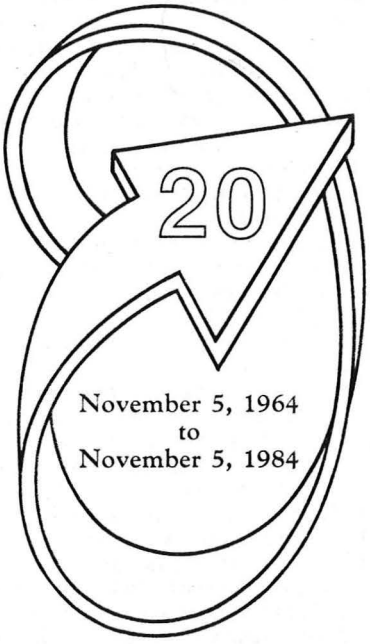
RENAL TRANSPLANTATION 1984

IN CELEBRATION OF 20 YEARS OF TRANSPLANTATION AT PMH-SWMS



Medical Grand Rounds

October 25, 1984

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INTRODUCTION

Organ transplantation is the stuff of ancient myth and medieval legend, of battlefield necessity and medieval dreams, of laureate research and supreme professional courage, of popular titillation and media exploitation. It has created cannonized science, ennobled gentlemen, and scientific heroes. From ancient papyrus to Christian stories of the saints, from the vaunted skills of Arabian surgeons to the precise skills of Rennaisance Italian medicine and Restoration English medicine, from lonely experiments in horse sheds, chicken coops, cow barns, organ transplantation has emerged from the mists to the reality almost of the mundane. The University of Texas Health Science Center of Dallas in collaboration with the Parkland Memorial Hospital has participated in the flowering of organ transplantation in the twentieth century. From November 5, 1964, when our own champion, Dr. Paul C. Peters, Sr., performed the first transplant in the state of Texas and one of the first transplants west of the Mississippi, until 20 years later renal transplantation has become an everyday phenomenon. Almost two transplants are performed each week at Parkland Memorial Hospital. From the signal program in organ transplantation in our state has grown a network of transplant programs that has reached the number of 9 and is still growing. From that single transplant in 1964 the city of Dallas now does a transplant every other day. This grand rounds commemorates the twentieth anniversary of renal transplantation at our university and hospital and in the state of Texas. My purpose is not to be encyclopedic but to review a potpourri of relevant new issues in renal transplantation. I will initially review the history of transplantation in general and detail the history of the Southwestern Medical Program outlining the magnitude, the quantity, and the quality of our transplanting effort. The remainder of the rounds will center about a discussion of allograft rejection. Great strides have recently been taken in expanding our understanding of the immunologic processes which lead to allograft antigen recognition and development of the effector mechanisms which culminate in graft destruction during rejection. This review will deal with this new understanding in some detail. The diagnosis of rejection at its very inception has been a continuing research and clinical problem. Some of the newer attempts to make this clinical judgment will be discussed highlighting Southwestern Medical School's scientific contribution to this field. Continuing problems of patient selection and preparation to reduce the incidence and severity of rejection will be discussed, including the perennial argument concerning the efficacy of tissue matching and the importance of and role for blood transfusion effect. Some of the most important breakthroughs in organ transplantation in the last 10 years involve newer treatment strategies to prevent or reverse acute allograft rejection. Lastly, the rounds will discuss the theory and practice of some of these newer modalities including pharmacologic agents such as cyclosporin A, biologic therapy, such as monoclonal antibodies against lymphocytes, and mechanical protocols, such as plasma exchange, immunologic manipulation, again highlighting the contributions of our transplant team.

HISTORY OF ORGAN TRANSPLANTATION

The earliest recorded description of transplants, skin grafts in reparative surgery, is apocryphally ascribed to the ancient Egyptians emanating from papyrus Smith, Ebers, and Berlin. A more correct translation of the papyrus fails to indicate that the Egyptians had any knowledge of

transplantation skills. In the dawn of transplantation, rhinoplasty, the repair of ritually or medically mutilated noses, has been the impetus for working out skin transplant techniques. The earliest true mention of such techniques is in Sanskrit in the Sushruta Samhita about 450 A.D. but were perfected by the Italian late Medieval and early Renaissance schools of surgery. Gasparo Tagliacozzi wrote an extensive treatise on the use of isografts of skin to repair this ever increasingly popular form of mutilization. The true father of organ transplantation may have been John Hunter, the Scottish surgeon-anatomist who transplanted skin, teeth, and vascularized endocrine tissues. His experiments raised the techniques of allograft transplantation from the mythical (e.g., the Italian love of the miracle of saints Cosmos and Damien who were said to have transplanted the leg of a black man to a patient whose own leg was recently amputated), to the modern world of actual whole organ transplantation.

In the Twentieth Century, disparate disciplines brought pieces of the transplant puzzle together. The world of cancer research and skin transplantation led to the discovery of the major histocompatibility complex, the laws of transplantation immunity and an initial understanding of rejection. From the experiments of Peter Gore, who discovered the mouse histocompatibility complex; of George Snell, who produced strains of inbred mice useful for research and transplantation; of Jean Dausset, who discovered the histocompatibility genes in man; of Sir Peter Medawar and Rupert Billingham, who applied an understanding of skin transplantation and rejection to a first hypothesis of the rejection process and of tolerance to the seminal surgical advances made by Alexis Carrell, whose Nobel Prize winning feat allowed for the technical capacity to do vascularized organ transplantation, transplantation became a clinical reality.

For transplantation of the kidney, two equally important technical feats were essential. The first was the discovery of the artificial kidney by Wilhem Kolff, which allowed for maintenance of life until a transplant could be performed and the sustenance of life until the transplant could be working

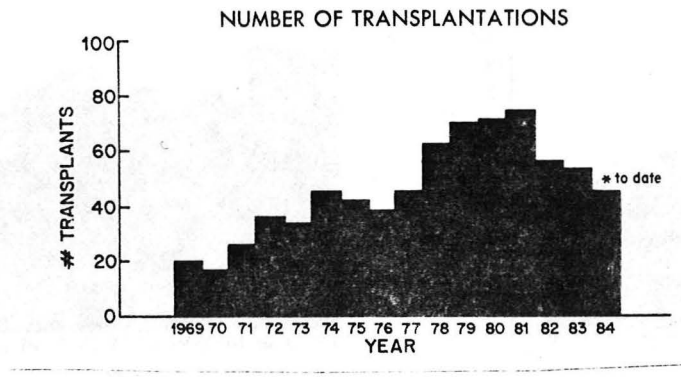


Figure 1

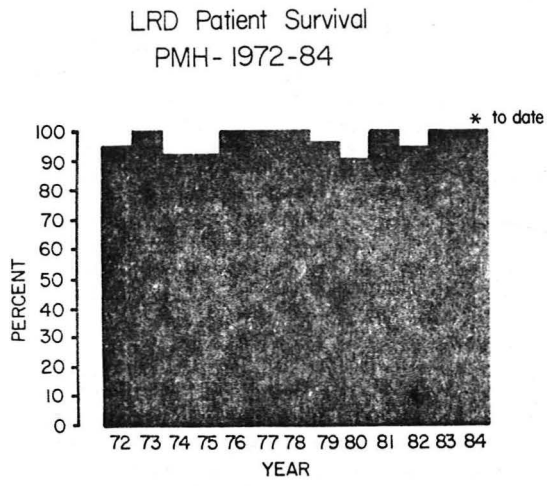


Figure 2

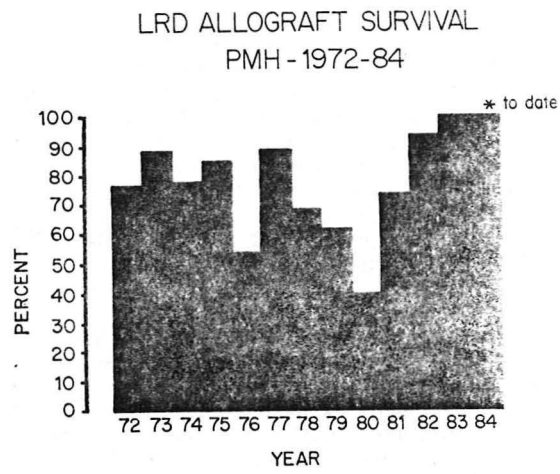


Figure 3

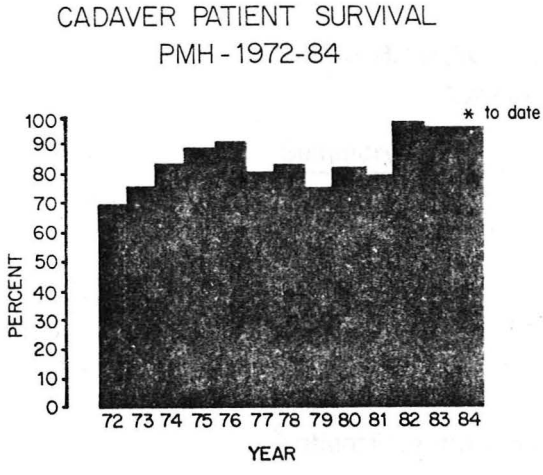


Figure 4

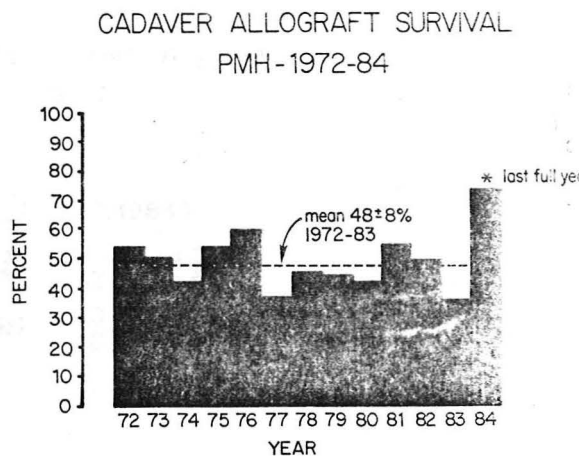


Figure 5

at full capacity. Another very important advance was the discovery of pulsatile perfusion and organ preservation by Folkert Belzer which allowed for organ storage until appropriate recipients could be found. With these forces taken together Simonsen and others successfully performed allografts in dogs culminating in the first successful transplant in man at the Peter Bent Brigham hospital in Boston, Massachusetts in the mid-1950s.

The history of transplantation at Parkland Memorial Hospital began on November 5, 1964, when Paul C. Peters with the help of the Nephrology Service performed the first renal transplant in Texas. Our own program has grown steadily through the Seventies and early Eighties achieving a peak of activity of 74 transplants in 1981. In 1981 a competing program of renal transplantation was opened across town in part responsible for an initial drop in transplant activity for the next several years. This calendar year the trend toward diminished transplant activity has been reversed. At the time of this writing 50 transplants have been performed with an estimate of 65 transplants expected by the end of the calendar year. I can truly say that transplantation, despite the headline of an article from one of the Dallas papers in 1965, is no longer a rare "surgical event" in the city.

