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Disparity in HLA-DR Typing and Mixed Lymphocyte Culture Reactivity

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ABSTRACT

Prospective HLA-DR matching has been shown to enhance renal graft survival. It has also been demonstrated that the major stimuli in the one-way mixed lymphocyte culture reaction (MLR) are the DR rather than DQ class II histocompatibility antigens. A two antigen DR match between the potential recipient and donor is usually associated with a negative MLR. The findings of two family studies are reported in which HLA-DR identity between the potential recipient and donors did not correlate with the MLR reactivity. These findings further strengthen the need for performing the MLR in conjunction with DR/DQ typing since splits have been described for some of the DR antigens which may not be detected using the currently available commercial typing trays.

Introduction

The D region of the HLA complex is comprised of at least three different loci, namely, DR, DP (SB) and DQ (DC, DS, MB).[3,15] Gonwa et al[8] found that adherent mononuclear cells (AMC) expressing DS (DQ) were the most effective cell population as antigen presenters for T lymphocytes compared to AMC lacking this class II gene product. Both populations of AMC expressed DR antigens and were equally effective as stimulator cells in the one-way mixed lymphocyte culture reaction (MLR). More recently, Navarrete et al[16] using monoclonal antibodies specific for DR and DQ, determined that DR antigen expression was necessary for the stimulator cell population in order to elicit a strong MLR. However, blocking the MLR with the DR specific monoclonal antibody did not block the generation of cytotoxic T cells. The addition of monoclonal antibody specific for DQ did not block the MLR, but it did impair the generation of cytotoxic/suppressor cells. The conclusion of Navarrete et al[16] was that strong reactivity in the MLR was a direct function of DR expression on the stimulator cells and not DQ.

The one-way MLR is an *in vitro* model of allograft antigen recognition and potential rejection when disparity exists

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in the D region.[4] Pineda et al[17] noted that increased graft survival times were associated with a low stimulation index (SI) in the one-way MLR. Berg and Ringden[5] and Radvany and Vaisrub[18] showed that a strong correlation exists between DR identity and MLR compatibility. Using a stabilized relative response, donor specific one-way MLR, Langhoff et al[10] noted that this procedure provided a better indication of graft survival than DR typing.

The results of two family studies are reported in which donor DR compatibility did not correlate with the one-way MLR results, further emphasizing the importance of performing the mixed lymphocyte culture reaction.

Materials and Methods

Family #1 (Caucasian): The son (potential kidney recipient) and both parents (potential donors).

Family #2 (Caucasian): The son (potential bone marrow recipient), mother, maternal grandparents, and maternal uncle (potential donors).

Preparation of Mononuclear Cells for HLA-A, B, DR, and DQ Typing

Heparinized tubes were used to collect venous blood from all individuals being tested. Purified lymphocytes were isolated using density gradient centrifugation* and carbonyl iron as described by Mittal et al.[14] The B cells were isolated from the purified lymphocyte preparations by nylon wool separation according to the method of Fotino and Menon.[7] Cell viability in all cases was >95 percent.

HLA-A, B, DR and DQ Typing

The microlymphocytotoxicity assay as described by Hopkins and MacQueen[9] was followed for the HLA typing. For the HLA-A and B typing of both families, Terasaki Third HLA trays, Lot #5† were used. The HLA-DR and DQ typing were performed on the enriched B cells of Family #1 using Terasaki DRW Tray, Lot 20† and Pel-Freez HLA-DR Tray, Series DR-72-5, Lot #6.‡ Terasaki DRW Tray, Lot #19 and Pel-Freez HLA-DR Tray, Series DR-72-5, Lot #4 were used for the typing of the B cells from members of Family #2.

One-Way Mixed Lymphocyte Culture Reaction (MLR)

The procedure of Cerilli et al[6] was followed for the one-way MLR. Briefly, mononuclear cells were isolated from heparinized blood using density gradient centrifugation as described, including blood from three unrelated individuals to serve as a positive stimulation control. Following three washes in Roswell Park Memorial Institute (RPMI) 1640 medium,§ the respective cells were divided in half. One portion was resuspended in complete RPMI 1640 medium (one percent L-glutamine, 100 μg per ml gentamycin, and 20 percent heat inactivated AB serum) at 1×10^6 cells per ml, while the remaining cells were treated with mitomycin C (50 μg per 6×10^6 cells) for 30 minutes at 37°C. Following three washes in RPMI 1640 medium, the cells were counted and resuspended as described previously. One-tenth ml of each of the respective cell populations was added to a 96 well Costar microculture plate‖ in replicates of five as follows:

* Histopaque 1.077, Sigma Chemical Co., St. Louis, MO.

