0041-1337/80/3002-0103$02.00/0
TRANSPLANTATION

Vol. 30, No. 2
Printed in U.S.A.

A HETEROPHILE SYSTEM IN HUMAN RENAL TRANSPLANTATION

IX. COMPARISON OF HETEROPHILE TRANSPLANTATION ANTIBODIES TO ANTIVASCULAR ENDOTHELIAL CELL ANTIBODIES[1]

MICHAEL S. ROHR, FRANK B. GELDER, LOUISE M. JACOBBI, AND JOHN C. MCDONALD

Department of Surgery, Louisiana State University Medical Center, Shreveport, Louisiana 71130

SUMMARY

Antibodies to heterophile transplant antigen (HTA) were tested for reactivity with antigens on human umbilical cord vein endothelial cells (VEC). Anti-HTA does not recognize antigenic specificities on isolated endothelial cells. Furthermore, there are antigens on endothelial cells that are distinct from HLA-A,B,C and from HTA.

It is concluded that the HTA and the VEC antigens are different.

HLA is a major histocompatibility system in man, and matching for HLA has led to improved results in clinical transplantation (*1*). Nevertheless, HLA matching has not solved the compatibility problem. There is increasing evidence to support the involvement of non-HLA incompatibilities in allograft rejection. The ABO blood group is the best established non-HLA histocompatibility system. Incompatibilities in this system (which is a heterophile system) are routinely avoided. Some evidence suggests that the Lewis blood group antigen may also be a histocompatibility determinant (*2*). For several years we have studied a group of non-HLA heterophile antigens and antibodies that are related to histocompatibility (*3–9*). We called the antigen, which is present on Wistar rat erythrocytes, HTA. Other publications have appeared relating anti-VEC activity to allograft rejection in humans (*10, 11*). It was reported that the presence of anti-VEC in the sera of recipients of renal allografts correlated better with the clinical courses of the allografts than did the presence of anti-HLA (*11*).

There are similarities between anti-HTA and anti-VEC. Both are apparently mediated by serum antibodies that are primarily IgG, and neither specificity is apparently active against lymphocytes (*4, 11*). The question arose as to whether or not these two systems were, in fact, identical but were being studied by different methods. The experiments reported herein were undertaken to determine the relationship, if any, between the HTA and VEC systems.

MATERIALS AND METHODS

Sera. Sixty-eight sera were selected for study. Twenty-four sera came from normal subjects who donated kidneys to relatives, 22 sera came from patients with functioning first-set renal allografts, and 22 sera came from patients who previously had rejected a renal allograft. All sera were stored in small aliquots at −70 C. No serum was frozen and thawed more than twice. All sera were absorbed with sheep erythrocytes to remove activity directed toward antigens shared by sheep and rat erythrocytes (*5*). Most sera were absorbed with human AB erythrocytes to remove anti-A and anti-B activity. They were then retested on human AB erythrocytes with a Coombs' reagent to ensure that all anti-A and anti-B was removed before use in this study.

Rat erythrocytes. Erythrocytes from Wistar rats were washed three times and suspended as a 2% solution in hemagglutination buffer for hemagglutination (*5*), or they were used for absorption as a packed suspension at a ratio of 1 volume of cells per 2 volumes of serum.

Vascular endothelial cells. Endothelial cells were harvested from fresh umbilical cords according to the method described by Cerilli (*11*). Briefly, the vein of each umbilical cord was filled with collagenase (Sigma Type VI) at a concentration of 500 units/ml in Hanks' balanced salt solution and then incubated at 37 C for 30 min. The endothelial cells were washed from the vein with a solution of TC-199 (Grand Island Biological Co.) containing fetal calf serum at a concentration of 20%. The cells were then concentrated by centrifugation. The final suspension of cells was in 1.0 ml of TC-199 with 20% fetal calf serum. Smears of the cell suspension were made on clean glass microscope slides and the slides were air-dried, fixed in absolute methanol for 10 min, and then stored at −20 C until needed. The ABO blood type of the endothelial cells was ascertained by typing cord blood.

Endothelial cells from eight umbilical cords were collected as described above and used for absorption as a packed suspension at a ratio of 1 volume of cells per 2 volumes of serum.

Platelets. Three-hundred outdated platelet packs were obtained from a regional blood bank. The platelets were concentrated by centrifugation and stored in 1% sodium azide in phosphate-buffered saline until needed. The platelets were pooled and used as a packed suspension for absorption at a ratio of 1 volume of platelets to 5 volumes of serum. Platelet absorptions were repeated three times.

Umbilical cord vein lymphocytes. Umbilical cord vein blood was obtained from a placenta and the lymphocytes were isolated on a Hypaque-Ficoll gradient and used in a microcytotoxicity test to test for the presence or absence of anti-HLA-A,B,C activity in serum before and after absorption with pooled platelets. The endothelial cells of the same umbilical cord vein were harvested as described above and used as target cells for determining anti-VEC activity in appropriate sera.

Determination of anti-HTA activity. This was performed by agglutination of rat erythrocytes as previously described (*3*). A serum was considered to contain anti-HTA if it agglutinated rat erythrocytes in dilutions of 1:10 or greater after it was

This work was supported in part by National Institutes of Health Grant 2 RO1 AM21346-03 and by the Employees of OlinKraft in West Monroe-American Heart Association-Louisiana, Inc. Award. Known positive and negative control sera, kindly supplied by Dr. James Cerilli, were used in each experiment.