The clinical results of our transplant program have been laudatory. In discussing clinical results, one describes actuarial survival for both the patient and for the transplanted allograft. The goal is to have no mortality and 100% graft survival, a goal as yet not attained by any transplant program for cadaver transplantation. We are nearing this goal for the Parkland experience for living related transplantation. Graft survival for both one haplotype and two haplotype matched donor-recipient pairs has been nearly 100% while we have lost one patient in the last four years. Several years ago the Parkland program had experienced a remarkable reduction in patient mortality in the cadaver circumstance from a mean of approximately 12% over many years time to 5% or less per year. Through the Seventies and early Eighties this

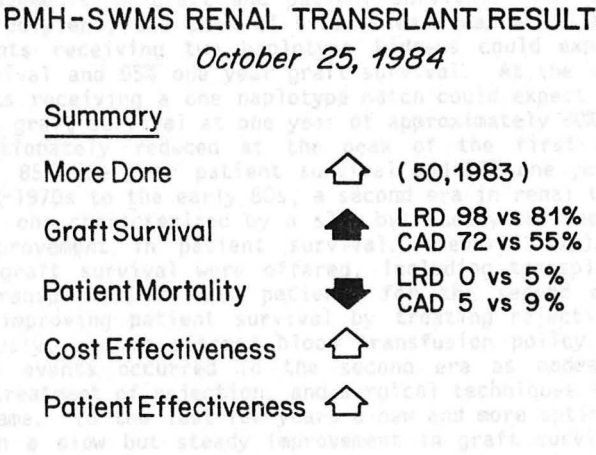


Figure 6

sharp and desired reduction in patient mortality was not matched by an improved graft survival with cadaver allograft actuarial one year graft survival being on the average of about 55%. More recently our results have sharply improved with the advent of newer approaches to diagnosis and treatment of rejection. We are now experiencing a greater than 70% graft survival, placing the UTHSCD-Parkland Memorial Hospital Transplant Program among the best programs in the nation.

Table 1

RENAL TRANSPLANTATION IN THE 20TH CENTURY
A Summary

ERA I	1953-64	The experimental period. Modest graft survival with high mortality.
ERA II	1964-82	The azathioprine era. Offered to more patients. Declining graft survival, improving patient survival.
ERA III	1982-?	The Cy A era. Individualized approach. Improving graft and very high patient survival.
ERA IV	?	The immunologists dream--no rejection; highly selective immune non-responsiveness.

One can divide the history of human renal transplantation into three eras. The first era, the initial 15 years of experience, was characterized by steady improvements in graft and patient survival. For the living related transplant recipient, the peak of success was reached in the early 1970s in which patients receiving two haplotype kidneys could expect more than 98% patient survival and 95% one year graft survival. At the apogee of the first era, patients receiving a one haplotype match could expect a patient survival of 90% and a graft survival at one year of approximately 80%. Both statistics were proportionately reduced at the peak of the first era for cadaveric recipients: 85% one year patient survival and 60% one year graft survival. From the mid-1970s to the early 80s, a second era in renal transplantation was experienced, one characterized by a slow but steady decline in graft survival with an improvement in patient survival. Several explanations for this decline in graft survival were offered, including transplantation of sicker patients, transplantation into patients for the second or third time, an attempt at improving patient survival by treating rejection less vigorously than previously, and an altered blood transfusion policy. Few clinical or intellectual events occurred in the second era as modes of prevention of rejection, treatment of rejection, and surgical techniques in general remained about the same. In the last few years a new and more optimistic era has been entered with a slow but steady improvement in graft survival accompanied by continued excellent patient survival. In many centers, total patient survival

at one year is 95% while groups utilizing one or another experimental program have improved their cadaveric survival rates toward 70% at one year. I am happy to report in this grand rounds that the UTHSCD-Parkland Memorial Hospital has been able to enter with the best transplant programs this third and new optimistic era. I think it appropriate to review some of the newer approaches to diagnosis and treatment of rejection that may have culminated in this more optimistic, third era of renal transplantation.

MECHANISMS OF TRANSPLANT REJECTION

The tumor transplant experiments of Cloudman and colleagues led to the view that transplantation immunity was an inheritable trait. The actual experiment in which tumors from mouse strain A were transplanted to tumors from mouse strain DBA suggested that many more than one transplantation gene governed the propensity for organ rejection. The early tumor work of Gorer and colleagues was reproduced by Medawar in his fertile laboratory in England with skin grafts leading to the view that histocompatibility gene products called alloantigens plan an essential role in rejection process. From the skin graft experiments of Prehn and Main, one could estimate 13 different gene loci important for transplantation rejection although one single locus, the major histocompatibility complex (MHC), called H2 in mouse or HLA in man, seemed to be most important for transplant rejection.

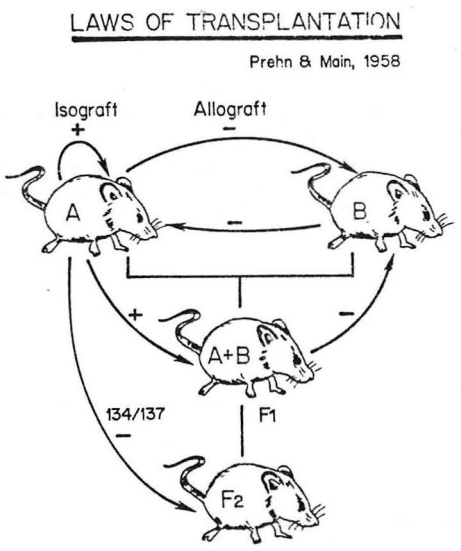


Figure 7

The genes of the major histocompatibility complex in man are found on the short arm of the sixth chromosome. An explosion of research has led to a precise characterization of the genes and the gene products on this portion of the chromosome and has been the subject of two recent grand rounds. A substantial review, therefore, is unnecessary. A cursory knowledge, however, of some of the features of this complex is essential for our discussion of the mechanisms of allograft rejection. The genes encode two different gene products distinguished by virtue of chemical structure and size. Genes A, B, and C encode glycoproteins called Class I proteins of approximately 45,000 daltons, intimately associated with a small molecular weight (12,000 daltons) protein encoded on another chromosome called beta II microglobulin. Twenty percent of these molecules exist transmembrane sequences and 80% remain outside the membrane. As pointed out by Dr. Capra there is great homology between constant regions of these

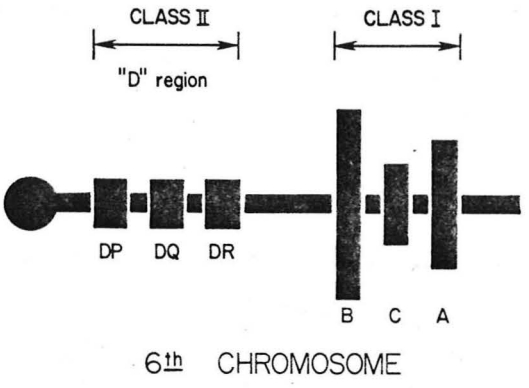


Figure 8

molecules from species to species with important overlaps with the domains of the immunoglobulin molecules as well. The highly polymorphic nature of the Class I proteins has led to a variety of antigenic specificities which is the basis for an approach to tissue matching for organ transplantation. The most recent biologic explosion has been in the area of understanding the Class II genes and gene products, homologues to the mouse I-A region. The gene

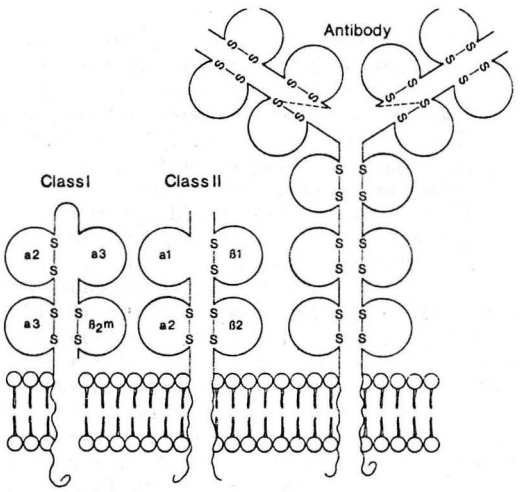


Figure 9

products of these Class II genes, commonly called D region genes, are comprised of two peptide chains, an alpha of 35,000 daltons and a beta of about 28,000 daltons, each with an intramembranous piece and substantial amounts of peptide extending from the membrane, with two distinct domains on each chain. Three separate gene regions have now been characterized with uniform nomenclature recently adopted at the last

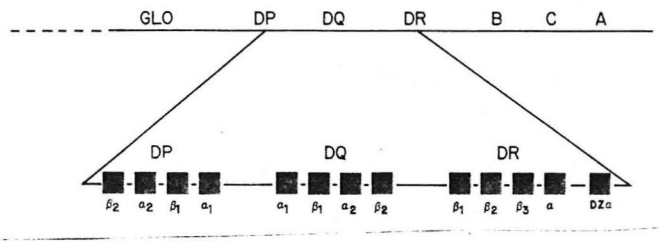


Figure 10

international workshop of the tissue typing association. These loci, the DR genes (homologues of the mouse IE), the DQ genes (homologues of the IA region gene), and the DP genes (the PLT locus), each may participate in governing the mixed lymphocyte culture reaction and be important in antigen recognition and transplantation immunity.

The rejection of vascularized organ grafts involves a polyphony of competing mechanisms: some antigen specific, some antigen non-specific; some involving immune cells, and some involving immune humors. One can view the recognition of the transplant as foreign tissue as a cascade of immunologic events initiated by recognition of Class II antigen differences by allosensitive T lymphocytes generally of the helper precursor pedigree. There is substantial evidence that the initial or primary recognition event involves recognition of Class II bearing blood borne cells called originally by Guttmann "passenger leukocytes" based on the tissue distribution of Class II antigens. In many species Class II antigens are not found on parenchymal cells but are confined to subsets of hemapoietic cells. While the importance of passenger leukocytes for transplant immunization for the rat may be proved, for man such a role is more controversial. In man Class II antigens are found on endothelial cells in the kidney and these sessile cells may be the means by which the allograft sensitizes the host. Moreover, it has recently been appreciated that during inflammation, the release of gamma interferon of activated lymphocytes may permit parenchymal cells devoid of Class II antigens to code for, synthesize, and express Class II antigens on their surface, thus amplifying the antigen-presentation phase of allograft rejection.

