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A Comparison of Flow Cytometry and Microcytotoxicity for the Evaluation of Alloimmune Therapy in Patients With Recurrent Spontaneous Abortions

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PROBLEM: There is substantial data that support the efficacy of paternal leukocyte immunization (PLI) for the treatment of alloimmune mediated miscarriage; however, there is confusion regarding the laboratory test that should be performed to determine levels of maternal anti-paternal leukocyte antibodies (MAPLA).

METHOD: Popular methodologies employed include: 1) microcytotoxicity (MCX), 2) mixed lymphocyte culture (MLC), and 3) cell flow cytometry crossmatch (FCXM). Cell flow cytometry crossmatch correlates well with the more difficult MLC assay although the former proves the more sensitive study. This work compares the MCX assays with FCXM. The study group consisted of ten women who had a history of three or more spontaneous abortions (SABs). All ten had very low levels (<10%) of MAPLA as measured by FCXM. Following PLI all subjects demonstrated elevated levels (>50%) of MAPLA by FCXM. At 12 weeks gestation, sera were simultaneously measured for MAPLA by MCX and FCXM.

RESULTS: Although all ten patients had very high levels of MAPLA by FCXM during pregnancy, five of ten had antibodies to HLA Class I and two of ten had antibodies to HLA Class II paternal antigens by MCX. Furthermore, all patients who were positive by MCX to paternal Class I antigens were also positive to Class I antigens not seen in either parent. Both patients who were positive by MCX to paternal Class II antigens were also positive to maternal Class II antigens. Notable is that all ten women eventually delivered healthy infants.

CONCLUSION: Based on this preliminary study, the MCX assay is neither sensitive or reliable enough to determine the need and/or to monitor the effectiveness of PLI. Flow cytometry should be the modality of choice when determining the need for alloimmunotherapy and to monitor the effectiveness of treatment.

Key words: recurrent spontaneous abortions, flow cytometry microcytotoxicity

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INTRODUCTION

The fetus is an allograft to which the mother must remain immunologically tolerant in order for the fetus to survive. Much interest has been focused on

the immunology of recurrent spontaneous abortion (RSA). Up to 50% of RSA may be mediated by the immune system via inadequate maternal anti-paternal antibodies (which protect the fetus from the mother's immune system)[1] or the presence of autoantibodies-e.g., antiphospholipid and antinuclear. These are the basis for immunotherapy with paternal leukocyte immunization (PLI), which has a reported success rate of 70% to 89% when performed and monitored properly.[2]

There is confusion regarding the choice of laboratory test that should be performed to determine levels of maternal anti-paternal leukocyte antibodies (MAPLA). The methodology employed may have a large impact upon the selection criteria and adequacy of response to treatment. It is likely that the variations in methods used to determine MAPLA has accounted for the differences in success rate in various studies. The most popular methodologies employed include: 1) microcytotoxicity (MCX), 2) mixed lymphocyte culture (MLC), and 3) cell flow cytometry crossmatch (FCXM). Each measures slightly different responses-e.g., MCX measures only antibodies that fix complement, whereas FCXM measures both complement and non-complement fixing antibodies. FCXM has been shown to correlate well with the more difficult MLC assay although the former proves the more sensitive study [3]. Several studies have used either FCXM or MCX to measure MAPLA .[4,5] This work compares the MCX assay with FCXM.

MATERIALS AND METHODS

Patient Population

The study group consisted of ten women who had a history of three or more spontaneous abortions (SABs). All ten had very low levels (<10%) of MAPLA as measured by FCXM, and all were negative for antibodies as measured by MCX. Following PLI all subjects demonstrated elevated levels (>50%) of MAPLA by FCXM.

Maternal Antipaternal Leukocyte Antibodies

The anti-paternal antibodies were determined preconception and at monthly intervals through week 24 of pregnancy via Becton-Dickinson FACScan flow cytometer using standard methods. The results were calculated on the HP 340 computer system. A patient was considered to have positive antibody formation if her antibodies coated 30% of the husbands T and B lymphocytes.

Microcytotoxicity Antibodies

The microcytotoxicity antibodies were determined preconception and at 12 weeks gestation using Lambda Cell Trays (One Lambda, Canoga Park, CA). Briefly, one lambda of the patients sera was added to each well on the cell tray. The tray was then incubated for 30 min at 25°. Five lambdas of complement (either T -cell or B-cell, depending upon the tray) was added to each well, and the trays were again incubated at 25°C for 60 min. Stain-Fix (One Lambda) was then added to the trays, which were subsequently analyzed for microcytotoxicity antibodies to HLA.

Paternal Leukocyte Immunization

Blood was taken from the husband and the buffy coat was subjected to a Ficoll-Hypaque gradient centrifugation with Histopaque 1077 (Sigma, St. Louis, MO). The mononuclear cell interface was extracted and treated with ammonium chloride to lyse any remaining red blood cells. Platelets were washed out (three times) with Hank's Balanced Salt Solution. PLI, consisting of 40 million cells, was injected intradermally into six sites on the forearms. Immunization was repeated 4 weeks later. One month following the second immunization, a flow cytometric analysis was performed to ascertain maternal response. If the response was suboptimal, additional immunizations of 80 million paternal white cells were administered. Couples were instructed to attempt conception only if the anti-paternal antibody levels were positive. When necessary, PLI was continued through mid second trimester.