† One Lambda Inc., Los Angeles, CA.
‡ Pel-Freez Clinical Systems, Brown Deer, WI.
§ M.A. Bioproducts, Walkersville, MD.
‖ Hyclone, Logan, UT.

R × Rm, R × Dm, R × Um, D × Dm, D × Rm, D × Um, U × Um, U × Rm and U × Dm in which m = mitomycin C treated, R = recipient, D = donor, and U = unrelated control. The cells were cultured for five days at which time 0.5 uci of ^{3}H-thymidine (6.7 Ci per mM) was added to each well. Following an additional 18 hours of culturing, the cells were harvested onto glass fiber filters and prepared for liquid scintillation counting. The data are presented as both the stimulation index (SI) and percent of relative response (% RR) as follows:

$$SI = \frac{\text{cpm of responder} \times \text{stimulator (m) treated}}{\text{cpm of responder} \times \text{responder (m) treated}}$$

cpm = counts per minute
m = mitomycin C treated

$$RR = \frac{\text{cpm of R} \times \text{Dm} - \text{cpm of R} \times \text{Rm}}{\text{cpm of R} \times \text{Um} - \text{cpm of R} \times \text{Rm}} \times 100\%$$

m = mitomycin C treated
R = potential recipient
D = potential donor
U = pooled mononuclear cells of unrelated individuals

The mixed lymphocyte culture reaction (MLR) data are reported routinely as both the simulation index (SI) and percent relative response (RR), since some investigators prefer both calculations. The % RR determination is based on both the MLR results of the recipient and donor and recipient and pooled cells of the unrelated individuals and, as such, is thought to be a better means of presenting the mixed lymphocyte culture reaction data.

As a control to insure the competency of the cells being tested in the one-way MLR, the mononuclear cells from the respective individuals were cultured with phytohemagglutinin* (25 μg per ml). At two days into the culture period, 0.5 uci of ^{3}H-thymidine (2.0 Ci per mM) was added, and the cells were cultured for an additional 18 hours prior to harvesting. The PHA data are presented as the SI of cpm with PHA/cpm without PHA.

* (PHA), Sigma Chemical Co., St. Louis, MO.

TABLE I

HLA Phenotyping of Family #1

Family Member	*HLA-A, B*	*HLA-DR, DQ*
Son	A1,30; B7,14	DR1,4,W53; DQW1,W3
Mother	A30,X; B14,X	DR1,4,W53; DQW1,W3
Father	A1,31; B7,40	DR1,4,W53; DQW1,W3

TABLE II

HLA Phenotyping of Family #2

Family Member	*HLA-A, B*	*HLA-DR, DQ*
Son	A1,24; B8,16	DR3,W6,W52; DQW1,W2
Mother	A1,24; B8,16	DR3,W6,W52; DQW1,W2
Uncle	A1, 2; B8,16	DR3,W6,W52; DQW1,W2
Grandfather	A1,11; B8,X	DR3,W6,W52; DQW2,X
Grandmother	A2,24; B16,X	DR3,W6,W52; DQW1,W3

Results

The data in tables I and II present the HLA phenotyping of families #1 and #2.

The results of the one-way MLR and PHA stimulation using the isolated mononuclear cells from the members of Families #1 and 2, including the three pooled unrelated controls, are presented in tables III and IV. Only the SI and % RR are being presented. The standard deviations for each run were always less than 15 percent of the respective means.

Discussion

The mixed lymphocyte culture reaction (MLR) is thought to be an *in vitro* model of allograft antigen recognition and rejection when disparity is present at the HLA-D region between the potential allograft recipient and donor.[4]

A low MLR and no DR incompatibility between recipient and donor are correlated with increased graft survival.[2,5] Madsen et al,[12] Middleton et al,[13] and Lucas et al[11] found that DR matching for cadaver renal allografts greatly enhanced the one year graft survival rates.