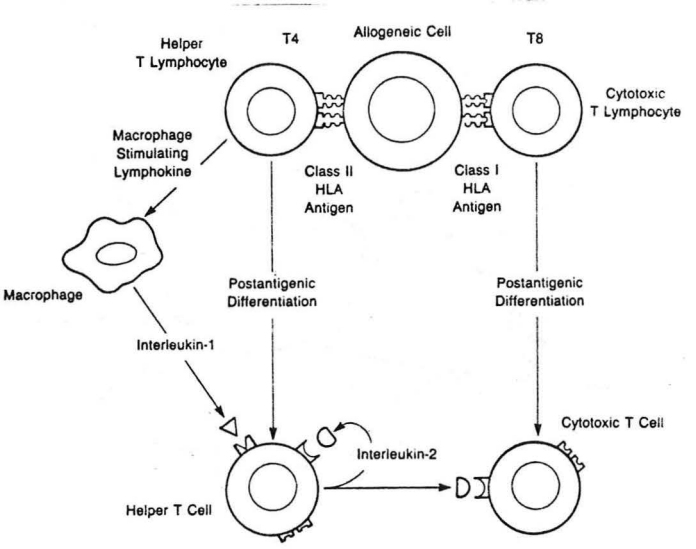


Figure 11

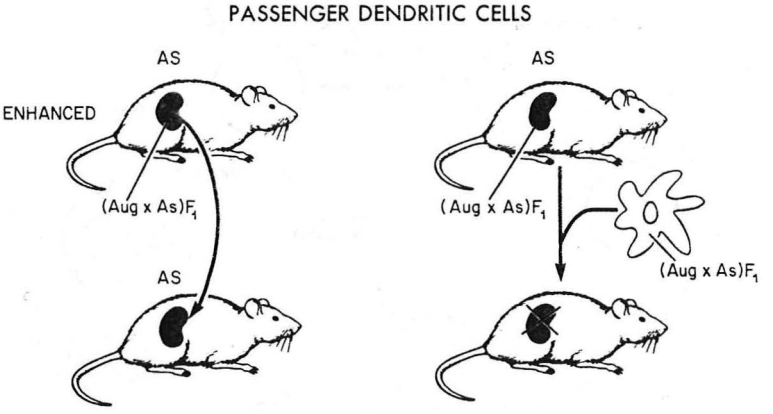


Figure 12

INFLAMMATION INDUCES CLASS II ANTIGEN SYNTHESIS OF THE KIDNEY

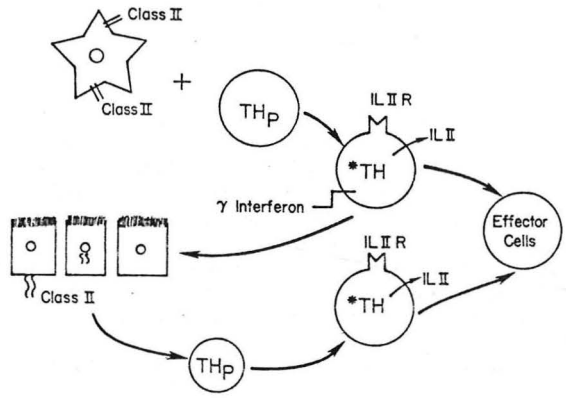
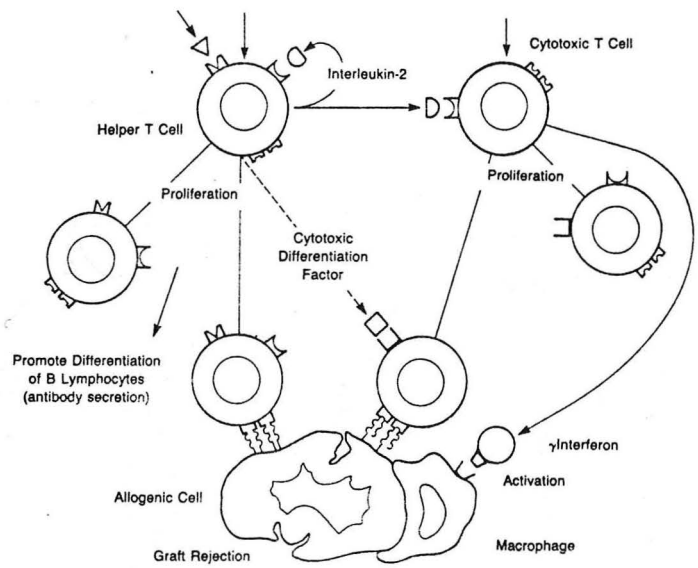


Figure 13

Once activated, the helper precursor cell either in situ in the allograft or in draining lymph nodes undergoes post-antigenic differentiation. A number of activation-induced surface receptors are synthesized such as that for Interleukin II, an important lymphokine which can amplify further the

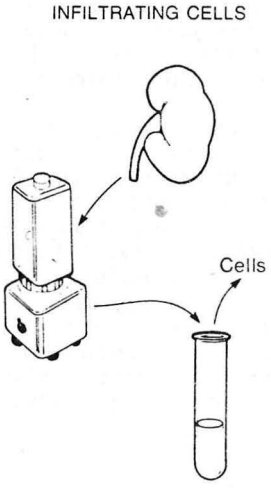


differentiation of activated lymphocytes. The activated lymphocyte not only synthesizes the Interleukin II receptor but also releases interleukin itself which, binding to the receptor, enhances and completes antigenic differentiation of the activated cell leading to clonal expansion of the helper subset of lymphocytes.

A second wing of activated lymphocyte amplification occurs via macrophages. The initial activating event of antigen recognition by the helper precursor cell leads to the release of the lymphokine, Macrophage Stimulating Factor (MSF), which activates macrophages directly. Part of the role of the activated macrophage is to release the macrophage product, Interleukin I, which further stimulates the differentiation of activated helper T cells. Taken together the antigen stimulated lymphocyte undergoes clonal expansion and can provide help for the sensitization of the cytotoxic T cell which has been stimulated by interaction with a Class I HLA antigen. Thus, the expanded helper clone of cells lead to the development of effector cells capable of inducing a transplant rejection by providing help for B cell differentiation in order for such lymphocytes to synthesize antibodies which can assault the allograft and by providing help for development of cytotoxic T cells which are directly capable of destroying the transplant.

The exact nature of the cells which mediate allograft rejection has also been the source of fertile research in the last few years. The classical and Nobel Prize winning observations of Medawar showed that rejection could be adaptively transferred and was antigen specific. Billingham and colleagues demonstrated the fact that cells transferred from sensitized animals to naive recipients unmasked accelerated rejection. The studies of the nature of effector cells and the role of humors in rejection has re-opened the question of the mechanism of rejection.

Upon presentation of graft antigen to antigen responsive cells, proliferation and clonal expansion proceeds along the lines just described.



	MHc Ag Specific	Ag Non Specific
T helpers	+ + +	+
T suppressors	+ +	±
T killers	+ + +	+
Mo	–	+ + +
Natural killers	+ +	+ +
Antibody	±	±

Figure 15

Sensitized cells developed in the allograft traffic to the draining lymph nodes and are released into the circulation. They then stream back to the allograft, ultimately leading to its destruction. A classic original experiment attempting to discern the nature of the effector cells causing rejection in the allograft were performed by Strom and colleagues. Irrevocably rejected human renal transplants were used as the source of infiltrating effector lymphocytes which were characterized morphologically and functionally. In the inflammatory infiltrate of these rejected kidneys were found macrophages, B and T lymphocytes. Natural killer cells were also identified. In the infiltrate cells were predominantly antigen specific, that is, targeted to destroy the class I bearing cells of the donor allograft. But equally important was a substantial contribution in the infiltrate of nonspecific natural killers and cytotoxic cells for which the given allograft was not the target. One could argue that the local inflammatory response initially instituted by effector lymphocytes in their reaction to the allogeneic sensitizing tissue called forth a nonspecific inflammation enhancing tissue destruction. The same laboratory group demonstrated that the effector cells entered the transplant as early as the fourth postoperative day, a finding later confirmed by the fine needle aspiration cytology procedure of von Willebrand and Häyry. These investigators showed that there is a second phase of clonal expansion of the effectors in situ with further release of lymphokines and other inflammatory mediators thus establishing the nonspecific nature of the inflammation. Hall and Dorsch thus conclude that "lymphocytes entering the graft at the time of rejection are a functionally heterogeneous population and identification of a given subtype of lymphocytes within a rejecting graft does not necessarily indicate that participation of that cell type in either the specific interactions which precede rejection or the rejection process itself."

This nonspecificity of the cellular infiltrate has made the characterization of the specific effector player quite difficult. Since the time of Medawar and Billingham it has been assumed that a cytotoxic T cell was the principle actor in graft rejection. The classic cytotoxic T cell is antigen specific and requires contact between the killer cell and the target to mediate cell death. Three stages of cytolysis have been characterized: 1) target cell recognition; 2) target cell binding, and 3) target cell death, a cascade generally thought to operate in the absence of lymphokines, other cells such as macrophages, or humors, although an event which can be modulated by hormones and neurotransmitters. Killer T cells are both generated against and utilize as targets the Class I the antigens in contradistinction to the means by which helper T cells are activated.

A second cell type has recently been promulgated as the important effector cell. This cell type has characteristics that suggest that it participates more in delayed type hypersensitivity reactions (DTH) and can be distinguished from classical cytotoxic T cells by virtue of surface markers bearing the helper identifying surface antigen (T4, Leu III) in contradistinction to the cytotoxic marker (T8, Leu II). The potential role of DTH cell as the major effector in rejection was posed initially by Simpson when he demonstrated in his experiments of immune surveillance that the temporal appearance of DTH cells correlated in plasma during rejection was more precise than the appearance of the cytotoxic T lymphocyte. The DTH cell does not directly destroy the target cell, but utilizes lymphokines to attract phagocytic cells such as macrophages to attract other cells such as natural

Table 2

EFFECTOR CELL IN REJECTION
T_c Versus DTH

DTH	T_c
1. Rejection temporally related to DTH cells	1. Ag specific T_c cells always present in rejecting grafts
2. T8 removal does not block rejection	2. Adoptive transfer of sensitized T8 causes rejection
3. T4 removal = no rejection	3. Ag specific nature of rejection in some assays
4. Ag nonspecific nature of rejection in some assays	4. Humoral manipulation of T_c function can regulate rejection

killer cells and phagocytic macrophages to effect graft destruction. The phenomenon may be antigen nonspecific in that the last cell in the effector chain, the macrophage, is totally nonspecific in this setting.

In order to discern the relative contributions of cytotoxic T cells and DTH cells to allograft rejection, investigators have returned to the adoptive transfer experiments originally described by Billingham and Medawar using highly characterized cell innocula in lethally radiated hosts. These experiments revealed that peripheral mature T cells are capable of restoring graft rejection to the irradiated animal and that removal of cells of the helper pedigree from the innoculum diminished or abrogated the capacity to reject allografts. This piece of information was taken as evidence to support the primacy of the DTH cell. Removal of T8 bearing cells did not diminish the capacity of the innoculum to induce allograft rejection leading to the conclusion that the cytotoxic cell was unnecessary for final effector function. A more balanced view as to the nature of the effector cells mediating allograft rejection has recently been put forth in a series of papers presented at the last international meeting of The Transplant Society. The importance of helper cells to initiate clonal expansion of effectors can explain the importance of the adoptive transfer experiments of isolated pedigrees of lymphocytes. The presence of antigen specific cytotoxic cells in infiltrates is not to be ignored but is part of the entire rejection process. The fact that natural killers and nonspecific effector cells such as activated macrophages can be present and in certain experimental circumstances sufficient to cause rejection cannot deny an important role to the cytotoxic T cell in clinical renal allograft rejection. Thus, one can envision the transplant rejection event as one which defies reduction to the simplistic and indeed is best characterized by that complex series of events in which cascades of antigen recognition leads to generation of a range of different varieties of effectors cells and humors which are both antigen specific and

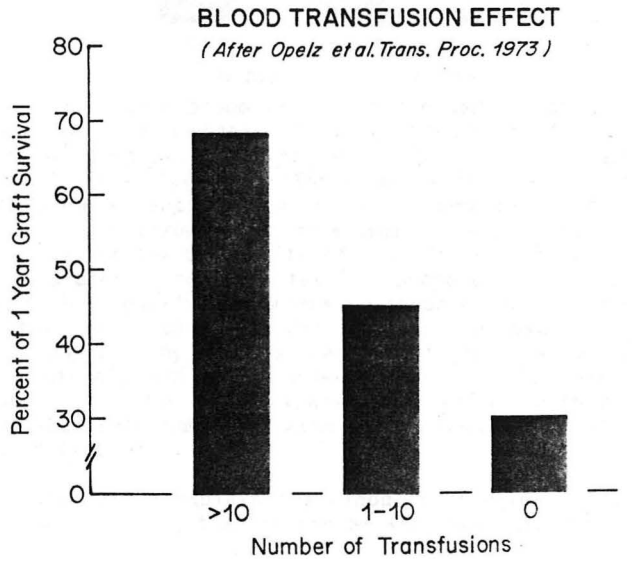
nonspecific. Taken these cascades together lead to allograft destruction. It is this complex view which has allowed a more scientific approach to creation of treatment modalities to prevent rejection or to treat rejection once in place, topics which will be reviewed in further sections of these rounds.

