Transpl Int (2000) 13: 453–454

LETTER TO THE EDITOR

Francesca Poli
Mario Scalamogna
Loretta Crespiatico
Elena Longhi
Viviana Sioli
Sara Frison
Elena Monfardini
Girolamo Sirchia

Comparison of serological and molecular typing for HLA-A and -B on cadaver organ donor lymphocytes

Received: 27 January 2000
Accepted: 22 May 2000

Sir: To date, several papers have been published dealing with the comparison of serological and molecular typing for HLA class I on cadaveric organ donors [1, 5, 6] lymphocytes. These analyses unanimously demonstrated an impressive advantage of the DNA technology over serotyping to the point that it is practised routinely today by many laboratories. The above mentioned studies referred to serological typing data performed in many different centers participating in the Collaborative Transplant Study or involved a limited number of cases.

We wanted to address the question of how often cadaver organ donors are mistyped in our setting, where all HLA typing tests are centralized in a unique laboratory. This question is more than academic, due to clinical and ethical implications. In a system like ours [8], where HLA matching is an important parameter for kidney allocation, errors in HLA typing can change the recipient selection and have a negative impact on the transplant outcome, especially in re-transplants and sensitized patients.

With this aim, we repeated with DNA techniques HLA-A, B cytotoxicity tests of 340 cadaveric organ donors referred to the North Italy Transplant program (NITp) [8] from 20th November 1996 to 20th October 1997. HLA-A,B molecular typing was performed with PCR-SSOP on DNA isolated from peripheral blood [4] whereas serotyping was done on T-lymphocytes isolated from lymphnode or peripheral blood using immunomagnetic beads (Class I Dynabeads, Dynal A.S., Oslo, Norway). For microlymphocytotoxicity test we used a locally assembled 60 well typing tray and a commercially available tray (72 wells, Pel-Freez, Clinical Systems, Brown Deer, WI 53223, USA). PCR-SSOP was carried out using primers amplifying exons 2 and 3 and a total of 66 probes (28 for A locus and 38 for B locus) supplied by LIFECODES Corporation, Stamford, CT, USA. Molecular typing was carried out in blind fashion. HLA results were considered discrepant if broad specificities were different or missed even if due to clerical errors. The discrepancies detected with the 2 techniques were resolved with PCR-SSP.

Sixteen typing results were discrepant with the 2 methods (serology and PCR-SSOP) (Table 1). Six discrepancies were detected at A locus, and 10 at B locus. In one case (sample 5762) the discrepancy was due to a clerical error. All PCR-SSOP data were confirmed by PCR-SSP.

Table 1 HLA-A,B determinations discrepant with the 2 methodologies (serology/PCR-SSOP). The discrepancies are underlined in *bold*

Sample	HLA-A, B serologic typing	HLA-A, B genomic typing
5487	A1, **32**; B7, 8	A*1, **3**; B*07, 08
5627	A11, **28**; B35	A*11; B*35
5662	A2, **3**; B38, 50	A*2; B*38, 50
5674	A1, **19**; B17, 55	A*1; B*57, 55
5789	A2, **11**; B51, 35	A*2; B*51, 35
5951	A24; B51, 35	A*24, **32**; B*51, 35
5460	A1, 2; B51	A*01, 02; B***18**, 51
5671	A2, 3; B51	A*02, 03; B***15**, 51
5672	A3; B7, **54**	A*03; B*07
5746	A2, 24; B51, **53**	A*02, 24; B*51
5759	A2, 32; B35, **61**	A*02, 32; B***48**, 35
5762	A2, 26; B37, **37**	A*02, 26; B*37, **38**
5781	A2; B**18**, 52	A*02; B*52
5935	A24, 32; B**39**, 61	A*24, 32; B*61
5939	A2, 31; B13, **47**	A*02, 31; B*13
5921	A1, 3; B**51**, 35	A*01, 03; B***78**, 35

This comparison analysis shows that in our setting, 4.7% of cadaver donor HLA-A,B typing results are incorrect. This percentage is lower than that revealed by other studies [1, 5, 6]. The difference could be attributable to the fact that in our series all donor HLA determinations are carried out in the laboratory of the Reference Center. We also noted that the HLA specificities were more often falsely assigned (false positive), rather than missed, and that the discordant results, except in the case of B*78, did not involve alleles difficult to detect serologically.

In conclusion, the error rate in serological definition of HLA-A,B molecules argues for the use of a rapid DNA technique in addition to serological typing. PCR-SSP method [2, 3, 7] is particularly appropriate, being fast, sufficiently simple in interpretation for most people with the minimum of training, and not influenced by personal interpretation. For the time being, we think it is prudent to maintain both methods. Consequently we have started a prospective study for HLA Class I

typing of all cadaver kidney donors with serology and PCR-SSP to evaluate the feasibility of this approach and the possible improvement offered by the combined use of the 2 methodologies.

Acknowledgements This study was supported by a grant from „Ricerca Finalizzata" Ospedale Maggiore, 1997.

References

1. Adorno D, Canossi A, Papola F, Ozzella G, Piazza A, Di Rocco M, Monaco I, Liberatore G, Maccarone D, CU Casciani (1997) Comparison between HLA Class I PCR-ARMS and serologic typing in cadaveric kidney transplantation. Transplant Proc 29: 1423–1425
2. Bunce M, O'Neill CM, Barnardo MCNM, Krausa P, Browning MJ, Morris PJ, Welsh KI (1995) Phototyping: comprehensive DNA typing for HLA-A, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 46: 355–367
3. Dupont B (1995) "Phototyping" for HLA: the beginning of the end of HLA typing as we know it. Tissue Antigens 46: 353–354
4. Gustincich S, Manfioletti G, Del Sal G, Schneider C, Carninci P (1991) A fast method for high-quality genomic DNA extraction from whole human blood. BioTechniques 11: 298–302
5. Mytilineos J, Lempert M, Middleton D, Williams F, Cullen C, Scherer S, Opelz G (1997) HLA class I DNA typing of 215 "HLA-A, -B, -DR zero mismatched" kidney transplants. Tissue Antigens 50: 355–358
6. Mytilineos J, Lempert M, Scherer S, Schwarz V, Opelz G (1998) Comparison of serological and DNA PCR-SSP typing results for HLA-A and HLA-B in 421 Black individuals: a Collaborative Transplant Study report. Hum Immunol 59: 512–517
7. Olerup O and Zetterquist H (1992) HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39: 225–235
8. Sirchia G, Poli F, Cardillo M, Scalamogna M, Rebulla P, Taioli E, Remuzzi G, Nocera A (1998) Cadaver kidney allocation in the North Italy Transplant Program on the eve of the new millennium. In: Cecka and Terasaki (eds) Clinical Transplants 1998. UCLA Tissue Typing Laboratory, Los Angeles, California, pp 133–145

F. Poli (✉) · L. Crespiatico · E. Longhi
V. Sioli · S. Frison · E. Monfardini
G. Sirchia
Centro Transfusionale e di Immunologia dei Trapianti,
Ospedale Maggiore Policlinico IRCCS,
Via Francesco Sforza 35, 20122 Milano, Italy
e-mail: nitpmi@polic.cilea.it
Tel.: + 39-02-55034239
Fax: + 39–02–55012573

M. Scalamogna
Servizio per il Prelievo e la Conservazione di Organi e Tessuti, Ospedale Maggiore Policlinico IRCCS,
Via Francesco Sforza 35, 20122 Milano, Italy