0041-1337/82/3305-0478$02.00/0
TRANSPLANTATION

Vol. 33, No. 5
Printed in U.S.A.

A HETEROPHILE SYSTEM IN HUMAN RENAL TRANSPLANTATION

X. HTA SENSITIVITY INCLUDES SENSITIVITY TO HUMAN B LYMPHOCYTES[1]

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

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

Antibodies that react with heterophile transplantation antigens (HTA) have been shown previously not to react with HLA-A, B, or C antigens. This paper presents evidence that anti-HTA does react with a subpopulation of human lymphocytes which is comprised primarily of B cells.

Anti-HTA reactivity was removed from sera by absorption with each of three different human B lymphocyte cell lines, but it was unaffected by absorption with platelets or thymocytes. Selected high titer anti-HTA sera absorbed with human platelets, human blood group type AB erythrocytes, and sheep erythrocytes caused lysis of a lymphocyte subpopulation principally composed of B lymphocytes. Absorption of these sera with rat erythrocytes removed both lympholytic activity and anti-HTA activity. Antibody recovered by affinity purification with rat erythrocyte membrane preparations contained both lympholytic and anti-HTA reactivity.

These data, considered with previous studies, seem to establish that B cell sensitization may be acquired by a substantial segment of the population by natural immunization from enteric flora and/or by infections with enteric bacteria.

Previous publications from these as well as other laboratories (*1-7*) have focused attention upon some heterophile reactions in human sera which are pertinent to transplantation. We have called this system the HTA system (*8*). It is distinctly different from other well studied heterophile systems such as the Forssman, Paul-Bunnel, Streptococcal associated, or Haganusi-Dieschi systems.

It has been established that some recipients of renal allografts produce antibodies which are "heterophilic" since they cross-react with antigen(s) present on rat and bovine erythrocytes (*2, 3, 9*). A rising titer of these antibodies in a kidney recipient frequently correlates with the onset of acute or accelerated acute rejection (*10, 11*). The antigens which stimulate the production of these antibodies are also present on enteric bacteria and some individuals are sensitized "naturally" by their enteric flora (*10, 12*). Since the antigens are present in some human beings and not others, they comprise an allogeneic system in man (*8*).

As we understand the system at present, the homologous antigens are on some enteric bacteria. Individuals who do not carry the antigens as autogenous components of their tissues become naturally immunized by their enteric flora (*12*), which can be boosted by infections with some enteric bacteria, as well as by renal allografts which contain the antigens.

We were unable, in earlier experiments, to detect HTA on human lymphocytes and we assumed that HTA was distinct from HLA (*11*). Later experiments excluded cross-reactivity of anti-HTA with HLA-A, B, or C, but were insufficient to exclude a relationship to HLA-D (*1*). This paper records evidence that HTA is found on a subpopulation of human lymphocytes which is comprised principally of B cells.

[1] This work was supported by NIH Grant 2ROI AM21346-05.

MATERIALS AND METHODS

Sera. All sera used in this study were selected, highly reactive anti-HTA sera (titer > 1:40). They were obtained from renal transplant recipients undergoing graft rejection concurrent with a rising anti-HTA titer. All sera were heat inactivated at 56 C for 30 min and absorbed three times with washed, packed sheep erythrocytes, and three times with washed packed human blood group AB erythrocytes at a cell to serum ratio of 1:2, unless otherwise stated in the text. Serum absorption was monitored with the hemagglutination assay as previously described (*8*) with the addition of an antiglobulin (Coomb's) test to assure complete absorption of unwanted specificities. Platelet absorptions were performed with a pool of platelets obtained from 300 donors as previously described (*1*).

Erythrocyte membrane preparation. Whole blood from Wistar rats was obtained by cardiac or venipuncture and anticoagulated with heparin. The erythrocytes were recovered by centrifugation at 500 *g* and washed three times with phosphate-buffered saline (PBS) (pH 7.3). Erythrocytes were hemolysed by the addition of 10 parts of distilled H_2O and mixed for 20 min at room temperature. Erythrocyte membranes were collected by centrifugation (5000 *g* for 10 min), resuspended by vigorous pipetting, and washed until the absorbance of the supernatant was less than 0.01 at 280 nm. The pellet was then suspended in an equal volumn of PBS by sonication.

Affinity purification of anti-HTA reactivity. One milliliter of high titer anti-HTA serum was mixed with 50 μl of a 50% erythrocyte membrane suspension and incubated for 30 min at 37 C with mixing. During the incubation period antibodies directed against erythrocyte membrane antigens were specifically bound to their antigenic determinants. Following the incubation, the membrane-serum mixture was centrifuged (500 *g* for 10 min). The resultant supernatant was found to be void of erythrocyte agglutinins, indicating complete absorption of antibody reactivity by the membrane preparation. The membrane pellet was extensively washed with PBS to remove trapped serum proteins. After the last wash (supernatant absorbance less than 0.01 at 280 nm), the membrane pellet was resuspended in 0.5 ml of 0.1 M glycine-HCl buffer (pH 2.2) to dissociate bound antibody. The mixture was immediately centrifuged and the supernatant collected, neutralized with 0.1 M NaOH, dialyzed against PBS, and tested for antibody reactivity by the hemagglutination assay. In all cases, 50 to 75% of the antibody reactivity was recovered in the glycine-HCl elutate.

Cytotoxicity testing. A two-color fluorescent cytotoxicity test was used to evaluate samples for the presence of cytotoxic