PREVENTION OF REJECTION

The Blood Transfusion Effect

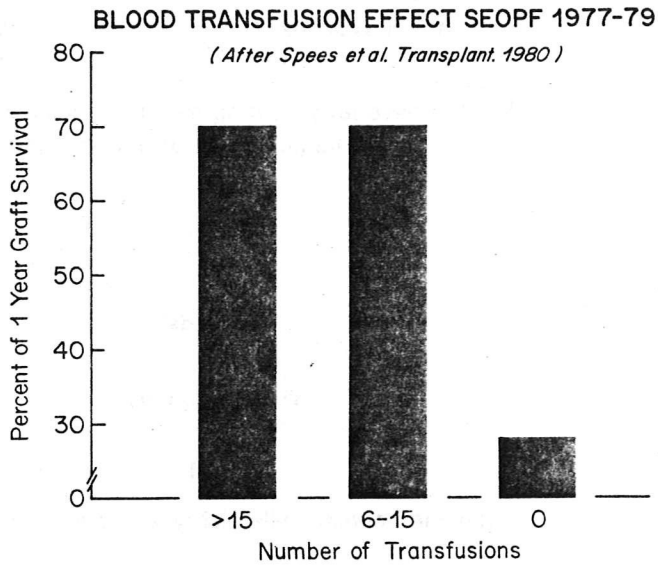
Classical immunologic teaching would assert that providing substantial alloantigenic loads such as contained in blood transfusions would be detrimental to the potential transplantability of patients with end-stage renal failure. Patients would mount an immune response against the alloantigens rendering them crossmatch positive to virtually all transplants available. Based on this orthodox theory recommendations with respect to blood transfusions in the Sixties and the early Seventies minimized the number of units to any given patient. Indeed, patients thought excellent transplant candidates were left untransfused unless dire medical emergency supervened. Clinical practice, however, forced a revision in such orthodoxy, an example which bedside observation challenged prevailing scientific notions and forced an experimental reevaluation of basic scientific premise. When Opelz and Terasaki analysed the effect of blood transfusions in several thousand transplants, the experience of which had been sent to the UCLA transplant registry from throughout the United States, a surprising finding was reached. Cadaveric allograft survival in patients who satisfied attempts to deny transplants to potential recipients was decidedly worse than for patients who, for medical or surgical reasons, had received a number of blood transfusions. Since that initial observation the same group has repeated their analysis often with similar findings. Subsequently, virtually every group that has evaluated this issue has demonstrated that blood transfusions confer a substantial, perhaps as much as a 40%, advantage in cadaveric allograft survival over nontransfused control individuals.

Figure 16



The thrust of recent research in this area has been to define the most advantageous timing of transfusions, the most efficacious form of blood product which renders patients least sensitized but maximally resistant to renal rejection, and the most appropriate timing for the transfusion. A detailed explication of each of the various approaches to the blood transfusion effect is beyond the scope of this review. The best synthesis of these data suggest that any form of blood transfusion in which red blood cells are the major element given at least within 6 months of transplantation will markedly enhance graft survival. The best estimate of the most appropriate number of units seems to be 5 at this time which has led to standard practice in many transplant centers.

Figure 17



While the number, timing, and major blood product for blood transfusion continue to be areas for research, the mechanism by which blood transfusions enhance allograft survival is of perhaps equal interest to the clinician and of more interest to the basic investigator. Three mechanisms had been proposed for the blood transfusion effect: 1) selection, separation of high and low immunologic responders; 2) immunologic enhancement; and 3) clonal deletion. Flechner and colleagues at the University of Texas at Houston using a battery of immunologic laboratory and skin testing propose that blood transfusions induce a state of low reactivity perhaps by inducing nonspecific suppressor T cells. The simple notion that high and low responder patients a priori can be selected is not supported by the best recent evidence. A number of immunologic enhancement protocols using red blood cells or white blood cells may be taken as models for the blood transfusion effect although patients have not yet been deliberately immunized with white blood cells alone in protocols similar to that of Carpenter.

The most interesting hypothesis to explain the blood transfusion effect has been promulgated by Terasaki himself, the individual who first discovered

Table 3

SELECTION HYPOTHESIS

For

1. Positive crossmatches developed consequent to transfusion precludes transplant.

Against

1. Small fraction of patients become sensitized by a transfusion.
2. Blood transfusions increase graft survival even in highly sensitized patients who receive allografts.

Table 4

ENHANCEMENT

For

1. Calculable reduction in in vitro assays of cell-mediated immunity in transfused patients.
2. T_s are activated by blood transfusions.
3. Presence of antiidiotypic antibodies after transfusions.

Against

1. In many experiments effect seen only when immunosuppression is later used.
2. Transfusions given simultaneously with immunosuppression prolong transplants.
3. Increased graft survival extends beyond period when enhancement effect is measurable.

this seeming paradox, as set forth in a recent editorial in the journal Transplantation, which I would like to review at some length. The Terasaka hypothesis states that the primary function of the transfusion indeed is to immunize directly patients in order to actually expand sensitized clones of lymphocytes which later can be eliminated by an introduction of immunosuppression. The blood transfusion, he feels, neither selects patients as low

Table 5

CLONAL DELETION HYPOTHESIS

1. The transfusion effect most often seen in combination with immunosuppression only.
2. Transfusions under the cover of immunosuppression are effective.
3. Transfusion effect may be "permanent".
4. Donor specific blood with immunosuppression is strongly effective.
5. Parous women have statistically better graft survival than nulliparous.

responders nor induces tolerance or enhancement. To support his hypothesis several pieces of evidence are marshalled. First, although at the time of transplant all patients must be crossmatch negative against the kidney donor, if one does deliberate transfusion studies in laboratory animals and assay frequently over time the majority of animals will mount an immune response that is measurable according to Soulilliou et al. When patients are analyzed for increased sensitization against a random panel several weeks after transfusion the majority cannot be shown to have enhanced sensitization from the blood transfusion probably because of the time of the analysis. The transfusion effect, Terasaki points out, requires the combination of blood transfusions and later immunosuppression. Several experimental models attempting to transfuse patients and place allografts without further immunosuppression were doomed to failure. On the other hand, the addition of blood transfusions to an immunosuppression regimen which prolongs allograft survival can in several experimental circumstances lead to permanent acceptance, thus, evidence for an important enhancing effect of the blood transfusion atop of immunosuppression. Terasaki then points out that one can also administer a blood transfusion simultaneously along with an immunosuppressive such as azathioprine and obtain a salutory effect toward graft survival. The simplest hypothesis, Terasaki argues, is that immunosuppression deletes clones of cells that are capable of reacting against a graft which are initially expanded by virtue of a blood transfusion. The immunization by blood induces lymphoblast formation, cells undergoing rapid turnover and thus amenable to removal by cytotoxic drugs. He summarizes the blood transfusion

effect by providing evidence that a blood transfusion is an immunization, the provision of immunosuppression during immunization unveils the salutory effect of transfusion, and the graft survival improvement that follows the blood transfusion plus cytotoxic drug regimen perforce is the result of deletion of antigen reactive clones of immune cells.

DR Matching

From the discoveries of the laws of transplantation by Gorer, Snell, and Prehn and Main, it has been assumed on immunogenetic grounds that organ transplants between individuals who shared the majority of histocompatibility complex gene products would fare better than in mismatched individuals. Such an assumption has been the basis for a tremendous amount of activity by the tissue typing fraternity. It is indeed true that this assumption can be supported in human organ transplantation when one examines the outcome of transplant events within families. The problem concerning the importance of tissue matching arises when one examines the clinical responses of individuals receiving cadaveric allografts with respect to tissue matching. At the dawn of human immunogenetics, Professor Daussett, a recent Nobel laureate for this work, characterized but one gene locus and gene product. Attempts to match for this single product was not met by enhanced clinical results. As outlined earlier in the section on the mechanisms of graft rejection a veritable panoply of genes now have been characterized which fill the short arm on the sixth chromosome of man with histocompatibility genes and complicate the statistical analysis of the problem of the importance of tissue matching. More than 15 years of research and clinical observation has failed to indubitably demonstrate that matching for class I (HLA, A, B, C antigens) confers major advantage for successful allograft transplantation. That is to say that the value of class I matching remains exceedingly controversial, even the subject of a recent report in the medical literature. The most recent analyses of large series fails to provide the clinician or the reader with a distinct answer about the importance of class I antigen matching. For example, Sanfilippo et al, analyzing 3811 transplant events from the 42 institutions that comprise the Southeastern Organ Procurement Foundation Program performed from 1977 through 1982, argued for a strong effect of HLA matching that is progressively revealed beyond one year of transplant experience. Matching both alleles of the A and B loci conferred a 64 ± 4% graft survival at 6 months and a 44 ± 7% graft survival at four years in matched individuals as compared to 55 ± 2% and 18 ± 4% respectively for unmatched individuals. This striking long-term effect has not been generally appreciated in previous studies. The European experience is similar as reported by Festenstein et al at the recent Xth International Congress of the Transplantation Society when he found a 70% one year graft survival when three or four alleles are matched as opposed to 42% at one year when 0 or one allele is matched of the class I antigens. Festenstein's group also underscored the important long-term differences with a 20% difference between the well and poorly matched groups observable after 14 years on an actuarial curve. At the very same time that these two groups have been finding importance of class I matching for outcome an equally large and heterogeneous group headed by Professor Gerhard Opelz running a collaborative transplant study of 191 transplant centers with more than 6000 transplant events to analyze could find "essentially no effect of A or B matching with one year graft survival being approximately 70% for all comers". One must conclude that the cadaver transplant circumstance is quite complex in that class I antigen matching may

in some centers contribute to graft success but in other centers additional factors may override the importance of class I matching.

One also has to point out the importance of the treatment era in which the particular set of data were accumulated to analyze this issue. In the second era of transplantation when results were stagnant at the 55% graft survival range, data obtained may no longer be relevant to what is experienced by almost all good centers at the present time. This may be one objection of the Sanfelippo set of data in which conventional immunosuppressive medications were exclusively used and in which maximum graft survival was on the order of less than 65% for one year. Class I matching for prediction of allograft survival has been hard enough to demonstrate that as many as one-third of tissue typing laboratories whose very economic survival may depend on such matching, have felt in a survey conducted by the American Association of Histocompatibility Testing Society to not believe in the importance of class I matching.

Our deeper understanding of the mechanism by which an allograft sensitizes the host might allow one to create an argument for lesser role matching for class I antigens by themselves may play in outcome prediction. Indeed, we now understand that it is differences at the class II alloantigens which initiate the sensitization of the helper wing of T lymphocytes absolutely essential for the clonal expansion of sensitized cells and the ultimate expansion of cytotoxic effectors for allograft rejection. One could even explain the confusing data with respect to class I matching in that one might be observing an indirect effect of such matching. The power of Class I matching may entirely be based on the linkage disequilibrium between class I antigens and class II antigens, matching for which might be the most strongly predictive of allograft success rates.

