February, 2000

DEPARTMENT OF SURGERY

BASIC SCIENCE COURSE

TRANSPLANTATION

Presented by:

John C. McDonald, M.D.

Professor and Chairman

Department of Surgery

This lecture is intended to bridge the gap between the basic sciences and clinical transplantation. By transplantation, I refer primarily to the transplantation of human organs which are designed to retain their function in the new host.

The study of any discipline begins with an understanding of the terms commonly used in the field.

TABLE 1

Grafts are termed isografts, allografts, and xenografts. These are nouns referring to the graft. An isograft is any graft between individuals that are genetically identical. In the human the only isografts are those between identical twins. Such grafts are common in the experimental laboratory which uses many inbred strains of murine species in which all members of each strain are genetically identical. The adjective relating this noun is isogeneic. An allograft is any transplant between members of the same species that are genetically different, while a xenograft is any transplant between different species. Historically allografts were referred to as homografts and xenografts as heterografts. You can see the appropriate adjective. An autograft is a transplant in which tissue is moved from one place to another in the same individual, such as skin autografts.

TABLE 2

Grafts are also often referred to as vital or static depending upon whether or not the graft is expected to retain metabolic activity or not. Static grafts are

those grafts such as lyophilized bone and blood vessels which are expected to act as struts or conduits, but are not alive. Orthotopic or heterotopic refer to whether or not the graft is anatomically placed in its normal position. For example, most liver transplants are precisely described as orthotopic vital allografts, kidney transplants are heterotopic vital allografts, while transplantation of bone would most commonly be a heterotopic static allograft.

In the natural course of events, isografts and autografts are, of course, not recognized as foreign by the host and are simply treated by the host as any other genetically identical material. The only abnormality of function might relate to the absence of nerve supply or the fact that the structure is not being used for its original intent, such as in a colon interposition to replace the esophagus which is a heterotopic vital autograft.

TABLE 3

All transplants that are genetically different from the host will be rejected if the host's immune response is unmodified.

TABLE 4

Rejection may be considered the process by which the host destroys the foreign material contained within the transplant just as microbes are destroyed. Rejection may be defined in many ways, but the most common and time-honored way of referring to rejection is by the clinical course that the graft follows. This slide shows rejection as being divided into hyperacute, acute and chronic. These are clinical syndromes which usually occur within specified time

intervals. Hyperacute rejection usually begins within minutes to hours after implantation and the graft is destroyed within 24 hours. The etiology of this rejection is that of a B-cell humoral response which occurs as a consequence of either natural immunization in the case of xenografts or prior sensitization in the case of allografts. Prior sensitization may occur through immunization of the female by the paternal genetic components of the fetus. It may be acquired by infusion of leukocytes along the blood transfusions, and it be acquired by the rejection of previous transplants.

Acute rejection is the normal response which occurs when an individual is presented with a new graft to which there has been no previous exposure. In general, the earlier the onset of acute rejection the more severe the genetic disparity between donor and recipient and the more difficult the rejection will be to control. The response is a T-cell cellular response which involves specific cytotoxicity and is a part of the delayed hypersensitivity cellular immune mechanism. Under ordinary circumstances, acute allogeneic rejection occurs within 7-21 days after implantation and in the unmodified or untreated patient is completed within 3-4 days. Chronic rejection is a smoldering gradual destruction which may begin months to years after implantation - it is characterized by both humoral and cellular responses and may require months to years for complete destruction of the graft.

TABLE 5

The pathology of hyperacute rejection is that of the activation of

complement mechanism and thrombosis of the graft. Biopsies of such tissue will show the margination of leukocytes along vascular channels. Thrombosis of the vasculature and necrosis of the graft will follow depending upon how long after vascular thrombosis the specimen is obtained. Acute rejection on biopsy will show infiltration of lymphocytes initially in a perivascular or peritubular fashion, but as time passes, the entire graft will be converted into a mass of infiltrating lymphocytes. The pathology of chronic rejection is one of cellular dropout, fibrosis, and intimal or subintimal hypertrophy with small vessel stenosis. Ultimately occlusion of vessels occurs from the intimal or subintimal hypertrophy. Antibody and immune complexes can be isolated from these vascular lesions. These organs on final examination will show atrophy, fibrosis, atherosclerotic-appearing blood vessels that are rigid and very stenotic or occluded.

