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HUMAN HETEROPHILE TRANSPLANTATION SYSTEM

The Chairman, Fellows and Guests: Today I hope to summarize the status of our studies of a Human Heterophile Transplantation System which we call the HT-system. Details of methodology can be found in previous publications or in two papers soon to appear in Transplantation.

Slide 1

In a previous study of several patients we noted that irreversible, acute renal allograft rejection was consistently associated with a rise in titer of the human anti-rat heterophile hemagglutinin. During the same period of time, recipient's lymphocytes were unable to undergo blastogenic transformation upon exposure to donor lymphocytes or to other antigens to which they had been sensitive pre-operatively. This study suggested that acute rejection in immunosuppressed patients involved humoral immunity. Moreover it confirmed the original work of Rapaport and associates.

Slide 2

Subsequently we monitored the titers of these heterophile antibodies following transplantation in selected patients, and noted that 15 of 16 patients with acute rejection produced an eightfold or greater rise in titer while 20 of 28 who had no rejection episodes failed to do so. Four of the 8 "false positive" changes were associated with gram-negative

infections. This latter observation was our first clue that similar or identical antigens reactive in this heterophile system were present in gram-negative bacteria as well as in human kidneys.

Slide 3

That study also demonstrated that careful monitoring of this serum response could, under appropriate conditions, be useful in the serologic diagnosis of acute rejection. Note the close correlation between the heterophile titer and serum creatinine in this patient.

Slide 4

Following the clue from transplantation patients with infection, we determined the heterophile titer in sera from 41 patients with monomicrobial gram-negative infections and compared them to titers in sera from 68 normal adults, 84 patients in chronic renal failure and 22 patients with acute rejection. Note the increasing mean titers among these groups indicated by the horizontal bars. 91% of patients with acute rejection had titers of 1:80 or greater. 45% of sera from patients with gram-negative infections had such high titers. This suggested that some individuals might be sensitized to the antigens under study as a consequence of gram-negative infections as well as by human kidneys. Further, this could account for the higher incidence of high-titered sera observed in serum from patients with chronic renal failure than was seen in sera from normal adults.

Concurrently, we noted in studies of several pre-transplantation and post rejection sera that acute rejection induced a change in the specificity of the sera as shown by their reactivity with sheep and rat erythrocytes.

Slide 5

Slide 5 shows such an example. Note that absorption of pre-transplantation serum with either sheep or rat erythrocytes removes both the homologous and the heterologous agglutination; however, in the acute rejection serum absorption with sheep erythrocytes no longer removed the anti-rat hemagglutinin although absorption with rat erythrocytes still removed the anti-sheep activity. In other words the rejection serum appears to contain a new specificity, for an antigen on rat erythrocytes. This can be visualized as follows.

Slide 6

When simple anti-rat hemagglutination is performed, the titer is determined by antibodies with two specificities. One specificity reacts with an antigen common to sheep and rat erythrocytes we called the Heterophile X or HX antigen and now believe it to be that originally studied by Iwasaki, Talmage, and Starzl. The other specificity reacts with an antigen we called the Heterophile Transplantation antigen or the HT antigen. It is the HT antigen which appears related to acute rejection. To determine the presence of the anti-HT specificity it is necessary to absorb a serum with sheep erythrocytes and then determine the residual anti-rat hemagglutinating activity: If all agglutinins are

removed the serum is anti-HX. If some activity remains, it is anti-HT. The presence or absence of anti-HT activity in over 370 sera has now been determined in this way.

Slide 7

Slide 7 shows the relationship between the titer of anti-rat hemagglutinins and the presence of the anti-HT specificity in 316 sera. The higher the titer the more likely the serum is to contain anti-HT activity. This accounts for the correlation we have previously observed between titer and rejection.

Slide 8

Slide 8 is a scattergram showing the absorption data from the same 316 sera. The sera are grouped according to source on the horizontal. Anti-rat hemagglutinin titers are on the vertical. The solid circles are sera containing the anti-HT specificity and those sera can be considered immune. Note: (1) Some sera with hemagglutinin titers of only 1:10 or 1:20 are immune. (2) Patients in chronic renal failure are more likely to be immune than are normal individuals possibly because they are more likely to have had gram-negative infections. (3) Sixteen of the 41 patients with gram-negative infections were immune to the HT antigen. (4) Twenty of the 25 patients who had acute rejection were immune <u>during</u> the rejection period. (5) Only five of 27 patients who were not immune pre-transplant and who had no rejection, developed the antibody post-transplantation, and 3 of that 5 had gram-negative infections.