For more than 5 years it has been known that the in vitro correlate activity in the mixed lymphocyte culture, transplant immunity a consequence of class II alloantigen mismatches between donor and recipient, could predict prospectively a successful outcome after renal allograft placement. Positivity in the mixed lymphocyte culture is governed by the general gene region that has been called HLA-D. The mixed lymphocyte culture in man takes approximately 7 days to read so that matching using the MLC test itself cannot be employed to improve graft survival clinically. Recently, it has been shown that B lymphocytes and monocytes bear antigens on their surface that are encoded in the HLA region very close to the MLC stimulating locus or part of the same locus called HLA-DR. This form of matching may be performed in serologic tests similar to routine HLA A, B, C matching at the time of transplant and requires no more additional time than that for separation of B lymphocytes from T lymphocytes. Data from Europe, where there are homogeneous populations, and from centers in the United States in which recipient populations tend to be white and of European ancestry, have demonstrated the importance of matching HLA-DR. In one prospective study of 170 transplants, cadaveric graft survival in which no DR compatible genes were identified was 83% at two years. When two DR antigens were shared regardless of class I typing, cadaveric graft survival at two years was 88% approaching that of living related transplantation. As American experience has been gathered, almost all centers find an important DR matching effect. Ayoub and Terasaki reported from their multicenter study group 75% one year graft survival if two DR alleles were matched as opposed to 47% graft survival if 0 DR alleles are

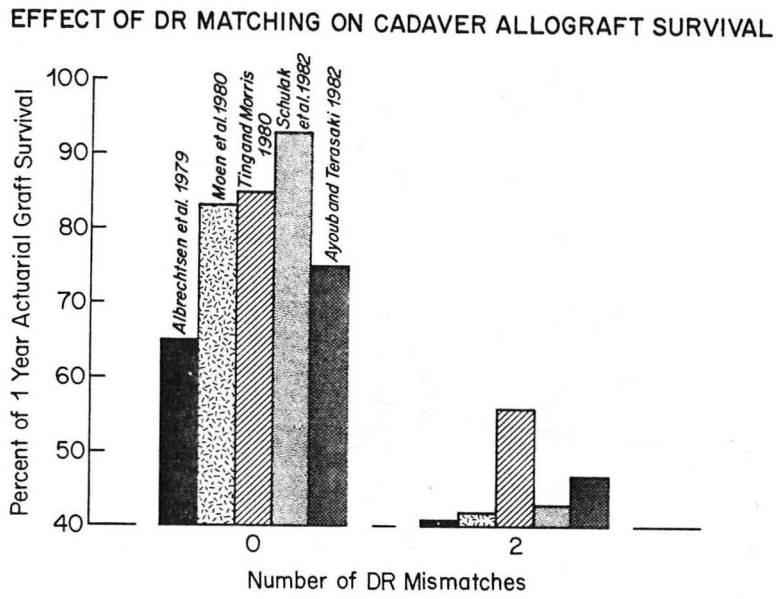


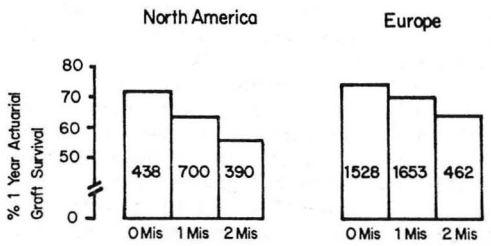
Figure 18

matched. Our own data is equally strong with respect to DR matching. At Parkland from 1982 to 1984 zero mismatches gave 85 one year graft survival as opposed to two DR mismatches which gave 40.

As discussed recently by Dr. Capra, two new sets of genes in the class II region of the HLA chromosome have been characterized. It might be possible for even more precise matching to allow for results as good as two haplotype living related kidneys. At this time such precise matching is not clinically possible in that wide numbers of well characterized tissue typing sera are not yet available for the DQ and DP gene products and the relationships between monoclonal antibodies to the gene products and the polyclonal antibodies in multiparous or multitransfused individuals is not yet clear. These technical aspects of tissue typing are critically important for the future development for this form of tissue matching. But more important to use the fruits of such efforts, it will be imperative to have national and international cooperation concerning organ sharing. It would be statistically impossible for any one small organ sharing unit to be able to identify donor and recipient combinations in which all these newer gene products are matched and to allocate organs accordingly. Initiatives along the lines of legislation recently considered in Congress will be essential.

If one follows this line of tissue matching argument to its logical conclusion, one can see that instead of performing a large number of transplants in randomly matched individuals, one might be doing many fewer transplants in highly matched individuals for whom excellent results may be

DR EFFECT ON CADAVERIC
ALLOGRAFT SURVIVAL



(from Opelz Collaborative Transplant Study 6184)

Figure 19

DR MATCHING AT PMH
1981-1984

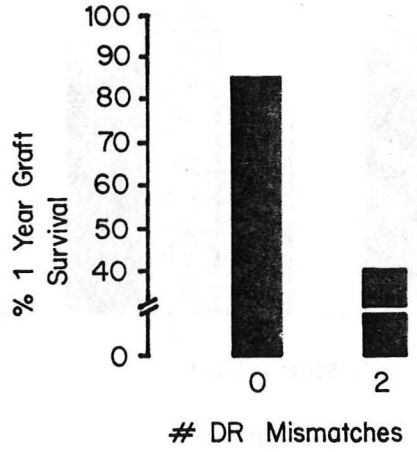


Figure 20

expected. Indeed, many transplant programs had actually moved to this point of view doing fewer transplants that were either two DR matched transplant events or zero mismatched events at the conclusion of era II of renal transplant history. This protocol consigns larger pools of patients to dialysis with that social and economic drain on our society. But, more recent experience has been teaching that the same excellent results achieved by precise DR matching may be experienced using newer therapies to prevent or to treat rejection in DR unmatched pairs. Some of these newer strategies will be reviewed in great depth later in this text, but I want here to highlight some of the published data that support this view, especially with respect to the new medication Cyclosporin A. Taylor from the University Hospital in Pittsburg analyzed the effect of cyclosporin A on DR matching and found that zero mismatched individuals under the cover of Cy A had 75% graft survivals, equal to that achieved in unmatched recipients receiving Cy A. An important report has recently been published by Harris et al from Portsmouth, England, who previously had reported on a strong DR matching effect. Cy A clearly overcomes the DR matching effect in Harris' hands. The largest such series over many years time, has been conducted by Dr. Barry Kahan and colleagues at the University of Texas Health Science Center at Houston, a sister transplant program in the state which has contributed much to our understanding of the new drug cyclosporin A. In Dr. Kahan's experience, the hitherto important DR matching effect when prednisone and azathioprine were used to prevent rejection was completely abrogated by the use of cyclosporin A on the

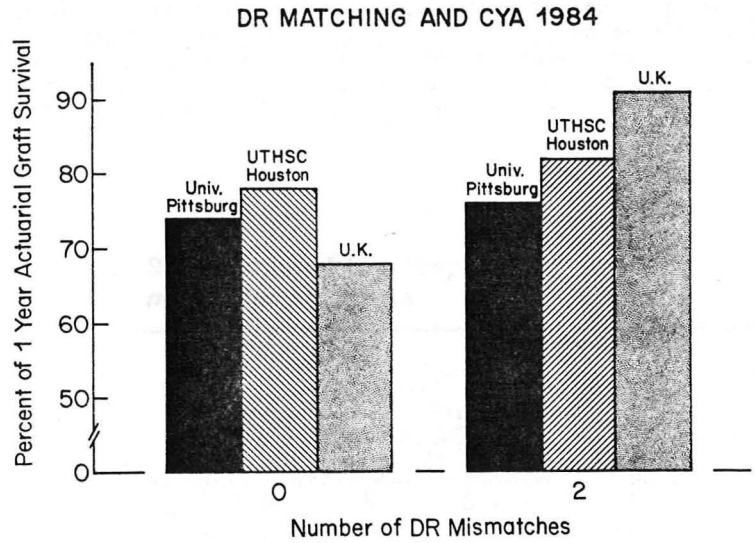


Figure 21

background of low dose prednisone therapy. Individuals mismatched for two DR alleles experienced 82% one year actuarial graft survival while individuals who were not mismatched at all for DR alleles experienced a 78% one year graft survival, not statistically significantly different and both excellent results in over 100 transplant patients followed for two years.

At Parkland Memorial Hospital we have been conducting a study using a combination of DR matching and cyclosporin A to maximize our cadaver allograft survival results. For individuals receiving their first allograft, an attempt will be made to find two DR matched kidneys. When such an attempt is successful then patients will receive the standard prednisone azothiaprine combination for prevention of rejection. In our experience such individuals can expect a greater than 80% graft survival using these conventional drugs. In individuals for whom a two DR matched kidney could not be found, a transplant will be performed in any case under the umbrella of cyclosporin A and prednisone. This protocol has been proceeding for more than a year and a half and the excellent graft survival rate that has been reported in these rounds has been partly a consequence of this protocol. Our success to date using this protocol has given us impetus to continue this approach individualizing treatment protocols for each donor-recipient combination.

Cyclosporin A

Standard regimens of immunosuppression for preventing transplant rejection have been unchanged for at least 20 years since Professor Roy Calne demonstrated the efficacy of azathioprine in combination with steroids. Rowe and colleagues studying a new undecapeptide metabolic product of the fungi Cyclindrocarpon lucidum and Trichoderma polysporum originally synthesized as an antibiotic, discovered the important immunosuppressive properties and anti-lymphocytic activity of this new agent called cyclosporin A. Shortly thereafter Kastakis and Calne demonstrated the prolongation of an experimental

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Sandimmune™ (cyclosporine) molecular structure

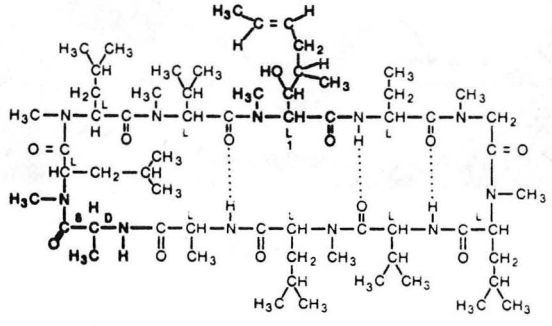


Figure 22

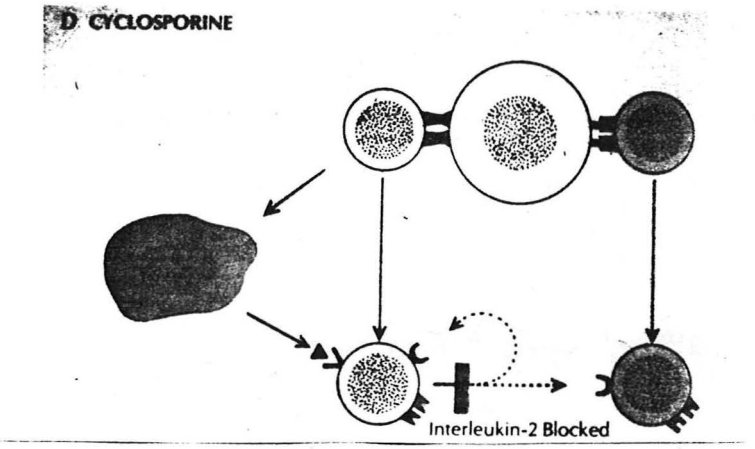


Figure 23

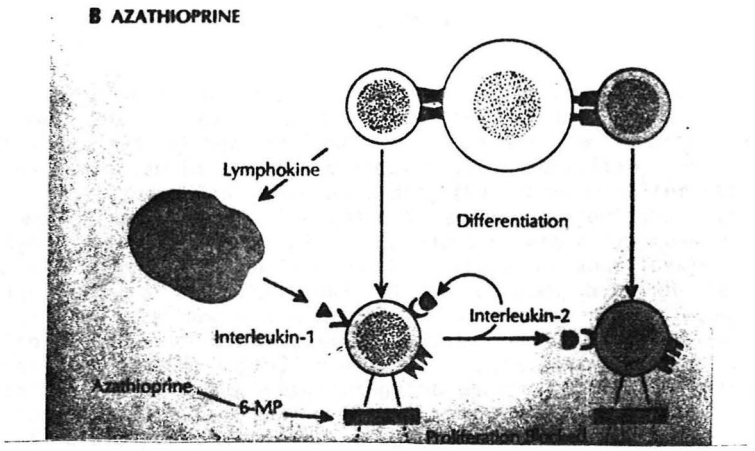


Figure 24

rat heart allograft model, confirmed later in dogs and pigs. Professor Calne and his group then embarked on clinical studies of this new and potent agent. Initially, the hope was that cyclosporin A could be given in short courses as the sole immunosuppressive agent. Thereafter, the recipient would accept an allograft. This initial dream has not been borne out by data from the clinic or the laboratory. On the other hand, cyclosporin A does seem to be a very powerful immunosuppressive agent that can reduce substantially the requirement for steroids.

