THE HLA SYSTEM IN FORENSIC PRACTICE

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The HLA system which represents the major histocompatibility complex of man encompasses approximately one thousandth of the human genome. It is governed by a gene complex situated on the short arm of chromosome 6 (between 6p21.1 and 6p21.3) and contains a series of closely linked loci (for review, see Histocompatibility Testing 1984):
- HLA-A, B and C coding glycosilated polypeptides with a molecular weight of 44000 daltons (on the cell surface, these chains are non-covalently bound to β_2-microglobulin, a polypeptide governed by chromosome 15),
- the HLA-D region with loci for α and β chains (glycosilated polypeptides with 34000 and 29000 daltons, respectively),
- at least 4 loci for proteins of the complement system (C2, C4A, C4B and Bf),
- loci for the 21-hydroxylase.

Out of the cell-bound HLA gene products, the alloantigens coded for by HLA-A, B and C present on all nucleated cells of the organism fulfil without doubts all the criteria which are demanded for the use of genetically defined markers in cases of disputed paternity: the mode of inheritance is known with certainty, the techniques of determination are reliable and simple, the phenotype reflects the genotype only, and the characteristics are developed at birth.
The loci HLA-A, B and C show a remarkable multiple allelism: the gene products of 19 HLA-A, 37 HLA-B and 8 HLA-C alleles can be defined for the moment. The analysis of family and population data demonstrates that not all specificities of these loci are yet serologically detectable. These "unknown" alloantigens are governed by alleles designated as AX, BX and CX, respectively. The alleles of HLA-A, B and C except the X genes are inherited in a codominant way; as the products of the

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X genes are not yet detectable, they formally follow a recessive mode of inheritance.
There exists a strong linkage disequilibrium between several alleles of the 3 loci, that means that in general the frequencies of the HLA-A,B,C haplotypes are not in accordance with the products of the corresponding gene frequencies.
Due to the large number of alleles in the 3 loci, there is a prodigious polymorphism in HLA-A,B,C: approximately 5×10^6 phenotypes and 23×10^6 genotypes. The most "common" phenotypes in Caucasoids are HLA-A1,3;B7,8;Cw7 and HLA-A1,2;B8,44;Cw7 with frequencies of less than 1%.

The tremendous HLA-A,B,C polymorphism and the low frequencies of the phenotypes are the reasons for the extreme usefulness of this system in solving problems of parentage: the investigation of the HLA-A,B,C gene products gives a chance of exclusion in false accusations of paternity of 96%. Together with the routinely used non-HLA systems ABO, MNSs, P, Rh, K, Fy, Jk, Lu, Xg, Se, acP_1, AK_1, ADA, PGM_1, GPT, EsD, GLO, Hp, Gm, Km, Gc, C3, Bf, Tf and Pi a cumulative chance of exclusion of 99.94% can be reached.

The alloantigens of the HLA-D region which are present on B lymphocytes, monocytes, some epithelial and endothelial cells consist of one α and one β chain being non-covalently bound. With serological techniques, it is possible to define the polymorphism of the HLA-DR and the HLA-DQ gene products. Up to now, 12 DR alleles coding for serologically detectable specificities and one X allele (DRX) are known; a linkage disequilibrium exists between some HLA-DR and HLA-B alleles. The polymorphism of DQ encompasses only 4 alleles (DQw1, DQw2, DQw3 and DQX). Due to the extremely strong linkage disequilibrium between DR and DQ, the DQ polymorphism adds no further information to the DR typing results for paternity testing. The same applies for DRw52 and DRw53 which are products of the DR subregion and show an extreme linkage disequilibrium with the other DR alleles.

Taking into account the HLA-DR alleles in addition to HLA-A,B,C, the number of HLA phenotypes and genotypes rises to 4×10^8 and 4×10^9, respectively. The chance of exclusion of HLA-A,B,C,DR amounts to 98%; the cumulative chance of exclusion with the non-HLA systems routinely used reaches 99.97%.

The determination of the HLA-DR alloantigens in paternity testing (see e.g. Waltz et al., 1983) should be carried out only by specialised laboratories, as specific antisera in sufficient quantities are not available and some factors cannot easily be defined. In our opinion, the two-colour-fluorescence (TCF) method is preferable to the use of separated B lymphocytes due to the fact that the former technique can be performed with rather small volumes of blood (3-4 ml) which can be easily obtained from small children, while the amount of blood necessary for the preparation of B cells is significantly higher.

The results obtained by using the HLA system in affiliation cases are excellent (see e.g. Mayr et al., 1981), especially in problematic ones (cases with 2 men not excluded in non-HLA systems, cases without mother or cases in which only relatives of the putative father could be tested). The analysis of 1130 affiliation lawsuits from Austria and the German Democratic Republic (Mayr et al., 1981) for instance showed that the inclusion of HLA-A,B,C could reduce in cases with 2 accused men the number of problematic situations from 66% to 9%.
In this material, the frequency of non-HLA exclusions without HLA exclusion is 4%; this figure corresponds very well to the calculated chance of paternity exclusion of HLA-A,B,C (96%).
The inclusion of HLA-A,B,C also has a strong effect on the biostatistical computations: in the above-mentioned material, the plausibility of paternity according to Essen-Möller reached the W value of 99.8% without HLA only in 72 out of 832 cases (8.7%), while with HLA, $W > 99.8\%$ was observed in 464 out of 832 cases (55.8%). A similar increase of the chance of paternity exclusion in the single cases could be found.

In spite of this excellent applicability of HLA-A,B,C (and in some cases also HLA-DR), there are several pitfalls which can occur during the serological determination of these alloantigens. The problems arise in connection with the various reagents used in the lymphocytotoxic assay: lymphocytes (media for resuspension, viability, contamination with granulocytes or platelets, storage), antisera (cross-reactivity, gene dosage effect, synergism, non-HLA antibodies), rabbit complement, other reagents (mineral oil, formaldehyde) or atypical HLA alloantigens (variants, changes by diseases or drugs, blocking antibodies); for details, see Mayr, 1977. The knowledge of all these factors and the use of well-defined antisera which have been tested against a large cell panel, however, ensure correct and reproducible typing results.

For the forensic practice, the genetic systems investigated in affiliation cases should be tested in the following order: ABO, MNSs, Rh, HLA, and thereafter all the other markers. This modus procedendi which takes into account the high chance of paternity exclusion of HLA and the short life span of the lymphocytes used for the definition of the HLA alloantigens avoids multiple bleedings of the individuals and provides all the informations for the evaluation of a case in only one series of tests.

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