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# A HETEROPHILE SYSTEM IN HUMAN RENAL TRANSPLANTATION

## VIII. THE MORPHOLOGICAL DISTRIBUTION OF THE ANTIGEN IN RAT TISSUES[1]

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SUMMARY

The morphological distribution of heterophile transplant antigen (HTA) was determined in rat tissues using an indirect immunofluorescence technique. Human anti-HTA sera were used to localize HTA in rat kidney, liver, heart, skeletal muscle, spleen, and stomach. HTA was found in basement membrane and supporting stromal elements of all tissues studied. In the kidney, HTA was demonstrated in tubular basement membrane but not glomerular basement membrane. No evidence for cell surface antigen distribution could be ascertained except for erythrocyte membranes. HTA was not found on endothelium of rat blood vessels. We know of no antigens previously implicated in histocompatibility that are stromal in location.

A heterophile system related to human transplantation has been under study by our group for several years (*1–7*). We called this system the HTA system (*3*). The antigen was found on Wistar rat erythrocytes, Enterobacteriaceae, and in some, but not all, human kidneys (*1–3*). A study of natural immunity to the antigen (presumably stimulated primarily by intestinal flora) suggested that about 60 to 70% of humans carried the antigen in their tissues and 30 to 40% do not (*3*). The antigen appeared to be an intrinsic component of human kidney since it persisted through several passages in cell culture (*7*). It also is present in human heart, skeletal muscle, and liver, in addition to kidney (*7*). This system is biologically related to human renal allograft survival (*6*).

Because HTA is present in several human tissues, we wondered if it was not also present in rat tissues. We thought that a study of the morphological distribution of HTA in rat tissues might be important since it might serve as a basis for a similar study of human tissues. This study was therefore undertaken to determine whether HTA could be found in rat tissues other than erythrocytes, and if so, how it was distributed.

## MATERIALS AND METHODS

*Determination of Anti-HTA activity.* This was performed by agglutination of rat erythrocytes as previously described (*3*). All sera were absorbed with sheep erythrocytes to remove activity directed toward antigens shared by sheep and rat erythrocytes also as described earlier (*3*). A serum was considered to contain anti-HTA if it agglutinated rat erythrocytes in dilutions of 1:10 or greater after it was absorbed with sheep erythrocytes. This is an arbitrary determination.

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

*Sera.* Eight reagent sera were selected for study. Four sera contained anti-HTA and four did not. All reagent sera were absorbed with human AB erythrocytes to remove anti-A and anti-B activity. They were then retested against human AB erythrocytes with a Coombs' reagent to ensure that all anti-A and anti-B had been removed. The four sera containing anti-HTA came from patients who had low titers of anti-HTA before renal transplantation but who developed high anti-HTA titers after renal transplantation. The anti-HTA titer of three of these sera was 1:160 and the titer of the fourth serum was 1:320. Two of these patients subsequently lost renal allografts because of accelerated acute rejection. The other two patients had functioning allografts at the time of this writing. Three of the four sera that served as negative controls came from renal failure patients under our care. Their serum was sampled many times over several months both before and after transplantation. None of their serum samples ever demonstrated anti-HTA activity. The other negative control serum came from a cadaver kidney donor.

*Rat tissues.* Female Wistar rats were anesthetized with pentobarbital. The kidneys, heart, liver, spleen, rectus abdominus muscle, and stomach were removed. The tissues were cut into approximately 5-$mm^3$ blocks and frozen in liquid nitrogen in Tissue-Tek II OCT embedding medium. The tissue blocks were stored at −70 C until needed, at which time 4-$\mu$m sections were cut with a Cryostat microtome and collected on clean glass microscope slides. The sections were washed in three changes of phosphate-buffered saline at pH 7.4 and used immediately for the experiments.

*Fluorescein-conjugated antiserum.* Antiserum to human IgG was prepared in a goat. Normal human IgG was prepared from pooled plasma by standard techniques using ammonium sulfate fractionation, size chromatography on Sephadex G-200, DEAE-cellulose chromatography, and preparative disc gel electrophoresis. Human IgG preparations were shown to be free of other serum proteins by analytical polyacrylamide disc gel electrophoresis and immunoanalysis with monospecific and polyspecific antisera.

After hyperimmunization of the goat with human IgG, antiserum was recovered by plasmaphoresis, clotted by the addition of calcium, and decomplemented by heating at 56 C for 30 min. The antiserum was absorbed with equal volumes of human AB erythrocytes and Wistar rat erythrocytes at 4, 25, and 37 C. Antibodies to immunoglobulin light chains were removed by absorption with human $\kappa$ and $\lambda$ proteins immobilized on Sepharose 6B. The $\kappa$ and $\lambda$ proteins were isolated from urine obtained from patients with multiple myeloma.

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