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Medical Intelligence

Rejection of Human Renal Homografts:

Basic Mechanisms and Terminology

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MANY ANESTHESIOLOGISTS have not had the opportunity to participate actively in transplant procedures; however, the principles of management should be familiar to all. There have been several communications concerning various aspects of anesthesia for transplantation.[1-5] The purpose of this paper is to describe the methods for detection of graft–recipient compatibility, and to provide some basic understanding of the rejection phenomenon and the means of modifying rejection.

Graft–Recipient Compatibility

Rejection of a transplanted organ occurs when the host recognizes genetically-determined antigenic differences between it and the graft. In the case of ABO blood groups, two antigenic polysaccharide substances, A and B, have been identified. Group O erythrocytes lack both A and B substances. The ABO phenotype of an offspring is genetically determined by the contributions of both parents as the unpaired chromosomal material becomes paired after fertilization. Each chromosome contains loci (genes) responsible for a given trait, *e.g.*, blood type. The corresponding loci on a pair of chromosomes responsible for a given trait are called "alleles." Thus, there exist allomorphic genes (alleles) both responsible for a given characteristic (blood type). These alleles express themselves to create antigenic identity or antigenic differences between individuals of the same species (*e.g.*, the polysaccharide substances A and B or lack of them).

Hogman [6] and Kovarik *et al.*[7] have demonstrated the presence of A and B antigens in almost all human tissue. Organ transplantation between ABO-incompatible persons usually results in violent rejection, which often occurs within hours of establishing the transplant.[8] Identification of ABO-incompatible individuals by blood-typing and cross-matching procedures has eliminated this catastrophic form of rejection from the current transplant scene.

Another series of antigens has become recognized as probably the most important in the human species with regard to transplantation. A transplantation antigen is one whose presence in or on a graft may stimulate an immune response in the host. Current work has been devoted to investigation of the HL-A series of antigens, which are found at least in the cell membranes of most tissues and are thought to be mainly protein in nature. It is not clear whether the antigenic substance is glycoprotein, lipoprotein, or protein only. The antigens are determined by genes which reside on a single pair of chromosomes (autosomes), although it is not known precisely which pair of genes is responsible. The HL-A locus on each autosome contains at least two subloci, designated "LA" and "4," each sublocus controlling a separate or segregant series of antigens. Thus, the HL-A phenotypic description of each individual will usually consist of four antigens, two sets of (multiple) alleles. Two antigens will be from the first segregant series, the alleles of the "LA" sublocus, and two from the second series, the alleles of the "4" sublocus. A diagrammatic representation of the phenotypes of a pair of individuals, transmitted

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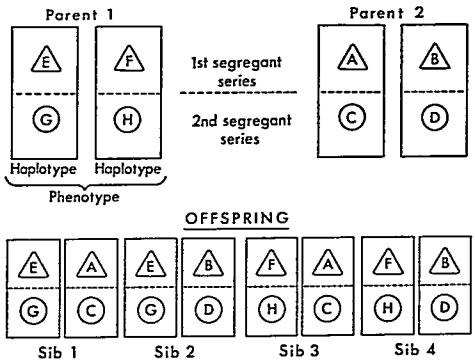

FIG. 1. Diagrammatic representation of HL-A antigen distribution and inheritance in a "family." The first segregant series is represented by the antigens (E), (F), (A), (B), the second series by the antigens (G), (H), (C), (D). The phenotype of Parent 1 consists of two haplotypes, each containing an allele of each of the two segregant series (E) (F) and (G) (H). Either haplotype (E) (G) or (F) (H) may be distributed to any offspring in the usual pattern of inheritance. Since similar reasoning can be applied to Parent 2, four phenotypes are possible in the offspring.

haplotypes, and the possible phenotypes of the offspring is shown in figure 1.‡

The number and nomenclature of the HL-A antigens is confusing. Some historical background and explanation of the evolution of the system is in order. Dausset [9] first described the presence of leukocyte antibodies in persons who had received multiple transfusions in 1954. In 1958, Payne and Rolfo [10] and van Rood *et al.*[11] described antileukocyte antibodies in pregnant women. These were directed against the antigenic component of the fetal leukocytes "donated" by the father. Also in 1958, Dausset [12] formulated the first description of a leukocyte antigen by studying the agglutination patterns of sera of patients who had received multiple transfusions against a panel of leukocytes. Since that time, numerous laboratories throughout the world have identified a multitude of leukocyte antigens by studying the reactions of sera from various sources including pregnant or multiparous women, persons immunized during blood transfusion, and volunteers injected with leukocytes. Each laboratory had its own nomenclature. A series of international workshops was established to study the HL-A systems. From these arose some standardization of methods, nomenclature, and matching of similar antigens from different laboratories. Presently, 11 HL-A antigens (HL-A 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13) have been "internationally recognized" and have been designated with the "HL-A" prefix as part of their nomenclature. Obviously, other antigens exist and are found commonly but have not yet been internationally recognized or do not meet the rigid criteria required by international standards.§ Terasaki, for instance, types for 25 antigens in his laboratory; the 11 HL-A antigens and 14 "Te" antigens (Te 40, 50, etc.).[13] These other antigens may be internationally recognized and given "HL-A" designations in the future.

