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Institute for Tropical Medicine Berlin, Berlin, FRG (Director: Dr. H. Bienzle) (1)
Dept. Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, FRG
(Director: Prof. Dr. H. J. Müller-Eberhard) (2)

*Human MHC class II DPB1*01011 and related alleles in West Africa*

Chr. G. Meyer[1], Dorothea Spauke[2], J. May[1], L. Schnittger[2]

Introduction

The genes of the human major histocompatibility complex (MHC) class II are located on the short arm of chromsome six. DR1, DQ1, and DP1 genes encode the so-called human leukocyte antigens (HLA) which are expressed on the surface of antigen-presenting cells. HLA-molecules are associated α/β (A/B) heterodimers which constitute the mature protein.

The overall function of HLA-molecules may conveniently be described as the capability of discriminating between self and non-self. HLA-molecules play a crucial role in immune responses: they bind processed foreign peptides to present them to the specific receptors of T-lymphocytes. This recognition results in T-cell activation and in the generation of an immune response. The qualitiy and the extent of the resulting immune response may vary and, partly, are dependent on involved HLA-types: protective immunity may result, but, in some cases, susceptibility to autoimmune disease as well.

HLA-elements have been associated with a number of autoimmune and infectious disorders. The value of studying HLA and disease associations consists in, firstly, the understanding of disease-underlying molecular mechanisms and, secondly, in defining a population which may be at risk for a particular disease.

The most striking feature of most expressed MHC class II gene families and their protein products is their high degree of allelic variation on the molecular level as detected and analyzed by modern techniques. The polymorphism is mainly displayed within the second (variable) exons of MHC-genes (2).

These exons encode the antigen-binding sites of the proteins and, thus, determine the possible spectrum of antigens to be bound. While polymorphism is comparatively high in both DQA1 and DQB1 gene families, the B genes DRB1 and DPB1 are much more variable than their A counterparts DRA1 and DPA1.

Molecular MHC-DNA typing techniques have greatly facilitated and contributed to the identification of the genetic polymorphism of MHC class II loci. Such techniques are mostly based on locus-specific DNA amplification of the second variable exons using the polymerase chain reaction (PCR) (15), followed by either restriction-fragment length polymorphism (RFLP) analyses or by hybridizations with sequence-specific synthetic oligonucleotide probes (SSOP) (4). Assessment of allelic variants may be complicated by ambiguity of RFLP- or SSOP-hybridization results, requiring definite resolution by DNA-sequence analysis. While RFLP- and oligonucleotide hybridizations of samples derived from Caucasians mostly allow an unambiguous assessment of alleles, unclear patterns occur more frequently in studies on poorly examined ethnic groups. Due to limited knowledge on allelic polymorphism and haplotypic linkages in such populations, yet unrecognized alleles may often be suspected (17). In addition,

DNA sequencing is advantegous for the characterization of those genetic regions which are not covered by either informative endonuclease restriction sites for RFLP analysis or by the oligonucleotide probes binding to the clustered variable regions.

In the past years, novel alleles have been identified for all variable MHC class II loci. Studies on evolution and diversification, however, have mostley focussed on the genetic regions DR and DQ (3, 6, 7, 8, 13). In the following, special attention will be paid to some related DPB1 genes, trying to put them into an evolutionary context.

Material and Methods

Frozen lymphocytes were obtained from individuals from Liberia and Benin, West Africa. DNA was extracted and purified according to standard methods (14). The second variable exon of the MHC class II DPB1 locus was enzymatically amplified using the polymerase chain reaction (PCR). Primers and PCR conditions were used following the recommendations as described in the protocols of the 11. International Histocompatibility Workshop (12). PCR products were monitored for the efficiency of the amplification procedure and the specific size on 1.7% agarose gels. PCR products were dot-blotted onto negatively charged nylon membranes and UV-linked. Sequence-specific oligonucleotide probes were labelled with digoxigenin and applied in stringent hybridization assays as described elsewhere (5).

