHC polymorphism between humans and chimpanzees. *Hum Immunol* 26, 107-21 (1989)

45. Andersson, G. Svensson, A. -C. Setterblad, N & L. Rask: Retroelements in the human MHC class II region. *Trends Genet* 14, 109-14 (1998)

Keywords: MHC, HLA-DR Haplotypes, Evolution

Send correspondence to: Dr Göran Andersson BioTransplant Inc, Charlestown MA, USA, Tel: 617-241-5200, Fax: 617-241-8780, E-mail: goran.andersson@vbiol.slu.se