The detection of the various HL-A antigens present in both donor and recipient systems is now a relatively rapid and reproducible process. The primary clinical method in use today is the detection of cytotoxic antibody activity. Originally described by Gorer and O'Gorman,[14] the microdroplet modification pioneered by Terasaki's group [15] utilizes specific antisera to the various HL-A antigens to detect their pres-

‡ There are probably still undiscovered antigens in each series, and strong evidence for even a third sublocus exists. This has clinical importance since in some individuals only two or three antigens may be discovered in performing histocompatibility studies.

§ While the international workshops have no true official status in any country, they are sanctioned and supported by N.I.H., W.H.O., and almost all investigators in this field. Except by agreement, international standards and nomenclature impose no obligations or requirements on persons or laboratories.

ence on the lymphocytes being tested. In this test a sample of purified peripheral lymphocytes (donor or recipient) is incubated in the presence of complement with a panel of antisera specific for the various HL-A antigens. The panels of HL-A-specific antisera are obtained in the United States from N.I.H. If the sought-after antigen is present on the lymphocytes being tested, the cell will be killed by the antigen–antibody reaction in the presence of complement. The dead cells, indicative of the presence on the cells of the antigen being tested for, are detected by adding a dye which will stain only dead cells. The antigenic patterns of the donor's cells and the recipient's cells may then be studied and compared. Additionally, preformed antibodies in the recipient (usually antibodies to leukocyte antigens from previous transfusions) may be detected by this method, using the recipient's serum and the donor's lymphocytes.

A second technique is that of the mixed lymphocyte culture.[16] This is based on the phenomenon of "blast" transformation of lymphocytes when they are stimulated by a foreign antigen, in this case a set of specially-treated lymphocytes. In this test lymphocytes from the donor and recipient are incubated together, one set having first been treated with Mitomycin C, which inhibits DNA synthesis. The treated set of cells is thus unable to undergo "blast" transformation, but is still able to stimulate the untreated cells, if they are antigenically foreign. The amount of stimulation can be measured by the uptake of tritiated thymidine, found only in DNA and an indicator of DNA synthesis, into the rapidly-transforming nucleus. The degree of incompatibility can then be assessed.

While there is controversy as to the degree of importance of histocompatibility testing in relation to the overall survival of allografts, most authors feel there are fewer problems with rejection in transplants between more closely matched individuals, at least in the case of related donors. Numerous systems of grading donor–recipient histocompatibility are in use. One commonly used in the United States is:

A match: The donor and the recipient are HL-A-identical.

B match: All the donor's HL-A antigens are present in the recipient; however, the recipient has antigens not present in the donor. This is still considered satisfactory since recipient antigen does not seem to affect graft acceptance.

C match: The donor has one HL-A antigen not present in the recipient.

D match: The donor has two or more HL-A antigens not present in the recipient.

F match: The recipient has preformed, circulating antibodies to the donor's HL-A antigens: a positive crossmatch.

A typical clinical situation may serve to illustrate the application of the material presented above.

Report of a Case

The patient, a 42-year-old married man with four children ranging in age from 16 to 22 years, was admitted to the hospital a year ago after fainting at work. He was found to be severely hypertensive and uremic. Further evaluation, including renal biopsy, revealed end-stage chronic renal disease, probably chronic glomerulonephritis. The patient was admitted to a chronic hemodialysis program and responded well enough to return to part-time work. Once on dialysis, he expressed a desire for a kidney transplant.

The patient was first blood-typed and tissue-typed. He was found to have blood type O, Rh+. His lymphocytes, run against a panel of specific antisera, were found to contain the antigens HL-A 1, 8, 2, and 12. Two brothers consented to give kidneys. One brother, found to be severely diabetic, was disqualified. The second brother had an ABO-incompatible blood type. The patient's one adult son consented. He had O, Rh+ blood, but his lymphocyte testing revealed the antigens HL-A 1, 8, 3, and 7, a two-antigen mismatch. The "D" match was considered a poor choice for this patient and he was urged to wait for a better match.

Six months later, a 22-year-old male patient was admitted to the hospital after having sustained a massive head injury in a motorcycle accident. When it became apparent that his brain damage was irreversible, his family was approached and consent was obtained for the use of his kidneys for transplantation at the time of his death. He was found to have blood type O, Rh+, and tissue-typing revealed the antigens HL-A 1, 8, 2, and 7, a one-antigen mismatch with our patient. The "C" match was considered satisfactory (especially

since there were no better matches among living related donors), and the cadaver kidney was successfully transplanted.