DNA samples reacting ambiguously in the hybridizations assays were reamplified and purified. After ligating them into plasmid vectors (pBS, pBlue Scribe, Stratagene), and cloning (DH5 alpha *E. coli* cells) the fragments underwent DNA sequencing applying the standard chain termination method (16) and automatic sequencing (Applied Biosystems).

Amplification, cloning, and sequencing were repeated to confirm novels allels.

Results

PCR-amplification yielded DNA fragments of expected size as documented by gel-electrophoreses. After hybridization with SSOPs, most PCR products were clearly assignable to one (homozygous) or two (heterozygous) of the currently published DPB1 alleles (1). DNA sequence analysis of two PCR products which reacted ambiguously in the oligonucleotide hybridizations revealed two yet unrecognized DPB1 alleles. The DNA sequences of these alleles are shown in figure 1, aligned to some related alleles. After submission of the sequences to the WHO nomenclature committee for factors of the HLA system, they have been designated DPB1*01012 (9) and DPB1*26012 (10). Derived amino acid sequences are given in figure 2. The homology of the novel sequences to those of some other alleles permitted the extension of a partial phylogenetic tree as depicted in figure 3.

Discussion

DPB1 polymorphism is characterized by six variable regions (VR A - F, see fig. 2) which are linked by conserved genetic elements identical in all DPB1 genes. A characteristic of DPB1 alleles is the absence of allele-specific sequences. Exceptions from this DPB1-specific feature is observed only in the allele DPB1*3801 and in several alleles which exhibit a silent mutation at codon position 43. Thus, a variable sequence element representing one of the six VRs, is, except from DPB1*3801, always found in at least two different alleles. Such alleles may differ otherwise partly or completely in the arrangement of their other VRs, leading to a polymorphic patchwork of DPB1 alleles. This pattern of variability does not result from allele-specific sequences but rather from the particular combinations of the first to sixth VR of their second exons. The generally agreed mechanisms leading to DPB1 allelic diversity are recombinations (reassortments) involving the six variable regions (VR) A - F of the DPB1 second exon (1) (fig. 2).