MICROSCOPIC SLIDES (1-4)

Discuss - 4 micrographs of rejection. (1) = hyperacute, (2-3) = acute, 4 = chronic.

There is no treatment for hyperacute rejection other than prevention. Once that process has been activated, the outcome is inevitable.

Acute rejection can be treated by a variety of immunosuppressive drugs with a high probability of successful reversal.

Chronic rejection is also untreatable. It is generally thought that those patients who are most likely to develop chronic rejection are patients who have

had early and severe acute rejection episodes. The patient has become sensitized and ultimately in spite of immunosuppressive therapy, there is gradual destruction of tissue with fibrous replacement and occlusion of small blood vessels. These forms of rejection occur in all allografts and the pathology differs only in detail.

The antigens (foreign molecules) which stimulate these rejection reactions are called histocompatibility antigens. As you recall, patients with blood type A have anti-B in their serum and blood type B have anti-A antibody in their sera. Blood type 0 has both A and B. These naturally acquired antibodies result as a consequence of the immunization to intestinal bacteria which cross react with A and B substances. Thus, essentially all patients except those that are blood group AB have a natural immunity to these allogeneic antigens. Transplants across such pre-existing immunity causes failure of the graft. In the case of kidney transplants, this results in a hyperacute rejection with thrombosis of the graft in the first few hours after implantation. In the larger liver transplant, complete destruction of the liver does not usually occur, but the antibodies are absorbed out of the serum, some parts of the liver do not re-perfuse. Such grafts may provide short-term, life-sustaining function, but long term success rates of livers across such incompatibility is poor. The rules of matching for the ABO blood groups are the simple rules of transfusion. The donor and recipient do not have to be compatible, but they must not be incompatible.

The third system of histocompatibility antigens is designated minor histocompatibility antigens. These antigens have not been identified specifically in the human species, although some have been identified in murine species. Minor histocompatibility antigens are inferred because all patients who are genetically HLA identical will reject in the absence of immunosuppression. These are less immunogenic antigens and the responses to them are readily controlled by standard immunosuppression.

The third histocompatibility system are antigens determined by the genes of the major histocompatibility complex or the MHC. They are called human leukocyte antigens or HLA. They are present on all nucleated cells of the human body. There is a major histocompatibility complex in every species thus far studied. That of the human is by far the most complex genetic region yet discovered.

TABLE 7

This subject was covered in detail by Dr. Gebel recently.

TABLE 8

The study of this complex may be confusing because the primary function of the genes which control these antigens is not to create a transplantation reaction. It turns out that the MHC and HLA are the mechanisms through which normal immune responses occur. This system is involved intimately in the control of immune responses to other foreign antigens. It is almost certainly involved with self identification and probably is involved in the maintenance of

self tolerance. So, this elaborate and complex system of genetics is an integral part of host resistance, immune response, and survival of each individual. However, in the transplantation of the organ, the antigens determined by these loci are recognized as foreign to the host and the principle immune response is generated against them.

TABLE 9

Study of these loci is further complicated by the fact that there are many different mutually exclusive alleles which may be present at each of 6 loci. For example, there are 50 known HLA-A alleles and the genes for any two of those may be present in any one individual. Similarly, there are 98 known alleles of the B locus, 42 of the C locus, etc.

TABLE 10

You may see from this slide that given the number of different genes which may comprise these six loci, there may be as many as 95 billion possible phenotypes which is some 15 times the number of human beings alive on this earth. Therefore, the probability of any unrelated individual being identical to another unrelated individual is about one in every 1.8 trillion. It should be apparent from these calculations that it is unlikely that any one individual will have another phenotypically identical unrelated individual alive in the universe at any one time. So, phenotyping for HLA identity is impractical. There is some hope for benefit in phenotyping because the HLA-C locus has never been shown to be a major histocompatibility antigen, and may be considered a minor

antigen. Further, the biologic importance of HLA Dp and Dq are not well established. Common clinical typing involves the serologic typing of HLA-A, HLA-B and HLA-Dr. By reducing the number of antigens tested, it is possible periodically to find individuals that are six antigen matches. This happens more commonly than expected because there is a linkage disequilibrium among the various alleles of this system. That is, some genes are much more common than others which increases the possibility of a random match between those particular genes. For example, HLA-A2 is present in some 50 percent of the Caucasian population. Several of the genes are present in 10-20 percent of the population, while some others are present quite rarely.