It should be noted that the main purpose of the mixed lymphocyte culture reaction is to detect disparity at the HLA-D region between the potential recipient and donor. Because certain variables are associated with this *in vitro* test system, a stimulation index of <five or a relative response of <20 percent is associated with MLR compatibility.[6,18] Some of the minor stimulation seen in the assay between HLA identical individuals is probably due to disparity at other non-HLA-DR loci of the HLA-D region. One variable of the MLR is the means by which the stimulator cells are processed to prevent their replication, namely, mitomycin C (m) treatment or irradiation. In our hands, the m treatment has been used successfully to inactivate the stimulator cells, but this method is not as efficient as irradiation. It has been found that m treated cells will exhibit a very limited degree of stimulation in the presence of PHA (SI of no more than five), compared to untreated cells which respond vigorously to this mitogen (SI > 100). Appropriate irradiation of the stimulator cells followed by the addition of PHA results in an SI of about two.†

Reinsmoen et al[19] described an anomalous reactivity in the MLR and DR typing in HLA-A,B,C, and DR identical siblings. The results in this study also indicate that DR identity does not always constitute a low MLR amongst blood relatives. In Family #1, the son (potential recipient) reacted vigorously to the DR/DQ identical cells of his father in the MLR (SI = 22.1, RR = 31.3 percent), while the converse MLR resulted in an SI of 5.9, indicating that the helper T cells of the son were seeing an additional determinant(s) on the DR antigens present on the paternal cells. This reactivity may be directed to splits related to the DR 4 antigen, since splits have not been described for DR 1.[1]

TABLE III

Phytohemagglutinin and One-Way Mixed Lymphocyte Culture Reaction Results of Family #1

	One-Way MLR			
Family Member	*Stimulator Cells (Mitomycin C Treated)*	*SI*	*% RR*	*PHA SI*
Son	x Mother	4.5*	5.3	152
	x Father	22.1	31.3	
	x Unrelated	68.5		
Mother	x Son	6.0*	24.5	128
	x Unrelated	21.6		
Father	x Son	5.9*	28.3	156
	x Unrelated	18.1		

*P < 0.05 compared to counts per minute of Responder x Responder (mitomycin C)
MLR = mixed lymphocyte culture reaction
SI = stimulation index
RR = relative response
PHA = phytohemagglutinin

Enhanced reactivity was also noted in Family #2 with the potential recipient's lymphocytes responding to the DR/DQ identical cells of his uncle (SI = 12.7, RR = 23 percent) and grandfather (SI = 11.7, RR = 22 percent). As in Family #1, the converse MLR results were SI of 5.6 and 4.1, respectively, again indicating that the helper T cells of the potential recipient were seeing additional stimulatory determinants, possibly antigens associated with one of the splits (DRW 13,14) of DR W6.[1] The interpretation of the results with the grandmother are less clear, mainly because the results of her DR/DQ typing do not completely correlate with the DR 3,W6 phenotype. Strong linkage disequilibrium exists with DR 3/DQ W2, and DR W6/DQ W1,[1] yet the grandmother was typed as a DQ W1,W3 rather than a

† Unpublished observations.

TABLE IV

Phytohemagglutinin and One-Way Mixed Lymphocyte Culture Reaction Results of Family #2

Family Member	One-Way MLR Stimulator Cells (Mitomycin C Treated)	SI	% RR	PHA SI
Son	x Mother	3.9*	6.0	124
	x Uncle	12.7	23.0	
	x Grandmother	38.5	75.0	
	x Grandfather	11.8	22.0	
	x Unrelated	50.9	-	
Mother	x Son	3.5*	3.3	170
	x Unrelated	75.3	-	
Uncle	x Nephew (PR)	5.6*	8.6	126
	x Unrelated	54.5	-	
Grandmother	x Grandson	23.0	36.5	146
	x Unrelated	61.3	-	
Grandfather	x Grandson	3.9*	4.7	115
	x Unrelated	61.8	-	

*P < 0.05 compared to counts per minute of Responder x Responder (mitomycin C)
MLR = mixed lymphocyte culture reaction
SI = stimulation index
RR = relative response
PHA = phytohemagglutinin

W1,W2. Although no DR reactivity other than the 3,W6 occurred, it is conceivable that she was not a DR3. The MLR results with the grandmother (SI = 38.5, RR = 75 percent) demonstrate vigorous T cell proliferation; however, in the converse MLR (grandmother × grandson), enhanced reactivity also resulted (SI = 23.0), which may indicate that the grandmother was not a DR3.

Although these are the only two cases in which type of MLR reactivity with DR compatibility has been seen, it further emphasizes the necessity of performing the MLR when the transplant involves a living donor, and points to the need for improvements in the DR typing to enhance the DR antigen/MLR correlation.