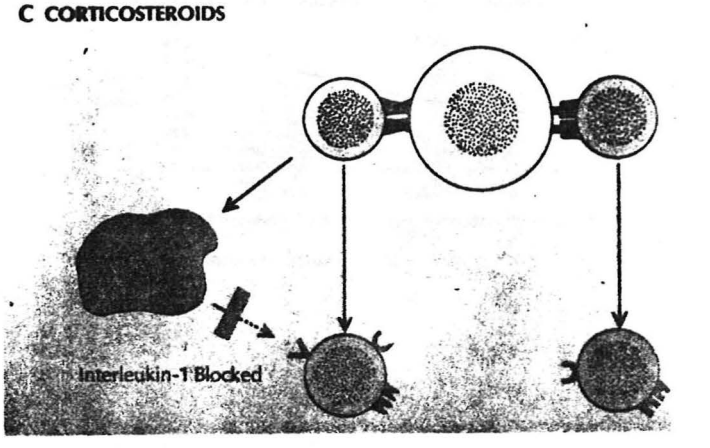


Figure 25

The mechanism by which cyclosporin A is immunosuppressive has recently been worked out in some detail. Returning to the schema of immunologic reactivity against allografts one should recall the important role of the lymphokines Interleukin I and Interleukin II in recruiting and amplifying the clone of sensitized cells, thus expanding the clone and alternately producing a large enough pool of effector cells to produce rejection. Cyclosporin has been shown to selectively inhibit the production and/or release of interleukin II from the activated T helper cells. There is additional data that the macrophage release of Interleukin I is reduced but not rendered zero. Additional new data now ascribes an important role of cyclosporin A to the generation or support of suppressor T cell function. The drug appears to specifically reduce the expansion of the T helper clones without affecting the suppressor precursor, thus permitting the generation of antigen-specific and antigen-nonspecific suppressor cells. There are certain immunologic dos and don'ts of cyclosporin A. The drug does reduce monocyte production of Interleukin I and abrogates activated T lymphocyte production of Interleukin II which aborts the differentiation of T cytotoxic precursor cells into mature cytotoxic lymphocytes. The drug does not prevent the cytotoxic cell from recognizing Interleukin II which has been previously synthetized or added exogenously nor does the drug reduce CTL function once generated.

Some initial variations in the precise protocol in which cyclosporin A has been employed has led to confusion about the role of cyclosporin A in inducing the improved graft survival that has recently been experienced by all good centers. It rapidly became clear to Calne and colleagues that short courses of cyclosporin A alone were not going to be the magic bullet for transplant survival. Thereafter three distinct protocols for the use of cyclosporin A have been tested with varying results. Merion et al writing for

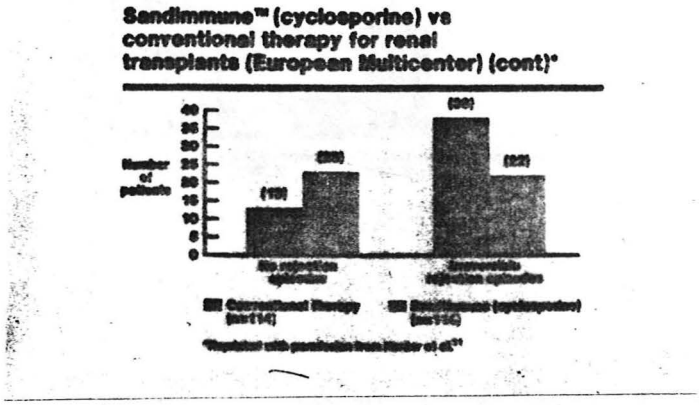


Figure 26

the Calne group reported their five years of experience with cyclosporin A used as the sole immunosuppressant. Used this way the actuarial graft survival at four years was 70% in the cyclosporin group and 62% in the conventional azathioprine/prednisone group, a difference which was not statistically significant although quite suggestive. The Canadian multicenter transplant study group reported their results in the New England Journal of Medicine using cyclosporin A in combination with every-other-day, low dose steroids. Data was not available for a full year of experience and predicted allograft survival was 80% for the cyclosporin receivers versus 64% using conventional drugs. Later, this same group reported their full year experience and the conventional group fared better. Indeed, there seemed to be no statistical difference between conventional drugs and cyclosporin A in most of the centers with a large difference at the Toronto center accounting for the trend that was reported. In contrast to these less than spectacular reports has been the world-wide experience reported in a multiplicity of abstracts and smaller papers concerning the use of cyclosporin A and daily low dose of steroids. Here, there seems to be absolutely no doubt that cyclosporin A confers a statistical advantage over the conventional immunosuppressive regimen. With more than 4 years of experience at hand the University of Texas Health Science Center at Houston program achieves an 83% graft survival in cyclosporin A and prednisone treated individuals as opposed to a 55% graft survival using the conventional immunosuppression regimen. It

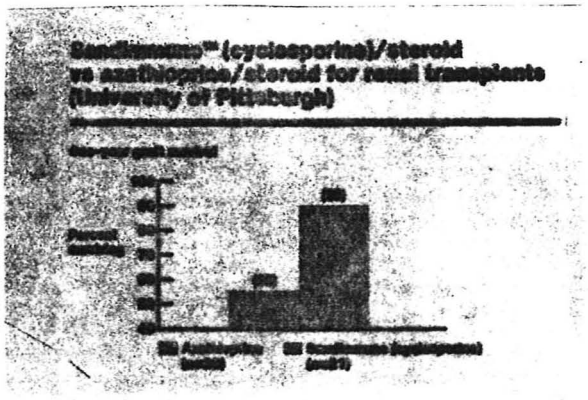


Figure 27

is this combined protocol which has been the most effective means of using cyclosporin A and has been that adopted by most programs, including our own. More recently, a triple therapy protocol has been tested using lower doses of cyclosporin A in combination with lower dose steroids and low dose azathioprine. There is no published experience with this latter protocol and time will tell if its goal of reducing the side effects of cyclosporin A without abrogating the important benefit of cyclosporin A will be forthcoming.

Despite the public relations hoopla concerning cyclosporin A, it has become clear that this extremely potent new immunosuppressive agent is not going to be a panacea. The agent has very real problems which make it difficult to use and potentially hazardous. Table 6 details a list of the important drug use complications of cyclosporin A. As a potent immunosuppressive agent the drug can be associated with infectious complications especially those produced by opportunistic organisms and viruses. On the other hand, cyclosporin A does not have bone marrow toxicity like azathioprine or cyclophosphamide and thus the importance of leukopenia in increasing the infection rate and complicating the management of an established infection is not present. A dose-related mild hepatotoxicity, characterized by real but minimal elevations in hepatocellular enzymes, is common but rarely leads to pathology and almost always rapidly responds to dosage adjustments. Other problems are being reported the relationship of which to cyclosporin A usage is as yet not completely clear. Significant hypertension in the absence of high dosage steroid use, renal failure, or transplant artery stenosis has been reported and appear to be resistant to management with a conventional moderate regimen of antihypertensive drugs. Some feel that best therapy for hypertension associated with cyclosporin A may be calcium channel blockers. An accumulation of isolated reports of unexplained hyperkalemia in the absence

Table 6

Principal adverse reactions

Reaction	Kidney (n=705) %	Heart (n=112) %	Liver (n=75) %
Hirsutism	21	28	45
Tremor	21	31	55
Hypertension	13	53	27
Nausea/vomiting	4	10	4
Gum hyperplasia	9	5	16
Mild and overt nephrotoxicity	25	38	37
Hepatotoxicity	4	7	4
Lymphoma	1	6	1

of renal insufficiency requires extensive evaluation. Hypertrichosis is rare and of modest magnitude and should not constitute an important enough problem to preclude its use. Initial reports of increased neoplastic events, particularly those related to lymphomas, were indeed quite worrisome, but as more extensive experience with cyclosporin A has accumulated, the neoplastic rate, although increased especially for those of formed elements of the blood, apparently will not be greater than that for other immunosuppressive drugs used for prevention of transplant rejection such as azathioprine.

The most important and vexing complication of the use of cyclosporin A to prevent rejection in organ transplants in general and in renal transplants in particular is the high propensity for the drug to be nephrotoxic. It is almost a paradox that the very agent used to maintain renal transplant function also causes renal transplant damage. When cyclosporin A is used to prevent rejection in heart or liver transplants, dosage schedules have called for higher drug levels than has generally been used for renal transplant. In those settings, important and severe nephrotoxicity has been experienced to the point that some fear that use of cyclosporin A will be sharply truncated. The Stanford cardiac transplant group reported recently in the New England Journal of Medicine that three patients of 22 with normal renal function actually required hemodialysis for cyclosporin A-induced nephrotoxicity.

For renal transplantation the use of cyclosporin A has a special set of problems in that altered transplant function may have a multiplicity of causes

which are difficult to discern on the background of cyclosporin A. In sharpest terms, it has been difficult to date to often discern the difference between subtle allograft rejection and cyclosporin A nephrotoxicity. This problem is worsened by the fact that cyclosporin A as a potent immunosuppressive agent has altered the clinical signs of rejection, which will be discussed in the next major section of these rounds, often making that diagnosis subtle even when drug nephrotoxicity is not present. To date, no set of symptoms, physical examination signs, or laboratory tests have been able to be used together to make the appropriate diagnosis. Although assiduously searched for, aspects of immune surveillance, needle biopsies of the renal transplant, nuclide scan tests, all which have been claimed by one or another group to be helpful, have not worked out as effective means of making this differential diagnosis. At present, most transplant groups using cyclosporin A proceed by feel.