RESULTS

Prior to therapy, all 10 patients tested negative for MAPLA as measured both by MCX and by FCXM. Following PLI all subjects demonstrated elevated levels (>50%) of MAPLA by FCXM. The 10 subjects became pregnant within 2 months of a positive MAPLA level, as measured by FCXM. At 12 weeks gestation, sera were simultaneously measured for MAPLA by MCX and FCXM. While all ten patients had very high levels of MAPLA to paternal B cells and paternal T cells by FCXM during pregnancy, only five of ten had antibodies to HLA Class I and two of ten had antibodies to HLA Class II paternal antigens by MCX (see Table I). There was a large variance in the variety of antibodies to paternal HLA, as some women had antibodies to only two of the father's HLA, whereas other women had antibodies to as

TABLE I. Antipaternal Antibodies

Patient	ABC Abs	DR, DQ Abs	Flow Cytometry Anti-T Cell Abs	Flow Cytometry Anti-B Cell Abs
R.W.	A2(H), A74 (N), B58(H)	None	Positive	Positive
C. M.	A11 (H), A23(N), A24(N), A26(N)	None	Positive	Positive
M. L.	None	None	Positive	Positive
N.G.	None	None	Positive	Positive
M.S.	None	None	Positive	Positive
L. R.	None	None	Positive	Positive
R.Z.	None	None	Positive	Positive
J. S.	A2(H), A33(H), A24(N)	DR4(W), DR14(N), DR15(H), DR53(N), DQ3(W), DQ6(N)	Positive Positive	Positive Positive
J.T.	A23(H), A24(N), A68(N), B8(H), B35(H), B7(W), C4(H)	DR1(W), DR17(H), DR52(H), DQ2(H) DQ6(H/W)	Positive Positive	Positive Positive
H.T.	A2(H), A68(N)	None	Positive	Positive
Totals	5/10 Positive	2/10 Positive	10/10 Positive	10/10 Positive

many as seven. Furthermore, all patients who were positive by MCX to paternal Class I antigens were also positive to Class I antigens not seen in either parent. Four of the five demonstrated antibodies to paternal, but not to maternal Class I antigens, whereas the fifth had antibodies to both paternal and maternal Class I antigens. Both patients who were positive by MCX to paternal Class II antigens were also positive to maternal Class II antigens. Notable is that all ten women eventually delivered healthy infants.

CONCLUSIONS

At first glance, there is a large discrepancy between the results of MAPLA as measured by MCX versus FCXM. Whereas all of these women demonstrated blocking antibodies by FCXM, few of them had antibodies by MCX, despite good pregnancy outcomes. Even more disturbing is that the antibody response as measured by MCX appears to be nonspecific, with a large number of antibodies to non-parental and to maternal antigens.

These results, however, are not surprising in an immunologic sense. Other workers have shown that the majority of antibodies found in the placenta are of the asymmetric, non-complement fixing variety.[6] In a model similar to paternal leukocyte immunization, MacLeod et al. reported the appearance of non-cytotoxic antibodies in transfused patients that reacted with the Fc receptors of B lymphocytes.[7] If the theory that MAPLA actually block the maternal immune response is correct, it would make more sense that these antibodies did not fix complement or initiate any specific immune response. This would explain why other workers have not shown a correlation of MCX with pregnancy outcome.[8] The advantage of using a flow cytometer to measure MAPLA versus MCX is that the machine is sensitive, the results are reproducible, and it measures non-complement fixing antibodies.

Based on this preliminary study, the MCX assay is neither sensitive or reliable enough to determine the need and/or to monitor the effectiveness of PLI. Flow cytometry should be the modality of choice when determining the need for alloimmunotherapy and to monitor the effectiveness of treatment.

REFERENCES

1. Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW Controlled trial of treatment of

recurrent spontaneous abortion by immunisation with paternal cells. Lancet. 1985; 1:941-943.

2. Beer AE, Semprini AE, Xiaoyu Z, Quebbeman JF. Pregnancy outcome in human couples with recurrent spontaneous abortions: HLA antigen profiles; HLA antigen sharing; female serum MLR blocking factors; and paternal leukocyte immunization. Expl Clin Immunogenet. 1985; 2:137-153.
3. Gilman-Sachs A, Luo SP, Beer AE, Beaman KD. Analysis of anti-lymphocyte antibodies by flow cytometry or microcytotoxicity in women with recurrent spontaneous abortions immunized with paternal leukocytes. J Clin Lab Immunol. 1989; 30:53-59.
4. Takakuwa K, Kanazawa K, Takeuchi S. Production of blocking antibodies by vaccination with husband's lymphocytes in unexplained recurrent aborters: The role in succcessful pregnancy. Am J Reprod Immunol Microbiol. 1986;10:1-9.
5. Cowchock FS, Smith JB, Savid S, Scher J, Batzer F, Corson S. Paternal mononuclear cell immunization therapy for repeated miscarriage: Predictive variables for pregnancy success. Am J Reprod Immunol 1990; 22:12-17.
6. Borel HM, Gentile T, Angelucci J, Pividori J, del Carmen Guala M, Biaghi RA, Margni RA. IgG asymmetric molecules with antipaternal activity isolated from sera and placenta of pregnant human. J Reprod Immunol. 1991; 20:129-140.
7. MacLeod AM, Mason RJ, Steart KN, Power DA, Shewan WG, Edward N, Catto GRD. Fc-receptor-blocking antibodies develop after blood transfusions and correlate with good graft outcome. Transplant Proc 1983; 15:1019-1021.
8. Smith JB, Cowchock FS. Immunological studies in recurrent spontaneous abortion: Effects of immunization of women with paternal mononuclear cells on lymphocytotoxic and mixed lymphocyte reaction blocking antibodies and correlation with sharing of HLA antigens and pregnancy outcome. J Reprod Immunol. 1988; 14: 99-113.