In this country, we have a national system of sharing all six-antigen matches in our clinic with approximately 200 patients on the renal waiting list, we get one or two six-antigen matches from the entire pool of cadaver kidneys in the United States annually, which is about 10,000. Studies throughout the world concerning the importance of HLA phenotyping to graft outcome are confined largely to renal transplantation. In summary, perfect six-antigen matched transplants certainly have better survival than any other combination. However, the difference in a one-antigen mismatch and a six-antigen mismatch are small and may be over-ridden by other factors such as the health of the transplant. The whole process is confused by the fact that different individuals have different intensity of immune response to the same antigen. That is, there are some individuals who are called high responders who will destroy any

kidney they receive with a single mismatch, while there are others who will not destroy transplants with a six-antigen mismatch. So far as can be determined, all of the alleles of the HLA loci are co-dominant and equally immunogenic.

TABLE 11

Because of the unique genetics of the MHC, it is possible to genotype within families, since all loci are located in close proximity on chromosome 6. In this slide, each bar in this pedigree represents one of the chromosome 6 pair present in each individual. Thus, with any one mating there will be two chromosomes to choose from in each parent. There will be four possible combinations from these two pair. The father's A chromosome here may be matched with either the C or D chromosome from the mother, and the B chromosome from the father may be matched with the C or D chromosome of the mother, and there are no other possibilities. Therefore, if one can identify a single different antigen on each of these four chromosomes, it is possible to infer which chromosome each progeny receives. By so doing, it is not necessary to mark all antigens, but to identify the individual chromosome. This can ordinarily be established, and it is possible to find genotypically identical siblings. In this schema all of the material present on any one piece of chromosome is called a haplotype so that siblings may either be two haplotype matches or identicals, they may be one haplotype match or haploidenticals, or they may share no chromosome and be a zero haplotype match.

TABLE 12

The other clinically important aspect of HLA typing is the pre-transplant crossmatch. A given individual may become sensitized to the HLA of other individuals through either pregnancy, blood transfusions, or previous rejected transplants. Each transplant patient has a final crossmatch from serum drawn immediately prior to the transplant and reacted with the cells of the donor. If this is positive, the transplant cannot be done between those individuals.

So, to summarize, this is a highly complex genetic system. It is in linkage disequilibrium. It is essentially impossible to find truly phenotypically identical tissues. The most important contributions of HLA typing clinically have been to identify the genotypically identical sibling which yields very good results, and to prevent hyperacute rejection by the pre-transplant crossmatch and to identify the six antigen match cadaver graft. For logistic reasons, HLA matching has been studied in detail only in renal transplantation.

The antigens that are involved in xenograft reactions are not even identified. What is known about xenograft reactions is that there is a naturally existing immunity between discordant species, all of which undergo rejection within minutes. Experimental efforts to cross this barrier have been devoted toward preventing complement deposition which prevents hyperacute rejection and enables the standard immune response of acute rejection to come into view. Another area of research at present involves efforts to transgenically modify other species to carry human HLA type genes rather than the genes indigenous to the species. There is much talk about xenografting at the moment

and I am reminded of the statement by Sir Roy Calne, Professor and Chairman of the Department of Surgery at Oxford University, who recently commented that "xenografting is right around the corner, and it always will be."

Since all allogeneic grafts fail, it was necessary to develop a means of suppressing the allogeneic immune response before clinical transplantation could develop. It has been the pharmacologic development of such agents which has allowed clinical transplantation to become a reality. There are several such agents in use today. I have prepared some information about 6 such agents which is in your handout, but which I will not discuss.

TABLE 13

The indication for transplantation today is essentially a patient with end stage organ failure who otherwise has a reasonable life expectancy and in whom the risk of the operative procedure is not

TABLE 14

prohibitive. Nevertheless, those patients who do receive kidney transplantations have in order of decreasing frequency: glomerulonephritis, diabetes mellitus, and hypertension.