Because most instances of cyclosporin A nephrotoxicity responds to dosage adjustment, one would have assumed a priori that careful assessment of drug blood levels would be helpful in distinguishing circumstances ripe for nephrotoxicity and instances of that clinical entity. Unfortunately, neither the radioimmune assay presently available, which uses an antibody which recognizes inactive metabolites as well as parent drug, or the high pressure liquid chromatography method of measuring the drug has provided any more than broad outlines as to therapeutic approaches. Moreover, what has been a therapeutic dose early in transplant may become a toxic dose later. Part of the problem is explained by the pharmacology of the drug. It is highly lipid soluble so that the tissue levels of the drug may not be reflected by the blood levels. Additionally, this orally administered drug has an important entero-hepatic circulation utilizing the P450 enzyme system of the liver for its metabolism. Biliary and hepatic disease strongly alter dosing schedules. Moreover, important drug interactions exist when one employs other agents which also utilize the P450 system. Lastly, there seems to be an interesting enhancement of GI mucosal absorption of the drug over time so that the one-third absorption rate present early after the initiation of therapy may climb to as high as 50% of the orally administered dose.

The drug complications and the difficulty of use has generated a tremendous energy among transplant immunologists and clinicians to learn by protocol study the most efficacious way of employing cyclosporin A in transplantation. My prediction is that such an explosion of investigation which has led to international meetings to report experiences with cyclosporin A alone will continue through the next ten years because of the very real advantages that cyclosporin A offers for effective transplantation. It is indeed an exceedingly potent immunosuppressive. Clinical trials already discussed have demonstrated even in the infancy of our understanding of how most appropriately to use the drug that results in cadaver transplant patients have improved to the 70-80% graft survival range. Infectious complications, although real, are much lower in rate than with the use of azathioprine or cyclophosphamide most probably because of the lack of bone marrow toxicity. Because only low dose steroids are employed, total steroid usage by transplant recipients may be importantly reduced lessening the potential side effects of that drug. Lastly, it appears that cyclosporin A can overcome some of the important matching features that were discussed under the DR section thus allowing many more individuals to be transplanted with equally good results than if one had searched for precise tissue matched donor-recipient pairs. It

has been the use of this new drug among other changes which has allowed the transplant world to enter a third optimistic era of clinical results which I believe will continue until more precise immunologic means of preventing rejection are discovered in the laboratory and tested in the clinic.

Total Lymphoid Irradiation

One of the dreams of transplant immunologists has been to develop a precise immunologic intervention which would allow a host to accept an allograft while maintaining the integrity of the immune sytem for all other antigens. This was the impetus that has led to the important basic work of Sir Peter Medawar, Rupert Billingham, Sir Leslie Brent, J. Wayne Streilein, Ron Guttmann, and C. Bernard Carpenter.

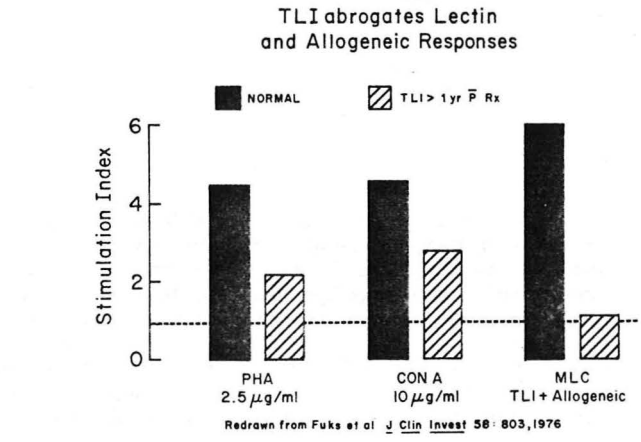


Figure 28

Recently, the Stanford chemotherapy group has demonstrated that total lymphoid irradiation (TLI), a radiation method used to treat patients with Hodgkin's lymphoma, induces a window of time during which one may be tolerant of new alloantigen. Slavin et al in a rodent model have demonstrated that bone marrow transfusion during this unique time frame induced by TLI has led to total chimeric reconstitution. These total chimeras will then accept allografts of organs bearing antigens relavant to the chimeric state. In the murine model, expected graft-versus-host reactions did not occur, a phenomenon ascribed to the induction of splenic and nonsplenic suppressor T lymphocytes. Unfortunately, an attempt to adapt the TLI plus bone marrow transplant for vascularized organ engraftment to larger mammals has been met an increased incidence of graft-versus-host reactions. Many further questions regarding ultimate neoplastic potential and the possibility of even inducing chimerism in humans remain before TLI can be applied in this fashion.

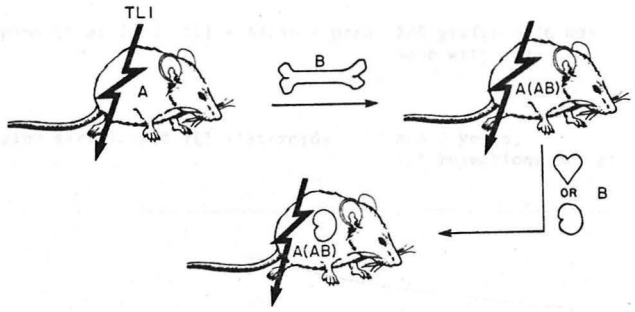


Figure 29

Because of the GVH potential and because of the original Slavin finding that only after chimerism has been induced can one see a benefit for other transplants, research in TLI has been confined to a few centers in Israel and California. Samson et al have employed TLI without bone marrow transfusion as long lasting immunosuppression in cadaver transplants. One hundred rads per treatment three treatments per week for six weeks for a total dose of 1800 rads were used with little side effects. At the completion of the radiation period an unmatched allograft was sought. In those individuals who received a short course of 6 daily doses of ATG and low dose (10-15 mg/day) prednisone, 8

Host Versus Graft Graft Versus Host

Figure 30

Table 7

CLINICAL TRIALS OF TLI FOR PREVENTION OF TRANSPLANT REJECTION

Sampson et al 1984	TLI + ATGx6 + pred	8/8 grafts 3-16 mos none with rej
Belgium series 1981	TLI + steroids	8/8 2 years; 1.5 rejections per pt

of 8 allografts survived for more than 16 months with no rejection episodes. Two of the individuals have been able to stop all immunosuppression without effects on their allografts. An earlier series from Waer Leuvene Belgium using a similar protocol was not so spectacular with 8 of 8 patients keeping their grafts but experiencing 1½ rejections on the average per patient. In the Belgium group, immune surveillance of the TLI treated individuals demonstrated that people treated with this modality for allograft placement have similar findings as do individuals treated for the lymphomas with diminished lectin response, mixed lymphocyte reactivity, and expansion of the T suppressor pedigree of lymphocytes. These promising reports beg for further study and expanded clinical trials.

DIAGNOSIS OF REJECTION

It makes clinical sense that success in reversing acute allograft rejection may be directly related to the time at which the diagnosis is made. Although this seems intuitively obvious, there have been no actual clinical tests of this notion. On the other hand, it has been the goal of transplant clinicians to devise evermore precise means of discerning rejection at its inception in order to intervene with least organ damage and the highest possibility of reversal. When clinical rejection becomes apparent patients have fever, complain of myalgias and arthralgias, have pain in the allograft site. They may become hypertensive, have diminished urine flow, have diminished glomerular filtration rate and raised serum creatinine, demonstrate increased protein excretion rate, and develop an active urinary sediment. When all of these signs and symptoms are present, tissue biopsy reveal the immune assault on the organ. Treatment at this juncture is most often successful in reversing the event especially if the patient is experiencing the first rejection. As will be discussed in more detail in the next section, conventional high dose steroids are successful in two-thirds of the cases at this juncture in allograft rejection reversal and treatments directed against T lymphocytes may be successful in as many as 90% of cases. Despite this high rate of success, it has been assumed that earlier detection of rejection prior to the time that the organ is grossly inflamed may lead to abortion of the event with less aggressive therapeutic intervention. This goal has been the impetus for major research efforts in the field of immune surveillance of

transplant function. I would like to review at these grand rounds two promising means of detection of allograft rejection that we have studied here at Parkland Memorial Hospital and one additional means which I feel will make important contributions toward accomplishing our stated goals.

Analysis of Beta-2 Microglobulin

Beta 2 microglobulin is a low molecular weight (11,800 daltons) globular protein detected on the cell membrane of all nuclated cells, including lymphocytes, found associated with the glycoproteins of the major histocompatibility complex. With a small Stokes radius, 16 A, and a seiving coefficient of 0.7 to 1.0, beta 2 microglobulins diffuses freely between intra and extravascular space and is freely filtered through the glomerulus, then reabsorbed (99.9%) and degraded by the proximal tubular cells. The kidney, the major site of the catabolism of beta 2 microglobulin can extract and metabolize beta 2 microglobulin even in the absence of glomerular filtration. Under normal circumstances, cellular synthesis, release, and catabolism of this protein varies little in an individual. Although certain inflammatory immunologic and neoplastic disorders can produce elevated serum levels in patients with normal renal function, the usual cause of an elevation in serum beta 2 microglobulin is renal insufficiency. Because of these properties beta 2 microglobulin is an excellent indicator of glomerular filtration rate. Moreover, in patients with recent kidney allografts who pass scanty amounts of urine, continued clearance of beta 2 microglobulin from the plasma may be taken as evidence of graft viability. These properties permit an analysis of the utility of serum beta 2 microglobulin measurements as a non-invasive laboratory assessment of renal transplant status. At present, serum creatinine is the standard non-invasive test available to evaluate renal function. However, creatinine levels can often lag behind the histologic changes observed in the rejection process and are of less use during hemodialysis or in the presence of post- surgical acute renal failure.

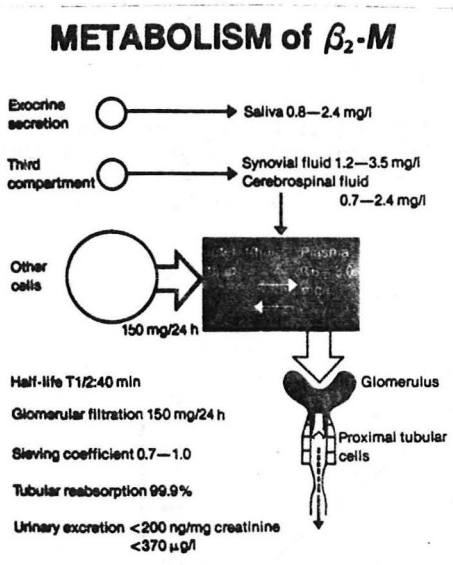


Figure 31

We studied 90 cadaver and 3 living related donor transplant recipients transplanted at Parkland Memorial Hospital, by the Southwestern Medical School team from 1980 through 1982. Material for study was available on 90

rejections and 30 periods of postoperative acute renal failure. Seventy-two patients had the clinical triad of graft swelling, graft tenderness, and fever at the time renal function declined. In all 90 instances of rejection the phenomenon was confirmed by arteriography, renal biopsy, or nuclide scanning (more of which will be discussed below). Our data allowed us to develop quantitative relationships of beta 2 microglobulin serum as listed in Table 8

Table 8

Quantitative relationships of beta$_2$-microglobulin in serum used to monitor transplant patients

Normal range	1.1 to 2.4 mg/liter
Mean daily variation in seven stable patients	0.06 mg/liter
Largest single change in stable patients	≤0.2 mg/liter
Pretransplant uremic range	20.4 to 48.4 mg/liter (34 ± 14.1)
Elevation when rejection diagnosed	6.13 mg/liter (range, 0.4 to 33.6)

with a normal range of 1.1 to 2.4 mg/L and a mean daily variation in 7 stable patients of 0.06 mg/L. The largest single change in stable patients was ≦0.2 mg/L. The mean elevation when diagnosis of rejection was made was 6.13 mg/L with a range of 0.4 to 33.6. Our data allowed us to develop a relationship

