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HLA typing strategies in a cord blood bank

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Background and Objectives. Although widely used, serological typing of HLA loci does not always produce uequivocal results. This may be particularly the case for cord blood since samples may be of small volume and poor quality, and contaminated.

Design and Methods. We typed 220 cord blood units (CBU) for HLA class I antigens using the serological technique. For those samples giving doubtful results we repeated the HLA typing by polymerase chain reaction with sequence specific primers (PCR-SSP).

Results. Results were satisfactory for 181 samples (82.3%). For the remaining 39 (17.7%) we had a doubtful antigen assignment for A locus in 9/39 cases (23.1%) and for B locus in 22/39 cases (56.4%). Eight of the 39 samples (20.5%) could not be analyzed by serology due to the high mortality of the cell suspension. Using PCR-SSP we obtained clear definition of class I antigens in all cases. All CBU were typed for HLA class II alleles by PCR-SSP with clear results in 100% of cases.

Interpretation and Conclusions. In our experience, PCR-SSP can resolve the limitations of serology but, at the moment, it cannot substitute the latter in routine practice. The best strategy, in cord blood typing, is to perform both serological and molecular typing in order to obtain an accurate and clear result.

Key words: HLA, serology, molecular biology, cord blood.

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Over the last 30 years, the microlymphocytotoxicity test, designed by Terasaki and McClelland in 1964,[1] has been used as the method of choice for HLA typing in transplantation. Since the serological technique relies on the use of human alloantisera, some problems, linked to the quality and the availability of the sera and the viability of the cells, can lead to results of doubtful interpretation. Fortunately, in recent years most problems linked to human alloantisera have been resolved by the use of monoclonal sera. Nowadays, molecular techniques can solve the ambiguities of HLA-A and HLA-B loci results produced by serological tests .[2-4] By comparing both methods, it was clear that two types of problems occurred: a) doubtful assignment of antigens belonging to a cross-reactive group; b) lack of antisera against rare specificities.

Although the serological test is almost satisfactory when analyzing normal lymphocytes, problems arise with cord blood cells that show a high background of dead cells and can be contaminated with erythroblasts. We analyzed the cord blood units (CBU) of our cord blood bank, typed by serology, and performed molecular testing for those considered doubtful in order to solve most of the problems. We found that using both methods gave us the best results.

Design and Methods

We typed 220 consecutive CBU for HLA class I antigens with human alloantisera using the standard complement-dependent microlymphocytotoxicity test according to the National Institute of Health (USA). Lymphocytes were isolated from 1 mL of CBU and placed in commercial serological typing trays (One Lambda, Biotest, GTI). After two incubations at room temperature, a mixture of acridine orange and ethidium bromide was added to each well and the trays were analyzed by fluorescent microscopy. The same units were analyzed by polymerase chain reaction with sequence specific primers (PCR-SSP) in order to detect HLA-DRB1 genes. Serological typings of all CBU were

Table 1. Comparative A-locus typing by serology and molecular biology of 9/39 CBU.

N	*Serology*	*Molecular biology*
1	A11/A19	A*11/A*74
2	A23/A19	A*23/A*32
3	A19	A*32/A*33
4	A3/A19	A*03/A*29
5	A2/A10	A*02/A*66
6	A1/A10	A*01/A*66
7	A2/A10	A*02/A*66
8	A11/A28	A*11/A*69
9	A2/28	A*02

considered *doubtful* when cross-reactions, false-positive reactions, unclear split assessment or poor cell viability occurred. All doubtful HLA class I typings were pointed out and repeated by PCR-SSP. DNA samples were extracted by the salting out procedure[5] and the PCR reaction, performed using commercial primers (Dynal, Essemedical), was carried out according to the manufacturer's instructions. Samples were placed in a PE GenAmp System 9700 and the PCR products separated by electrophoresis in a TBE 1% gel at 120 V for 30' followed by photography of the DNA products. The specificities were assigned by the presence or the absence of the band on the gel for each specific primer combination.

Results

The serological typing for HLA class I antigens of our CBU gave satisfactory results in 181/220 cases (82.3%). Thirty-nine of the 220 CBU (17.7%) had doubtful typings and needed a second analysis by PCR-SSP.

Nine of these 39 samples (23.1%) showed ambiguous determinations of HLA-A locus specificities. In 4/9 cases (#1-4, Table 1) we found problems in the assignment of the A19 splits while 3/9 cases (#5-7, Table 1), all A10, did not show the A66 specificity; in one case (#8, Table 1), serology did not allow us to define the A69, split of A28, and finally, a cross-reaction between A2 and A28 occurred (#9, Table 1). In 22/39 (56.4%) samples there were problems in assigning the HLA-B locus specificities:

- 7/22 cases showed cross-reactions between the cross-reacting group (CREG) B35-B51-B53 (#10-16, Table 2);
- 3/22 cases (#23, 25, and 28, Table 2) involved difficulty in the assignment of B40 (in two cases due to the low reactivity of the specific antisera and in one case to the cross-reaction between B40 and B47);
- 3/22 cases (#17, 20, and 27, Table 2) showed a low reactivity of the antisera with B15, 3/22 cases (#26, 29, and 30, Table 2) gave problems in defining the B58 antigen allowing only the detection of the broad specificity B17;
- 2/22 cases (#18, 19, Table 2) showed some extra reactions particularly with B7, in one case (#24, Table 2) it was impossible to discriminate between B7 and B81 antigens;
- 2/22 cases (#21, 22, Table 2) showed some extra reactions;
- one case (#31, Table 2), showed cross-reactions between B51 and B52.

