0041-1337/78/2603-0191$02.00/0
TRANSPLANTATION

Vol. 26, No. 3
Printed in U.S.A.

A HETEROPHILE SYSTEM IN HUMAN RENAL TRANSPLANTATION

VII. THE ANTIGEN IS AN INTRINSIC COMPONENT OF SEVERAL HUMAN TISSUES[1]

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SUMMARY

The presence or absence of the heterophile transplantation antigen was sought in renal tissue from stillborn infants, primary cell cultures, and several organs from adult human cadavers. The heterophile transplantation antigen was found in renal tissue at birth, was retained in human renal cell culture, and was present in human organs other than kidney. The most likely explanation for these results is that the heterophile transplantation antigen is an intrinsic component of many human tissues.

We have previously published considerable data concerning a heterophile transplantation antigen (HT-A) which appears to be a histocompatibility determinant in human renal transplantation (*2–7*). It has been demonstrated that HT-A is present in some but not all human kidneys and thereby appears allogeneic (*6*), that the same or cross-reacting antigens are present on rat erythrocytes and Gram-negative bacteria (*3*), and that the transplantation of a kidney that contains the antigen into a recipient whose tissues do not contain it adversely affects the survival of the allograft (*2*).

The purpose of this study was to answer two questions. First, is HT-A an intrinsic component of human tissue? Second, is HT-A present in human tissues other than the kidney? That HT-A is an intrinsic component of human tissue seemed likely because of evidence presented earlier that strongly suggested a genetic basis for its distribution (*6*). However, it seemed possible for HT-A to be bacterial fragments or residues that had been deposited in the kidney over the course of time. If a genetic susceptibility for deposition of bacterial fragments in the kidney existed, the fragments might stimulate an immune response which would appear allogeneic.

If HT-A is present in tissues other than the kidney, its recognition would be important in the transplantation of those tissues. Additionally, its presence in other tissues would increase the likelihood that it is an intrinsic component of human tissues.

MATERIALS AND METHODS

Tissues. Samples of various tissues were obtained at autopsy from six adult human cadavers. A total of 38 tissue samples were studied from these six cadavers. Kidneys were obtained from six fetuses who were alive at the onset of labor but were stillborn.

One adult kidney was removed operatively because of injuries sustained from blunt trauma. One-half of this kidney was used to prepare HT-A and the remainder was prepared as a primary renal cell culture.

Renal cell cultures. Three primary renal cell cultures (GIBCO P 1101, Flow 4000, and MA 147) were obtained from commercial sources. These were grown in RPMI 1640 media with 2% fetal calf serum. They were subcultured once into liter bottles, grown to confluence, and harvested. The yield from each was approximately 0.25 g of packed wet cells. These cells were used to prepare HT-A.

HT-A preparation. Tissue was homogenized in distilled water. The homogenate was covered, placed in a boiling water bath for 1 hr, and then allowed to stand at 4 C overnight. The mixture was brought to 85% ethanol by adding an appropriate volume of 95% ethanol. This ethanol suspension was allowed to stand overnight at 22 C, after which it was centrifuged at 20,000 *g* at 10 C. The pellet was discarded and the supernatant was evaporated to dryness at 56 C. The resultant solid material was weighed, resuspended in Difco hemagglutination buffer, and dialyzed against hemagglutination buffer overnight. Osmolality and pH were measured, and dialysis was continued until pH = 7.3 to 7.5 and osmolality = 300 mOsmol/liter ± 20. Some samples were again evaporated to dryness to determine the reduction in mass. These were again suspended in sufficient distilled water to restore osmolality and pH to physiological ranges. The solid material is referred to as antigen, although it is to be understood that probably only a small and possible varying amount of the material was active antigen.

Sera used. Five reagent sera were used. Each was obtained from a patient who had experienced an acute renal allograft rejection and who had produced a high titered anti-HT-A serum as a consequence.

Neutralization of anti-HT-A hemagglutination (macromethod). Each reagent serum was titrated on the day of the experiment against a 2% suspension of rat erythrocytes. The HT-A preparation tested was prepared in 0.1-ml aliquots by doubly diluting the original material from 1/2 to 1/256. Each aliquot of antigen was incubated for 1 hr at 37 C with 0.1 ml of serum containing 4 hemagglutinating units. Thrice-washed rat erythrocytes (0.1 ml of 2% suspension) were added and the mixtures again incubated at 37 C for 30 min. The suspensions were then centrifuged and inspected for hemagglutination after agitation. Agglutination was read as 0 to 4+ in the conventional way.

Neutralization of anti-HT-A hemagglutination (micromethod). These experiments were performed in Cooke U-shaped microtiter plates. A box titration of anti-HT-A against HT-A was performed using a 0.4% suspension of thrice-washed fresh rat erythrocytes in hemagglutination buffer stabilized with human AB serum at a 0.5% concentration. The AB serum had been previously absorbed with rat erythrocytes. Each well contained 0.5 μl of rat erythrocyte suspension, 0.5 μl of appropriately diluted antibody, and 0.5 μl of appropriately diluted antibody, and 0.5 μl of appropriately diluted antigen. Antibody and antigen controls were included in each tray.

The mixtures of antibody and antigen were incubated for 1 hr at 37 C on an automatic rotator before adding the erythrocyte

[1] Supported by National Institutes of Health Grant 7 RO1 AM21346-01 SGYB.