TABLE 15

The most common etiology of liver failure leading to transplantation in the pediatric age group is biliary cirrhosis. In the adult population cirrhosis, first secondary to alcohol, second secondary to hepatitis C. There are a number of other causes for end stage liver disease which lead to

transplantation such as primary biliary cirrhosis, stenosing cholangitis, some biliary cancers, and certain inborn errors of metabolism.

TABLE 16

The most common diseases leading to cardiac transplantation are ischemic cardiomyopathy.

TABLE 17

When rejection supervenes in a variety of transplants, it is well to remember that in the kidney, heart, and probably pancreas, the standard way of establishing that diagnosis is by biopsy.

TABLE 18 B, C, D, E, F, & G

The following series of six slides provides the most current national data relative to graft and patient survival for kidney, liver, and heart transplantation. Current results are believed to be somewhat better, reflecting the introduction of the new immunosuppressive agents.

On another subject, you should be familiar with brain death and the criteria for that diagnosis.

Table 19

These criteria include profound coma in the absence of any brainstem reflexes. The cause of the coma must be known, the patient must not be hypothermic, and some confirmatory test must be included in the clinical diagnosis. That may be either a period of observation or most commonly a technetium brain scan which shows no blood flow to the brain.

TABLE 20

By the absence of brainstem reflexes, it is meant that there should be no pupillary reflex. The pupils should be dilated and fixed. There should be no decerebrate or decorticate responses, no gag, or cough reflex. The oculocephalic reflex refers to what is called "Dolls Eyes" in that when the patient's head is moved, the eyes move with it. The oculovestibular reflex is tested by putting ice water in the ear which should cause nystagmus under normal circumstances. The patient should have no pain response and should be apneic. It is necessary to establish that there is an adequate PCO2 to stimulate respiration, and there is a specific test for doing that in which the patient is removed from the ventilator, placed on nasal oxygen, and the CO2 is allowed to rise until it reaches a level of at least 50.

TABLE 21

The leading causes of brain death in this country are: cerebrovascular accidents, motor vehicle accidents, gunshot wounds of the head, and brain tumors.

TABLE 22

Once organs are obtained, preservation is ordinarily by storage in cold solution. The standard preservation solution today is the so-called Wisconsin preservation solution which is modeled after intracellular fluid. Maximal preservation time differs from organ to organ with the least allowable time for heart transplants and the maximal time for kidneys. Preservation times for

kidneys can be extended up to 48 or even 60 hours by pulsatile perfusion, but this is usually unnecessary and is unduly expensive. In summary, the transplantation of all allogeneic vital organs is much the same. There is substantial variation in the technical difficulty and the surgical complications that are unique to each organ. There are mild variations in the preservation techniques and allowable ischemic times. There is even less variation in the immunosuppressive therapy and surprisingly little difference in outcome. Results around the world today would indicate that the transplantation of the heart is the most successful of all grafts, followed by the kidney, followed by the liver, the lung, and thereafter, the pancreas. Most allograft failure occurs within the first year with a stabilized but gradual decline in graft function thereafter. Currently you may consider that all transplants are palliative in nature in that they rarely sustain function for longer than 15-20 years. Nevertheless, I wish to emphasize to you that there are now approximately 50,000 individuals in the United States waiting for organ transplantation. All of these patients awaiting a heart or liver will die without an organ replacement. All waiting on kidneys will be condemned to permanent dialysis and early death without organ replacement. These people can be restored to nearly normal existence for many years with transplantation. Although the results are not perfect, they are very good considering the alternatives, and they improve annually.

There are about 12,000 people in the country who die each year who

would be appropriate organ donors. Only about 6,000 actually become donors. I believe it is no longer moral to bury organs that can be used to prolong the lives of others. Thus, I submit that everyone in this room should be willing to be an organ donor and should make arrangements to that end. Further, no matter what type of practice you perform you should be alert to the need and miss no opportunity to attempt to recruit organ donors. Whenever you observe someone dying, the question should arise.

Louisiana has a very good organ procurement agency called LOPA. Professional people are available 24 hours per day, 7 days per week to receive a call about a potential donor. All you have to do is to call them and answer a few questions. If the patient is a possible donor, they will do everything else.