

B LYMPHOCYTE ANTIBODIES ASSOCIATED WITH SUCCESSFUL RENAL TRANSPLANTATION AND SUCCESSFUL PREGNANCY

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Summary

This study shows that non-cytotoxic Fc receptor blocking antibodies occurred more frequently in pre-transplant sera when the recipient had been transfused over five units of blood. Moreover 7/12 previously untransfused patients developed Fc receptor blocking antibodies during an elective blood transfusion regime. Erythrocyte antibody-rosette-inhibiting antibodies were also found at significantly higher values in the sera of women during the first trimester of a normal first pregnancy than in women studied at the time of spontaneous abortion. Family studies showed that this activity was linked to the HLA gene complex. These results indicate that EA inhibiting antibodies directed against MHC linked antigens are associated with specific abrogation of the immune response.

Introduction

Studies on the rat renal transplant model have shown that the presence of Fc receptor blocking antibodies detected by the erythrocyte antibody inhibition assay (EAI), whether actively [1] or passively [2] induced correlated with prolonged allograft survival. These antibodies were subsequently shown [3] to be directed against antigens coded for by the rat MHC. We have already shown [4] that when EA inhibiting antibodies are present in pre-transplant sera in the human, transplant survival is improved. The mode of induction of these antibodies is unclear but since blood transfusions are also known to improve allograft survival we aimed to determine whether patients transfused over five units of blood are more likely to develop these antibodies than those given five units of blood or less and to monitor antibody development in 12 chronic dialysis patients during an elective blood transfusion regime.

Preliminary data [5], using a different assay technique have shown that during successful pregnancy, which may be considered a form of transplant, women produce detectable quantities of blocking antibody which are not found in women subject to recurrent spontaneous abortion. In this study therefore we

aimed to compare the development of EA inhibiting antibody in a group of successfully pregnant women and a group of recurrent spontaneous aborters and to determine by studying paternal family members whether such antibodies were directed towards paternal HLA linked antigens.

Materials and methods

Sera were obtained from (a) 35 recipients of cadaver donor renal transplants with known blood transfusion histories, within 12 hours prior to transplantation; (b) 12 previously untransfused chronic dialysis patients before each of three elective blood transfusions, at monthly intervals and after the last transfusion; (c) 10 normal primigravidae of < 16 weeks gestation; (d) nine women at the time of abortion who had never experienced a normal pregnancy and all but one of whom had experienced two or more spontaneous abortions; and (e) 10 normal multiparous women. All sera were heat inactivated at 56°C for 45 minutes, an aliquot was absorbed with pooled human platelets. The sera were ultracentrifuged at 100,000g for one hour prior to use.

The target lymphocytes used were (a) B lymphocytes from renal transplant donors, viable in 33 cases; (b) lymphocytes from a panel of six patients with chronic lymphatic leukaemia (CLL); (c) paternal B lymphocytes; and (d) B lymphocytes from members of six paternal families all of whose haplotypes had been determined by HLA-A and B typing and HLA DR typing where necessary.

EA inhibition was performed by a modification of a rosette technique [4].

Statistical analysis was performed using Fisher's exact test for four-fold tables and the Wilcoxon rank sum test.

Results

Significantly more patients in the group transfused over five units of blood showed pre-transplant EAI against both the donor ($p < 0.05$) and the leukaemic cell panel ($p < 0.025$) (Table I). EAI was present in the serum of only one patient, a multiparous woman, prior to transfusion and was directed against the cells of two members of the CLL panel. Antibodies developed against cells of a further two panel members during the course of the transfusion regime. A further six of the 12 cases after transfusion developed EAI against different numbers of panel members. This varied from two out of six in two cases to one patient who developed antibodies against all six panel members. Therefore in all but one case EAI was selective.

