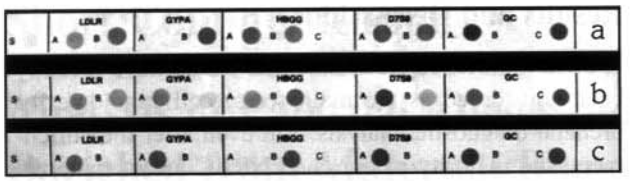

Fig. 1. Detection of paternal PM alleles in nucleated red blood cells enriched from maternal peripheral blood. Strip a) paternal blood. Strip b) $CD45^-/CD14^-/CD71^+$. Strip c) maternal blood. Paternal LDLR B, GYPA B, HBGG A, and D7S8 B alleles were detected in the $CD45^-/CD14^-/CD71^+$ fraction.

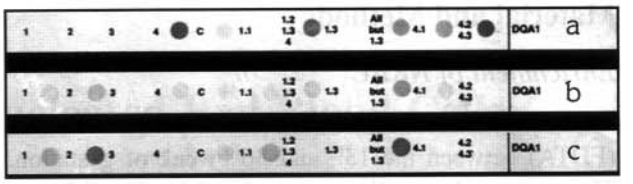

Fig. 2. Detection of paternal HLA DQA1 alleles in nucleated red blood cells enriched from maternal peripheral blood. Strip a) paternal blood. Strip b) $CD45^-/CD14^-/CD71^+$. Strip c) maternal blood. Paternal DQA1*0501 (4.1) allele was detected in the $CD45^-/CD14^-/CD71^+$ fraction.

(4.2/4.3). Our results showed that the combination of Polymarker System alleles with HLA antigen typing gives high probabilities of paternity.

In 6 cases it was impossible to demonstrate an allelic difference between the mother and foetus, because either the mother and the father shared the same DNA markers (3 cases), or we did not observe any PCR product which would indicate a paternal allele in the mixture of cells (3 cases). We suppose that the success of defining paternal alleles in the NRBC fraction depends on the concentration of DNA extracted from NRBC. PEP can increase possibilities of paternal allele detection by 50%.

Although we were successful in the identification of the PM system alleles, which was very simple and informative, we met some problems with analysis of HLA-typing results obtained using commercial kit LiPA. Foetal autosomal DRB1 and DQB1 alleles inherited from father were identified only in 6 cases definitely (using the DRB1 locus as a marker in 5 cases and using DQB1 in 1 case), because we could not use the software for HLA typing analysis. The probes on hybridized strips that corresponded to the third (paternal) allele were estimated by this program as false positive and results might not be received. We were forced to evaluate our results of HLA system typing manually using tables, which was frequently accompanied by technical and interpretation difficulties. The cause of these difficulties lay in the fact that not one but plenty of probes on a strip correspond with a particular HLA antigen at a low resolution level and the manual analysis of difficult tables can yield ambiguous results. Since HLA antigen typing in the NRBC fraction might be applicable in transplantation medicine, we intend to continue our study in this way.

Using different genetic markers located on different chromosomes, we have proved the presence of foetal cells in the mixture of enriched nucleated red blood cells separated from maternal peripheral blood from the 13^{th} week of gestation. Our results demonstrated that foetal cells enriched from maternal peripheral blood may be used as an alternative non-invasive source of foetal DNA for prenatal diagnosis, paternity testing and other application.

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