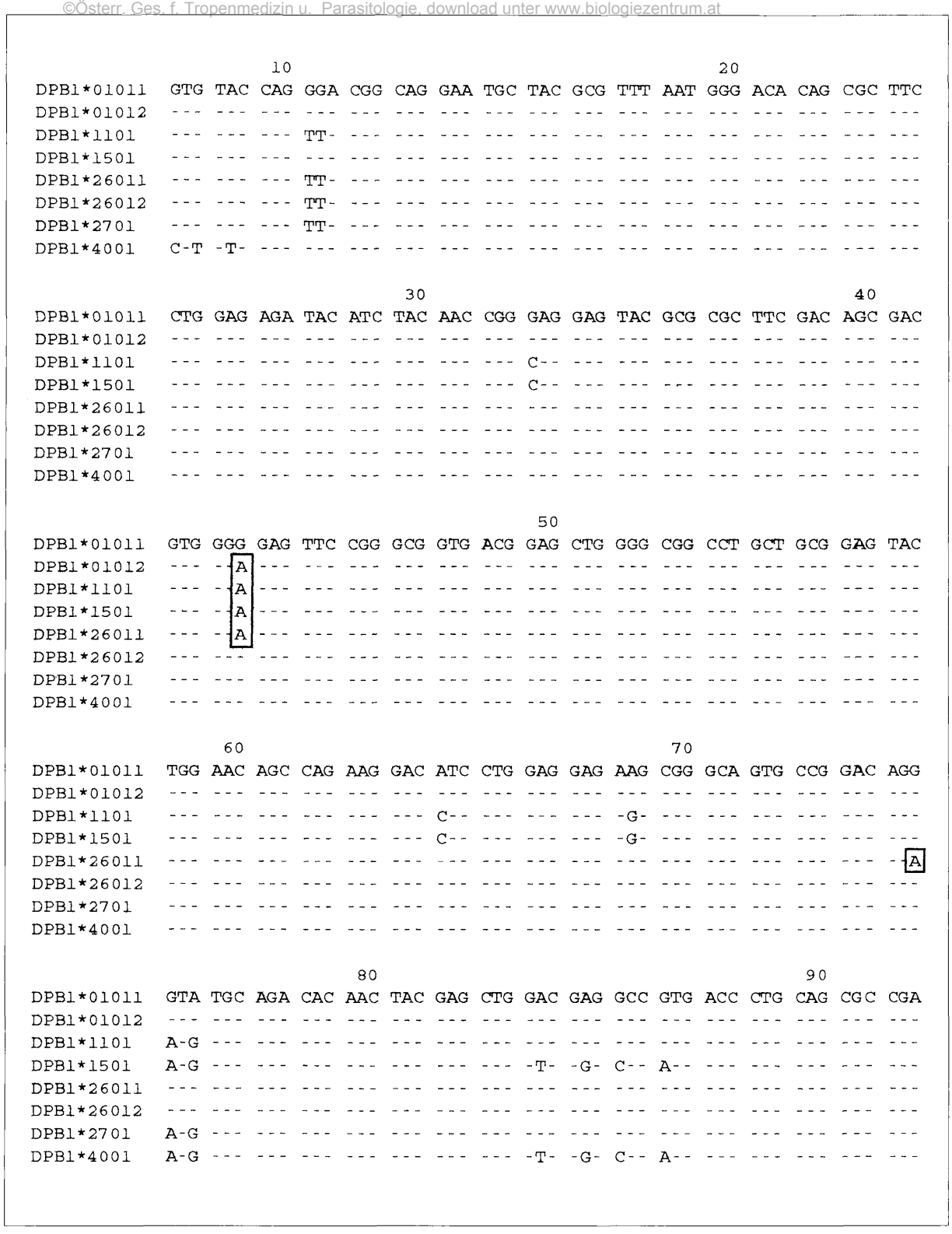

Figure 1:

DNA sequence alignment of the second exon of the DPB1 allele DPB1*01011 (consensus sequence) and those of the related DPB1 alleles mentioned in this report. PCR-amplification primers and their annealing sites are not shown. Dashes indicate nucleotide identitiy with the consensus sequence. Synonymous nucleotide exchanges are shown in boxes. Numbers above indicate positions of corresponding amino acids in the mature protein.

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                10        20        30        40        50        60        70        80        90
DPB1*01011      VYQGRQECYAFNGTQRFLERYIYNREEYARFDSDVGEFRAVTELGRPAAEYWNSQKDILEEKRAVPDRVCRHNYELDEAVTLQ
DPB1*01012      ----------------------------------------*-----------------------------------------
DPB1*1101       ---L--------------------Q---------*--------------------L---R------M--------------
DPB1*1501       ------------------------Q---------*--------------------L---R------M------VGPM----
DPB1*26011      ---L------------------------------*-----------------------------------------------
DPB1*26012      ---L-----------------------------------------------------------------------------
DPB1*2701       ---L-----------------------------------------------------------------M-------------
DPB1*2701       LF--------------------------------------------------------------------M------GGPM----
DPB1*2701       LF--------------------------------------------------------------------M------VGPM----
                ___                   ___                 ___       ___       ___       ___
                A                     B                   C         D         E         F
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Figure 2:

Derived amino acid alignment of the variable protein elements of the DPB1 alleles addressed in this report. The consensus sequence is that of DPB1*01011. Amino acids are given in the single letter code. Dashes indicate amino acid identity to the consensus sequence. Asterisks mark the codon position 43 which contains the synonymous nucleotide exchange in the corresponding nucleotide sequences.