The Rejection Phenomenon

The rejection phenomenon is the important manifestation of any incompatibility that exists between graft and recipient. Deodhar and Benjamin [17] have presented an excellent review of their experience in studying the histopathology of all the specimens associated with their renal transplant program in a six-year period. Their experience and that of others allow us to recognize four pathologic types of rejection:

1) *Chronic rejection:* This is characterized primarily by obliterative arteritis of the medium-sized and small renal arteries. Also seen are glomerular changes similar to those seen in glomerulonephritis and interstitial lymphocytic infiltrates. This is usually considered an irreversible form of rejection, although the processes may extend over a period of months to years before terminal renal failure ensues.

2) *Acute rejection:* This phenomenon is characterized by fibrinoid necrosis of the small vessels and interstitial and perivascular infiltrates of immunoblasts. The donor ureter may be involved by this process. If the reaction is not extensive or severe it may be reversible by increasing the immunosuppressive therapy.

3) *Hyperacute rejection:* This form is characterized by the presence of fibrin thrombi in the small vessels. Diffuse cortical necrosis is present. Hyperacute rejection is usually seen in recipients who have preformed or circulating antibodies to the donor's antigen system. It is usually rapid, occurring in hours to days, and results in destruction of the graft.

4) *Rejection due to ABO incompatibility:* This form of rejection is typified by renal-artery thrombosis and diffuse hemorrhagic necrosis involving the entire graft. It is rare now that ABO matching of donor and recipient is required.

Modification of Rejection

Two mechanisms of immunologic responses in the rejection process have been identified: the cellular response and the humoral antibody response. Circulating antibodies are thought to be produced by plasma cells, which originate from bone-marrow precursors. The cellular response is mediated by a group of small lymphocytes which are extremely long-lived. When these cells become stimulated (by a foreign antigen) they are transformed into immunoblasts, which invade the graft and come into contact with the foreign cell. Once there, they evoke a number of inflammatory and cytotoxic responses and, in an ill-defined manner, augment the antigen–antibody response. Although there are no known means of changing any preformed antibodies, immunosuppression is aimed at modifying the cellular and humoral responses to newly-introduced antigens, in this case the kidney homograft. The immunosuppressives in current use seem to work by three basic modes of action: by depressing the number of lymphocytes, by interfering with protein and/or nucleic acid synthesis, and through nonspecific anti-inflammatory action. The immunosuppressives most commonly used are corticosteroids, antilymphocyte globulin (ALG), and azathioprine (Imuran).

Corticosteroids act through all three mechanisms. Inhibition of protein synthesis interferes with the transformation of sensitized lymphocytes into "blast" forms necessary for the cellular response. Their anti-inflammatory action is well known. The steroids, through an unknown action, also deplete lymphocytes by hastening their destruction.

ALG is usually produced in horses which have been sensitized to human lymphocytes. It has been found to cause rapid reduction in circulating lymphocytes, as well as depletion of the small lymphocytes found in the paracortical regions of lymph nodes. ALG seems to have no effect on humoral responses. No decrease in granulocytes or erythrocytes accompanies the lymphocyte depletion, although platelets may be decreased in some cases. When used with prednisone, it allows the prednisone dosage to be reduced by half,[18] according to some authors, although not all agree.

Imuran, a 6-mercaptopurine derivative, is a potent antimetabolite. It inhibits incorporation of purine into nucleic acids. While broad effects include some generalized bone-marrow depression, the principal action is to curtail rapid synthesis of nucleic acid necessary for

the formation of immunoblasts, the sensitized "blast" forms of the small lymphocytes.

In general, immunosuppression has the undesirable side-effect of reducing total-body immunity against many infections. Among the well-known side-effects of steroids are cataract formation, osteoporosis, disorders of glucose metabolism, gastrointestinal bleeding, and, with high dosages, psychoses. ALG may cause serum sickness and a type of nephritis in which ALG may be found attached to glomerular basement membrane. Imuran, in addition to its bone-marrow toxicity, may cause alopecia, anemia, and hepatitis.

Conclusion

This paper has dealt with rejection of human renal homografts. Transplantations of other organs have been performed, but even less is known of their rejection patterns. The mechanisms of rejection and means of modifying them are being vigorously explored. Advances in this field lag behind the more technical (surgical) aspects of transplantation. There is little doubt that perfection of a safe means of coping with rejection will lead to the evolution of a broad new field of medicine, dealing not only with renal transplantation but with liver, lung, heart, pancreas, and bowel grafts, as well as autoimmune diseases and perhaps even the aging process.

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