The correlation between clinical outcome of 70 allografts and anti-HT immunity is shown in the next two slides.

Slide 9

Fourteen allografts were performed in recipients presensitized to the HT antigens. Nine experienced acute rejection, six began between the third and sixth post-operative day and proceeded rapidly to complete destruction of the graft--the so called "accelerated" acute rejection. Four grafts did not undergo rejection--possibly because they did not contain the antigens. Note that presensitization to these antigens was not associated with hyperacute rejection.

Slide 10

Fifty-six allografts were performed in non-sensitive recipients. Eleven of 16 patients who had acute rejection concomitantly produced the anti-HT antibody. Only 5 of 27 who had no acute rejection produced the antibody. And, as mentioned, 3 of those 5 were associated with infection.

Chronic rejection was not associated with immunity to the HT-antigens.

Slide 11

We have found that Klebsiella pneumoniae type 16 is particularly rich in these antigens. A soluble preparation from this bacterium designed to produce the Common Antigen of Enterobacteriaceae contains the HT antigens. They will adsorb to unmodified human O cells which

are then agglutinated by anti-HT sera. This activity can be absorbed by bacterial preparations, rat erythrocytes and dessicated renal tissue. The anti-rat hemagglutinating activity of the same sera can be absorbed by some gram-negative bacteria, rat erythrocytes and dessicated human kidney. As yet we have no means of producing a reaction between the sera and renal tissue so this aspect of the study has not been done.

If the chemical procedure used to prepare Common Antigen is used starting either with human kidney or rat erythrocytes rather than bacteria, soluble preparations are obtained which also contain the HT antigens. Such preparations from rat erythrocytes will adsorb to human O cells as does that from bacteria, but the preparations from kidney do not.

The same type of experiments shown in slide 11 were done using soluble antigen for neutralization rather than whole tissue for absorption with identical results. Thus the similarity of antigens derived from the three sources had been shown by two serologic techniques.

Soluble HT antigen from 13 human kidneys has been used to neutralize the rat hemagglutinating activity of six anti-HT sera.

Slide 12

Sera are on the vertical axis and kidney HT antigen preparations on the horizontal. Pluses indicate neutralization of rat hemagglutination. The circled data are instances in which the neutralization was performed

with antigens prepared from renal tissue autogenous to the serum produced. In no instance did autogenous material produce neutralization. This indicates that the response is not auto-immune. The sera react in random fashion on various kidneys as is expected of alloantibodies. This experiment offers strong evidence that the HT antigens are allo-antigens in the human.

Finally, appropriate absorption experiments with selected leukocytes have established that the HT-antigens are not on leukocytes and therefore are not HL-A. Further in this series there was no correlation between HL-A incompatibility and the occurrence of acute rejection.

In summary, a heterophile transplantation system has been described. These HT antigens appear to be alloantigens in the human, and similar or identical antigens are present on rat erythrocytes and some gram-negative bacteria. Patients can be identified who are pre-sensitized to the HT-A system, presumably by previous gram-negative infection. Allografts fair poorly in such recipients. In non-immune recipients acute rejection is usually associated with the appearance of anti-HT antibodies. Similar antibodies seldom appear in the absence of rejection unless produced by infection. These data strongly suggest that the HT-A system is another human histocompatibility system which appears to be involved in acute rejection but not in hyperacute or chronic rejection.

Slide 13

The final slide shows our operational concept of these antigens as I visualize it at present. Human kidney and human leukocytes possess the HL-A determinants which are histocompatibility antigens. HT-antigens are not present on human leukocytes or sheep erythrocytes, but are shared by rat erythrocytes, some gram-negative bacteria and some human kidneys. They also seem to be histocompatibility antigens. The HX-A can be present on all five tissues, but apparently is unrelated to histocompatibility. The Common Antigen is present on Enterobacteriaceae and also on rat erythrocytes. It is very similar to and may cross-react with the HT-antigens.