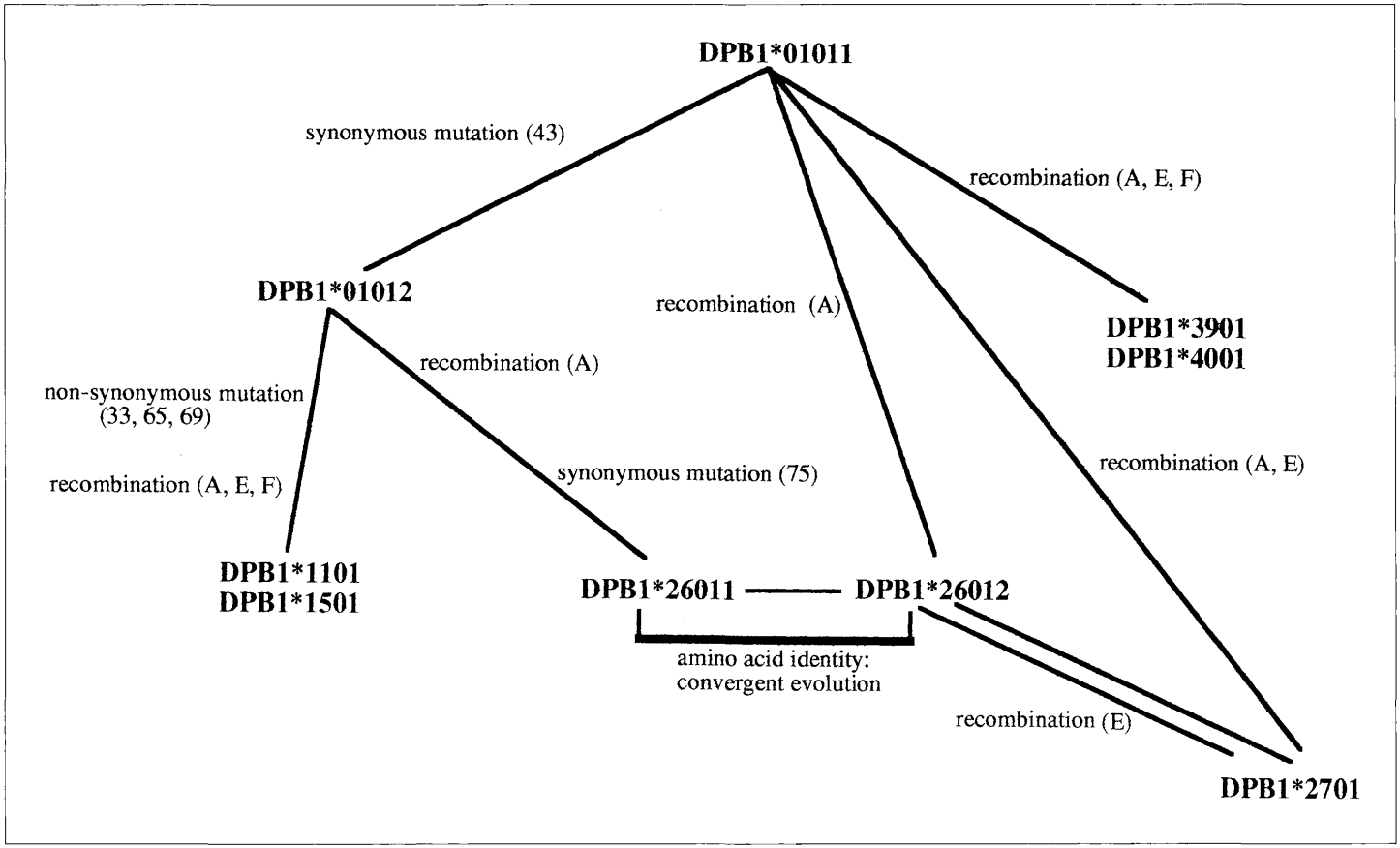

Figure 3:

A proposed phylogenetic tree for DPB1*01011 and related alleles corresponding to the amino acid sequences as shown in figure 2. Mechanisms leading to diversification are indicated. Numbers in parentheses designate codon positions, A - F in parentheses designate the regions of variability which are involved in recombinational reassortments.

