Simultaneous DNA analysis of HLA-DPB and -DQB loci from single hairs: a criminal case report

S. Pelotti, V. Mantovani*, G. Angelini°, F. Barboni* and G. Pappalardo

Institute of Legal Medicine University of Bologna, via Irnerio 49 40126 Bologna, Italy
*Tissue Typing Laboratory Malpighi Hospital, Bologna, Italy
°National Cancer Institute, Genova, Italy

INTRODUCTION

Polymorphic DNA sequences can be amplified over a millionfold with polymerase chain reaction (PCR), therefore samples can be typed even when the DNA is degraded or very small amount is available. The HLA Class II genes HLA-DR, -DQ, and -DP present a high degree of polymorphism and are suitable as genetic markers for individual forensic identification (Korman et al 1985). Higuchi et al (1988) typed for HLA-DQA single hairs by using PCR. We report a case concerning five hairs found on the hands of a murdered man. HLA-DPB polymorphism was analyzed from all the single hairs, for two simultaneously with HLA-DQB polymorphism. The typing was compared to that obtained from plucked hairs of the victim and the suspect.

MATERIALS AND METHODS

Morphological examination of evidence samples showed 5 human hairs of their natural colour, at different growth phases (one anagen with sheath, three catagen with residues of sheath, one telogen), four 6 cm and one 3 cm in lenght. The specimens were cut in the middle, where a small portion was taken, cross sectioned and typed for ABO by immunoenzymatic method with inconclusive result for individual diagnosis. Finally, the hairs were stored in glycerol until DNA extraction. Reference samples consisted of plucked hairs from both victim and suspect.
For HLA-DPB typing, DNA was obtained from the root and the proximal portion of longer shafts and from the whole shortest hair. For HLA-DQB typing, the half distal part of two specimens was separately extracted. The remaining two distal shafts were retained. DNA of reference samples was obtained from the roots.

DNA Extraction

Reference samples were rinsed in distilled water, followed by absolute ethanol. For evidence samples an additional washing with absolute ethanol preceded. The samples were digested in 0.5 ml TNE, containing 0.15 mg Proteinase K, 80 mM DTT, SDS 4%, 0.05 mg RNAse for 12 hr at 37°C and then for 24 hr at room temperature. DNA was obtained by phenol/chloroform-isoamylalcohol extraction, ethanol precipitation and finally resuspended in 30 µl TE (Maniatis et al 1982).

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

Oligonucleotide Primers and Probes

The oligonucleotide primers DB04 (5'-CAGGTACCCGCAGAGAATTAC) and PB03 (5'-CCCTCACTCACCTCGGCG) were used to amplify a 288 base pair (bp) region of the polymorphic second exon of the DPB1 gene. Sixteen oligonucleotide probes were 32P-end-labeled and used to distinguish 19 different alleles at the HLA-DPB locus (Bugawan et al 1990). Oligonucleotides for the analysis of this region were synthesized using a cyanoethylphosphoramidite method. Two primers were employed to amplify a polymorphic fragment of the second exon of the DQB1 gene. Seven sequence-specific radiolabeled probes were chosen to distinguish eight different HLA-DQB alleles. The oligonucleotide primers and probes used are described by Molkentin et al (1991).

PCR Amplification

Due to the small amount of DNA recovered from hair samples 30 cycles of amplification were followed by additional 30 cycles, the latter performed on 1/10 of the first amplification product. DNA amplification was performed in 50 µl reactions containing 50 mM potassium chloride, 10 mM Tris-HCl pH 8.4, 1.5 mM magnesium chloride, 200 mM of each deoxyribonucleotide triphosphate, 0.01% gelatin, 5% DMSO, 50 pmoles of each primer and 1.2 units of Taq-polymerase. The PCR amplification consisted in 1 min denaturation at 96°C, 2 min annealing at 50°C for the first 30 cycles and 54°C for the latter cycles, 1.5 min polymerization at 72°C. Opportune negative and positive controls were included in every stage of analysis.

Dot Blot Hybridization

Four microliters of amplified DNA were denatured in 0.4 N NaOH, 25 mM EDTA, then spotted on a nylon membrane and ultraviolet cross-linked for 5 min. Several replicate filters were done. For DPB oligotyping, membranes were hybridized and washed as previously described (Angelini et al 1989). For DQB oligotyping, the TMAC protocol according to Molkentin et al (1991) was employed.

RESULTS AND DISCUSSION

All the samples submitted to analysis were found to amplify efficiently for HLA-DPB and -DQB genes and all PCR amplification products resulted typable with oligoprobes (Fig.1). The hair roots of the five specimens showed the same DPB type: DPB1*0401/*0901; in addition, the two shafts analyzed for DQB showed the same type: DQB1*0603/*0201. Because the victim exhibited different HLA alleles (DPB1*0401/*0201, DQB1*05/*0201) and the suspect also showed different HLA typing (DPB1*0402/*0201, DQB1*03), we were able to eliminate both of them as donors of the five hair specimens.

These results indicate that, analyzing separately the root and the shaft, it is possible to simultaneously amplify and type two

different polymorphic and indipendent loci from a single hair. Actually, in our experience with PCR, the shaft of most individuals with natural hair contains enough genomic DNA to perform a molecular typing.
The high polymorphism of HLA-DPB and -DQB loci and the relative absence of linkage disequilibrium between them, make the simultaneous typing assay a powerful tool in the field of individual identity.

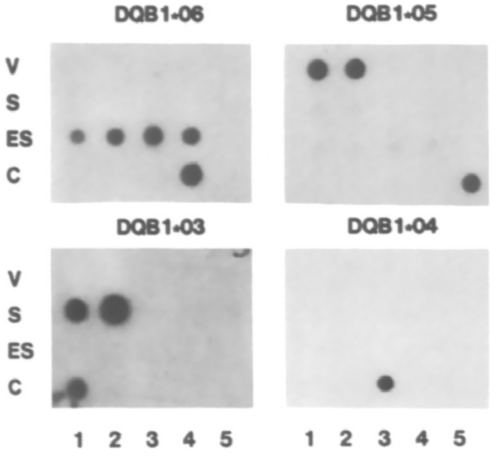

Fig. 1. Dot blot analysis of amplified DNA from the shaft of evidence samples (ES) and from reference samples of victim (V) and suspect (S), all in duplicate. Four oligoprobes are shown, specific for HLA-DQB1*06, -DQB1*05, -DQB1*03 and -DQB1*04 alleles. C: positive and negative controls

REFERENCES

Angelini G, Bugawan TL, Delfino L, Erlich HA, Ferrara GB (1989) HLA-DP typing by DNA amplification and hybridization with specific oligonucleotides. Hum Immunol 26: 169-177
Bugawan TL, Begovich AB, Erlich HA (1990) Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes. Immunogenetics 32: 231-241
Higuchi R, von Beroldigen CH, Sensabaugh GF, Erlich HA (1988) DNA typing from single hairs. Nature 332: 543-546
Korman AJ, Boss JM, Spies T, Sorrentino R, Okada K, Strominger JL (1985) Genetic complexity and expression of human class II histocompatibility antigens. Immunol Rev 85: 45-85
Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor New York
Molkentin J, Gorski J, Baxter-Lowe LA (1991) Detection of 14 HLA-DQB1 alleles by oligotyping. Hum Immunol 31: 114-122