EA inhibitory activity against paternal B lymphocytes was present in nine out of 10 multiparae, seven out of 10 primigravidae and none of the nine women at the time of spontaneous abortion. The mean levels of EAI in the three groups were 57.8 ± 28.9 per cent, 33.5 ± 12.5 per cent and 5.4 ± 7 per cent respectively. The difference in antibody activity between the groups of primigravidae and recurrent spontaneous aborters was statistically significant ($p < 0.001$). Maternal sera which shared EA inhibiting activity against paternal B lymphocytes were tested against cells from the other members of the father's family. In five out of the six families tested the EAI in maternal sera was directed only against family

TABLE I. EAI and number of pre-transplant blood transfusions

	EAI positive	EAI negative	
Donor			
> 5 transfusions	13	12	p < 0.05
≤ 5 transfusions	0	8	
CLL panel			
> 5 transfusions	17	9	p < 0.025
≤ 5 transfusions	1	8	

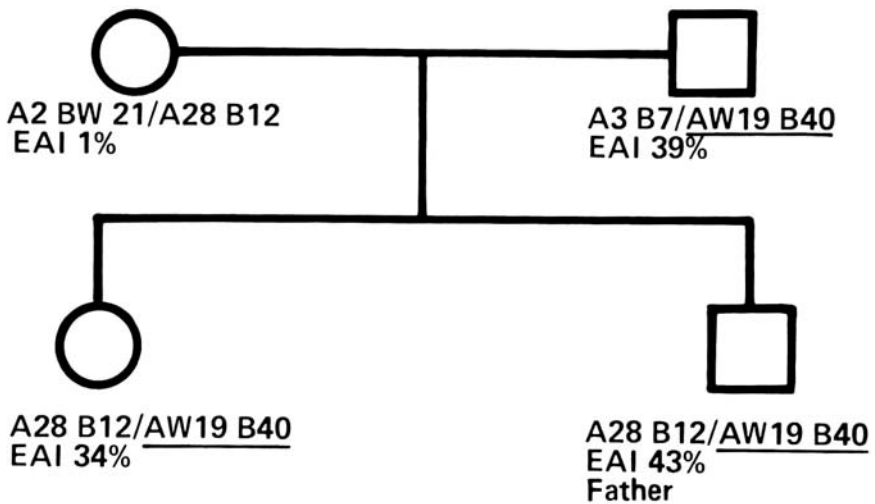


Figure 1. Paternal family with EAI versus maternal serum. Paternal haplotype is at bottom right. EAI is associated with AW19 B40 haplotype

members carrying a particular paternal haplotype (Figure 1). In one case, by tissue typing blood from the umbilical cord, it was shown that this haplotype had been inherited by the fetus. The probability that this occurred by chance was $p < 0.05$ (Lod score) which suggests that the antigens detected by EAI are coded for by gene loci linked to the HLA system.

Discussion

This report shows that Fc receptor blocking antibodies detected by the EA inhibition assay, which correlate with good renal transplant survival (a) occur more frequently pre-transplant in recipients transfused with over five units of blood; (b) can develop during a blood transfusion regime; (c) are present significantly more often in the sera of successfully pregnant women than in those

subject to recurrent spontaneous abortion; and (d) are directed against members of the paternal family sharing a similar haplotype.

The nature of the Fc receptor blocking factor is not yet defined but we have shown by DEAE column chromatography that it occurs in the IgG fraction of serum. Immune complexes may also cause EAI and they were therefore removed by ultracentrifugation. Throughout this study the EAI was selective against both the family and panel members which makes non-specific immune complex blocking unlikely. Since EA inhibiting activity was noted in sera absorbed with platelets, it suggests that the target antigens were not the standard HLA-A, B and C antigens as directed by the lymphocytotoxicity assay. We have shown in another study that selection was not based on the panel members HLA-DR tissue type [4]. Morito [6] has also shown that standard tissue typing sera are not directed against cells of a given tissue type in the EAI assay. The results of the studies on the six families however do suggest that these antibodies are directed to antigens coded for by the MHC. It may be therefore that the EAI assay detects antibodies against a further HLA linked antigen system on B lymphocytes and that these antibodies, which can be generated by both pregnancy and blood transfusion, represent a humoral method of suppression of the immune response.

References

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