Eight of the 39 samples (20.5%) requiring PCR-SSP analysis could not be typed by serology due to high cellular mortality. PCR-SSP yielded clear results in all cases, as shown in Tables 1 and 2.

Discussion

The increasing numbers of cord blood banks have led tissue typing laboratories to develop a valid strategy to achieve clear results with low costs. Initially, the policy of our laboratory was to perform HLA class I typing by serology and HLA class II typing by molecular biology. However, given the high percentage of typing that needs to be repeated because of the well-known problems associated with cord blood cells,[6,7] we are currently combining the molecular technique with the serological test. In fact, the polymorphism of HLA molecules is generally detected by alloantisera or monoclonal antibodies that have a wider specificity than synthesis reagents, primers or oligonucleotides; for this reason serology can lead, in particular cases, to incomplete information. Furthermore, serology is sometimes inadequate with samples from hematologic patients (low numbers of lymphocytes in peripheral blood, interference by pharmacologic therapies) or cord blood cells (poor quality and low volume of samples, background of dead cells, contamination by erythroblasts).

A recent study, performed on 1,644 cord blood units,[8] showed that 235 of these (14.5%) had a *doubtful* HLA class I typing. In this case, a second study by PCR-SSP revealed an incorrect assignment of HLA-A and –B antigens of the same cross-reactive group (CREG).

Table 2. Comparative B-locus typing by serology and molecular biology of 22 / 39 CBU.

n	*serology*	*molecular biology*
10	B51/B53/B37	B*51 / B*37
11	B51/B53	B*51
12	B51/B35/B53	B*51/B*35
13	B51/B35/B53	B*51/B*35
14	B51/B35/B53	B*51/B*35
15	B51/B35/B53	B*51
16	B51/B53/B35	B*51
17	B49/B15wk	B*49/B*1524
18	B57/B7/B27	B*57/B*27
19	B55/B41/B7	B*55/B*41
20	B44/B15wk	B*44/B*15
21	B44/BX	B*44
22	B57/BX	B*57
23	B51/B40/B47	B*51/B*40
24	B35/B7/B81	B*07/B*35
25	B57/B40wk	B*57/B*40
26	B17/B35	B*58/B*35
27	B18/B15wk	B*15/B*18
28	B51/B40wk	B*51/B*40
29	B17	B*58
30	B15/B17	B*15/B*58
31	B5/B18	B*52/B*18

In our experience, most of the difficulties in HLA typing occurred at the B locus. In fact, with regards to the A locus, all problems, except that of case #9 in which a cross-reaction between A2 and A28 occurred, concerned the split assignment; in particular, in 3 cases it was difficult to detect the same antigen (A66) always, while in one case it was impossible to detect the A74, because of the lack of the corresponding alloantiserum. With regards to the B locus typing, most of the problems concerned cross-reactions within the CREG group B5-35-53 (8 cases) and in one case a cross-reaction between B51 and B52 antigens occurred. In five cases we had some extra reactions, and, again, five cases showed low reactivity of the antisera, particularly of the anti-B15. In 3 cases the problems were due to the lack of single sera against B58 (2 cases) and B81 (1 case). It is relevant that 8/39 samples (20.5%) could not be typed by serology because of the high mortality (>30%) of the cells.

As mentioned before, all the molecular typings led to clear solutions.

Compared to serological typing, the molecular approach offers some advantages: greater accuracy, precise results, small quantity of blood, low costs, independency from biological reagents, cell viability and delivery times of samples to the laboratory. On the other hand, PCR-SSP for the detection of class I antigens, needs large number of PCR reactions to be set out and this is not applicable in routine use. Furthermore it sometimes requires deduction of information, such as splits that cannot be detected by molecular biology at a low resolution, from serology.

By using monoclonal sera, the problems linked to serology can be drastically reduced and it is possible to obtain a clear result. It is true that one can apply this to molecular biology only for doubtful cases or when a single antigen is detected at one locus by serology, but we think that the use of both techniques, serological and molecular, gives more accurate and reliable results, without the necessity of larger blood samples, if one performs serological typing on the cells obtained from a small amount (1 mL) of the cord blood that undergoes DNA extraction (5 mL). Finally, but not less importantly, we have to consider that overlapping results obtained from serology and molecular biology is a further control for confirming correct DNA extraction from the sample.

In our opinion, the strategy to obtain the best result in cord blood typing is to use both serology and molecular biology; with the combined use of the two techniques it becomes possible to obtain a clear result in nearly 100% of cases.

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All authors of the current communication directly participated in the conception, design, data analysis, article drafting, critical revision and final approval of the study.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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PEER REVIEW OUTCOMES

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Jordi Sierra, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Dr. Sierra and the Editors. Manuscript received May 4, 2001; accepted May 6, 2002.

What is already known on this topic

HLA-compatibility is more accurately studied by molecular techniques than by serological methods.

What this study adds

Molecular typing was necessary for HLA class I assignment of 17% of cord blood units. The combination of serological typing and, in doubtful cases, PCR-SSP allowed HLA class I identification in 100% cases.

Potential implications for clinical practice

The sequential use of serological and molecular methods seems reasonable for routine HLA-typing of cord blood units.

Jordi Sierra, Deputy Editor