The nucleotide sequence of the novel variant DPB1*01012 (9) differs from DPB1*01011 in one synonymous mutation at codon position 43 only. Based on the observation of this synonymous mutation it is suggested that DPB1*01012 might represent a genetic link between DPB1*01011 and those other alleles which carry the same synonymous mutation at codon position 43. These alleles are DPB1*1101, DPB1*1501, and DPB1*26011 (formerly DPB1*2601-DPB1*WA2; see Ref. 11). Since it is most unlikely that single mutations occur independently at identical sites of different alleles, those alleles which carry this synonymous mutation at codon position 43 appear to establish one distinct allelic lineage of DPB1. To date, DPB1*01012 has been found in five individuals from Liberia, West Africa.

The other novel DPB1 variant, DPB1*26012, points towards a further detail of DPB1 evolution (10). DPB1*26012 is identical to DPB1*26011 in its derived amino acid sequence but it differs in its nucleotide sequence by two synonymous mutations at codon position 43 and 75. DPB1*26011 is part of the allelic lineage which is charaterized by the presence of the synonymous mutation at codon position 43 whereas DPB1*26012 appears to relate directly to DPB1*01011, circumventing the genetic link of DPB1*01012. It appears to have evolved autonomously by a recombinational event involving the first of the six variable regions (VR A). Alternatively, DPB1*26012 could originate from another closely related allele, DPB1*2701 (the former DPB1*WA3, see Ref. 17) after recombinations involving the first and fifth VR (VR A and -E).

The differences in the nucleotide sequence of DPB1*26011 and DPB1*26012 imply two individual evolutionary routes of their generation. The independent generation of two functionally identical alleles via different genetic routes may be interpreted as an example for convergent evolution at the DPB1 locus.

The notion of convergent evolution of two DPB1 alleles in West Africa suggests that specific immunological needs driven by local pathogens of major significance are selecting forces which require the propagation of these DPB1 elements. This view is supported by the fact that many of newly identified West African alleles, including the alleles DPB1*3901 and *4001 (fig. 1, 2) are homologous in their second to fourth VR to DPB1*01011 (fig. 2) which is, by far, the most frequent DPB1 allele among negroids. The functional significance of DPB1*01011 and its related variants in negroid populations, however, has yet to be explained.

Summary A major concern of human histocompatibility complex (MHC) genetics is the characterization of allelic polymorphism in various ethnic populations. This includes research on local frequencies and distributions of distinct alleles and haplotypes in defined groups as well as studies on mechanisms of the origin, diversification, and maintenance of the extensive MHC allelic variability. The abundant frequency of the MHC class II DPB1 allele DPB1*01011 in West Africans recommends evolutionary assessment of partly homologous alleles.

Here we extend a preliminary phylogenetic tree of DPB1*01011 and some related alleles. The phylogenetic alignment comprises two recently identified DPB1 variants.

Key words MHC, HLA-DPB1, allelic polymorphism, DNA-typing, MHC-evolution.

Zusammenfassung

*MHC Klasse II DPB1*01011 und verwandte Allele in West-Afrika*

Die Charakterisierung der Variabilität des menschlichen Haupthistokompatibilitätskomplexes (MHC) in verschiedenen ethnischen Gruppen spielt in der genetischen MHC-Forschung eine entscheidende Rolle. Solche Studien umfassen neben den Beschreibungen von Häufigkeiten und Verteilung bestimmter Allele und Haplotypen Untersuchungen zu Ursprung, Diversifizie-

rungsmechanismen und Aufrechterhaltung der allelischen Vielfalt des Haupthistokompatibilitätskomplexes. Die Häufigkeit des MHC Klasse II DPB1-Alleles DPB1*01011 legt die entwicklungsgeschichtliche Zuordnung von verwandten Allelen nahe.

In dieser Arbeit werden zwei neue DPB1 Allele in einen vorläufigen phylogenetischen DPB1 Stammbaum integriert.

Schlüsselwörter MHC, HLA-DPB1, Polymorphismus, DNA-Typisierung, MHC-Evolution.

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Korrespondenzadresse: Dr. Christian G. Meyer
Institut für Tropenmedizin Berlin

Engeldamm 62 - 64
D-10179 Berlin · Bundesrepublik Deutschland