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References

1. ALBERT, E. D., BAUR, M. P., and MAYR, W. R., eds.: Histocompatibility Testing 1984. Munich, Springer-Verlag, 1984.
2. ALBRECHTSEN, D., BRATLIE, A., KISS, E., SOLHEIM, B. G., THORESEN, A. B., WINTHER, N., and THORSBY, E.: Significance of HLA matching in renal transplantation. A prospective one-center study of 485 transplants matched or mismatched for HLA-A,B,C,D, and DR antigens. Transplantation *28*:280–284, 1979.
3. AUFFRAY, C., LILLIE, J. W., ARNOT, D., GROSSBERGER, D., KAPPES, D., and STROMINGER, J. L.: Isotypic and allotypic variation of human class II histocompatibility antigen alpha chains. Nature *308*:327–333, 1984.
4. BACH, F. H., BACH, M. L., and SONDEL, P. M.: Differential function of major histocompatibility complex antigens in T-lymphocyte activation. Nature *259*:273–281, 1976.
5. BERG, B. and RINGDEN, O.: Correlation between relative responses in mixed lymphocyte culture, HLA-D and DR typing, and graft survival in renal transplantation. Transplantation *33*:291–297, 1982.
6. CERILLI, J., WILLIAMS, M. A., NEWHOUSE, Y. G., and FESPERMAN, D. P.: Correlation of tissue typing, mixed lymphocyte culture, and related donor renal allograft survival. Transplantation *26*:218–220, 1978.
7. FOTINO, M. and MENON, A. K.: Nylon wool separation of T and B lymphocytes. The AACHT Laboratory Manual. New York, The American Association for Clinical Histocompatibility Testing, 1981, pp. I-8-1-I-8-5.
8. GONWA, T. A., PICKER, L. J., RAFF, H. V., GOYERT, S. M., SILVER, J., and STOBO, J. D.: Antigen-presenting capabilities of human monocytes correlate with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. J. Immunol. *130*:706–711, 1983.
9. HOPKINS, K. A. and MACQUEEN, J. M.: Basic microcytotoxicity technique. The AACHT Laboratory Manual. New York, The American Association for Clinical Histocompatibility Testing, 1981, pp. II–1–1–II–1–8.
10. LANGHOFF, E., JAKOBSEN, B. K., PLATZ, P., RYDER, L. P., and SVEJGAARD, A.: The impact of low donor-specific MLR versus HLA-DR compatibility on kidney graft survival. Transplantation *39*:18–21, 1985.
11. LUCAS, B. A., JENNINGS, C. D., THOMPSON, J. S., FLANIGAN, R. C., MCROBERTS, J. W., and HOLLAND, N. H.: Prospective DR matching for first cadaver donor renal allografts and retransplantation. Transplantation *39*:39–44, 1985.
12. MADSEN, M., GRAUGAARD, B., FJELDBORG, O., PETERSEN, V. P., HANSEN, H. E., and KISSMEYER-NIELSEN, F.: The impact of HLA-DR antigen matching on survival of cadaveric renal allografts. Transplantation *36*:379–383, 1983.
13. MIDDLETON, D., GILLESPIE, E. L., DOHERTY, C. C., DOUGLAS, J. F., and MCGEOWN, M. G.:

The influence of HLA-A,B, and DR matching on graft survival in primary cadaveric renal transplantation in Belfast. Transplantation *39*:608–610, 1985.

14. MITTAL, K. K., FOTINO, M., and MENON, A. K.: Isolation and purification of lymphocytes from peripheral blood. The American Association for Clinical Histocompatibility Testing (AACHT) Laboratory Manual. New York, The American Association for Clinical Histocompatibility Testing, 1981, pp. I–2–1–I–2–11.
15. MOLLER, E., CARLSSON, B., and WALLIN, J.: Distinct or overlapping functions of human class II genes. Scand. J. Immunol. *20*:483–486, 1984.
16. NAVARRETE, C., JARAQUEMADA, D., FAINBOIM, L., KARR, R., HUI, K., AWAD, J., BAGNARA, M., and FESTENSTEIN, H.: Genetic and functional relationship of the HLA-DR and HLA-DQ antigens. Immunogenetics *21*:97–101, 1985.
17. PINEDA, A. A., MOORE, S. B., WOODS, J. E., OFFORD, K. P., ZINCKE, H., and TASWELL, H. F.: Mixed lymphocyte culture, phytohemagglutinin stimulation, and matching grade: clinical relevance in renal transplantation from living related donors. Transplantation 29:97–99, 1980.
18. RADVANY, R. M. and VAISRUB, N.: HLA-DR typing as a predictor of MLC compatibility. Transplantation *38*:347–351, 1984.
19. REINSMOEN, N. L., NOREEN, H. J., FRIEND, P. S., GIBLETT, E. R., GREENBERG, L. J., and KERSEY, J. H.: Anomalous mixed lymphocyte culture reactivity between HLA-A, -B, -C, -DR identical siblings. Tissue Antigens *13*:19–34, 1979.