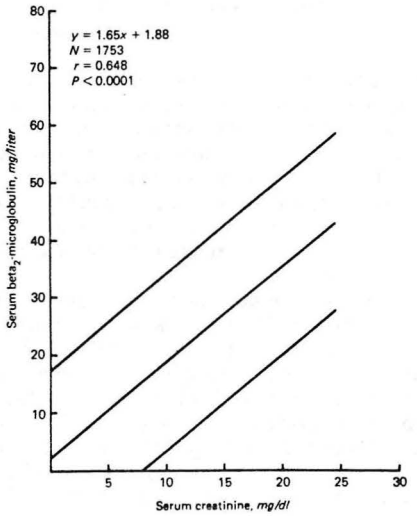
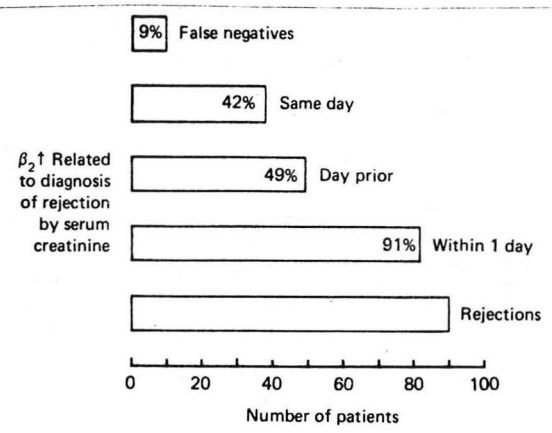


Figure 32

The relationship between serum creatinine and serum beta$_2$-microglobulin. The *regression line* is drawn as the best fit through 1,753 sets of data with high confidence ($P < 0.0001$). The *upper* and *lower* boundaries represent the 95% confidence limits of this relationship.

between the serum creatinine and the serum beta 2 microglobulin which was highly statistically significant indicating that for many patients the beta 2 measurement was an index of the GFR. Figure 32 reveals the utility of the assay to predict rejection prior to the elevation of serum creatinine. In 42% of the rejection episodes the beta 2 test became positive for rejection on the



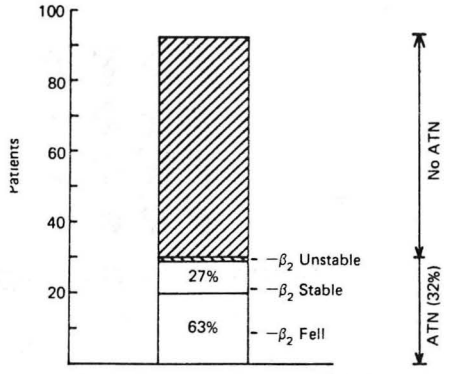
Assessment of rejection by serum beta$_2$-microglobulin analysis in relation to a diagnosis by serum creatinine.

Figure 33

same day the serum creatinine was elevated enough to make a diagnosis. In 49% the beta 2 test was diagnostic a day prior to the elevation in serum creatinine so that in 91% of the cases the beta 2 measurement was abnormal within one day of the time the rejection diagnosis may have been made from other signs or symptoms.

Equally interesting was the ability of the beta 2 microglobulin to discern post surgical renal failure. We experienced a 32% rate of ATN (30 of 93) which is slightly below the nationally reported value. In 19 patients (63%) serum beta-2 microglobulin continued to fall prior to the fall in serum creatinine indicating tissue viability and supporting the clinical suspicion of acute renal failure. In 27% of additional cases the beta 2 was stable. In less than 10% of the cases was beta 2 unhelpful in discerning the diagnosis of acute postsurgical renal failure.

Our study concluded that serum levels of beta 2 microglobulin reveal a highly significant correlation with serum creatinine and documents that a ≥0.4 mg/L increase in serum beta 2 microglobulin is both significant and sensitive in monitoring renal transplant function for the appearance of rejection. Daily measurement of serum beta 2 microglobulin is felt by our program to be a very effective technique in corroborating the diagnosis of transplant rejection and in supporting the diagnosis of potentially reversible post-transplant acute renal failure. This means of assessing the transplant is supportive only and to date has not been able to be of enough use to predict rejection many days before clinically apparent so that further means of



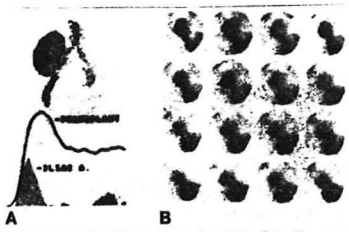
Serum β_2-microglobulin levels in patients with postsurgical acute renal failure. This complication was experienced by 32% (30 out of 93) of our study group. In 19 patients (63%) sβ_2M continued to fall prior to sCr indicating tissue viability and supporting the clinical suspicion of acute renal failure.

Figure 34

detecting immune activity against an allograft in the very early phase will have to be sought.

Nuclide Scanning

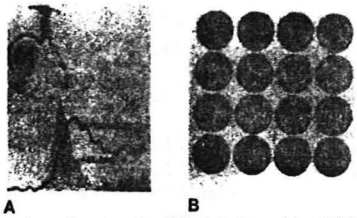
In addition to immune surveillance and analyses of molecules which are cleared by the kidney, such as creatinine and beta 2 microglobulin, various radiographic techniques to make the diagnosis of or confirm the diagnosis of rejection have been available. Since no single clinical finding or laboratory test has been pathognomonic for rejection to date, additional supportive studies would be helpful in allowing the clinician to choose the appropriate therapeutic course. The accuracy of radionuclide renal scans in distinguishing rejection from acute tubular necrosis or other problems of the allograft has been controversial. Although this technique cannot be assumed to be used to diagnose immunologic activity against the graft in a very early period prior to intragraft pathology, since it requires important perfusion changes for analysis, it certainly can be adjunctive and helpful in allowing the clinician to make the right diagnosis. The Parkland Memorial Hospital transplant program thus conducted a single blind study on the sensitivity and positive predictive value of computer generated, serial ^{99m}Technetium-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) renal scans for diagnosis of renal allograft dysfunction. We studied 28 consecutive transplants in December of 1981 through July of 1982 in a prospective manner. In order to reduce bias no patient was diagnosed as having rejection based on any of the scanning results. Rather rejection was diagnosed clinically by allograft tenderness, increase in creatinine, decrease in urine flow, fever, proteinuria, or decrease in glomerular filtration rate. Rejection episodes occurred in 25 of the 28 patients. Half of the patients receiving a living



A B

FIG. 2. Normal renal scan on day 1 in living related donor renal transplant recipient E. A. with serum creatinine of 0.7 mg./dl. and ^{131}I-iothalamate clearance of 60.5 ml. per minute. *A*, blood flow curves show excellent allograft perfusion. *B*, during excretory phase of study there is prompt visualization of renal calices, pelvis and ureter, and early drainage into bladder.

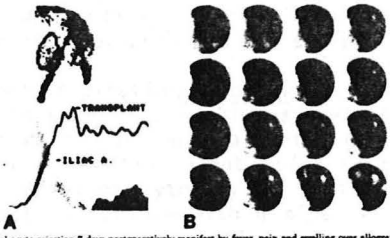
Figure 35. Normal



A B

FIG. 3. Baseline renal scan in cadaver renal transplant recipient K. H. with oliguric acute tubular necrosis and serum creatinine of 3.5 mg./dl. *A*, blood flow curves reveal only minor impairment of allograft perfusion (interval to peak activity and washout parameter are prolonged but general shape of curve is preserved). *B*, excretion is decreased markedly. These findings are typical of acute tubular necrosis.

Figure 36. ATN



A B

FIG. 6. J. T. had clinical acute rejection 7 days postoperatively manifest by fever, pain and swelling over allograft, decreased urine output, hypertension and proteinuria. *A*, allograft blood flow curve has deteriorated (interval to peak activity and washout parameter prolonged). *B*, excretion, which was poor initially owing to acute tubular necrosis, predictably remains low.

Figure 37. Rejection

related transplant escaped rejection. Baseline renal scan was obtained within the first 24 hours following the procedure. Thereafter scans were obtained in a serial manner every 3 to 4 days for the initial 3 week hospitalization in all patients retaining an allograft and upon hospitalization for allograft dysfunction. We performed 111 renal scans in 28 patients with a mean number of scans per patient of 4 with a range of 2 to 7. Time activity curves for the allograft and the ipsilateral iliac artery were generated by a computer after identification of appropriate areas of interest and subtraction of background activity.

We were able to characterize a number of discrete patterns of flow and excretion. In those patients with prompt and immediate function with no evidence of acute tubular necrosis and an excellent glomerular filtration rate, initial blood flow scans showed what we called a normal renal blood flow pattern with a rapid interval to peak activity, good washout parameters, and a short intrarenal transit time. In terms of the visual display we could see rapid appearance of radionuclide in the renal collecting system in bladder. In contrast, patients with acute tubular necrosis had only minor alterations in allograft blood flow but

had a 4-5 second delay between allograft to iliac peak activities and slight prolongation of intrarenal transit time in washout of the nuclide. Serial scans during the next 1-2 weeks in individuals with ATN showed resolution of these findings and normalization of the blood flow curve and improvement in the excretion patterns. The classic findings in acute rejection was marked deterioration in renal perfusion compared to a baseline scan even in the presence of underlying acute tubular necrosis. Prolongation of peak activity time, intrarenal transit time, and washout parameters were obvious in the majority of cases. Successful treatment of the rejection episode can result in the return of the flow curve to normal with normal excretion. Of the 52 scans performed in the instances in which clinical rejection was felt to be present, 47 of the routine studies were interpreted as positive for the clinical entitity for sensitivity of 90.4%. Five scans were interpreted as showing no change in renal perfusion from the baseline. Of the five false negative scans three were done less than 24 hours after clinical rejection and

Transplant Monitoring by Nuclide Scan

In 52 cases of clinical rejection, scans + in 47

Sensitivity = 90.4%

In 53 scans rejection was diagnosed, 47 instances of clinical rejection felt present

Positive predictive values 88.7%

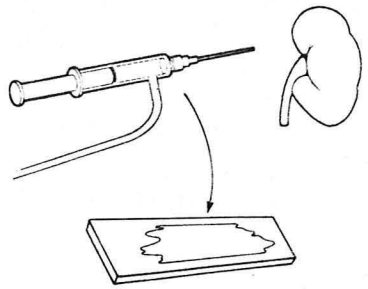
5 false negatives: all became positive by next scan
6 false negatives: all developed clinical rejection within 24 hours.

repeat scans later revealed the rejection. Clinical rejection was present during 47 of the 53 scans interpreted as showing rejection (a positive predictive value of 88.7%) and the remaining 6 cases with initial false positive scans clinical rejection rejection developed within less than 24 to 72 hours. Thus, the scan may be predictive before other clinical signs are available. Our team concluded that serial DCDTP renal scans are useful in differential diagnosis of renal allograft dysfunction. Semiquantitative analysis of sequential computer generated flow curves compared to the baseline scan provides a sensitive and specific indication of acute rejection. This test is particularly helpful in detecting rejection superimposed on oliguric acute tubular necrosis and can sharpen diagnostic acumen.

Fine Needle Aspiration Cytology

It is with fine needle aspiration cytology of the renal transplant that the best chance for accomplishing the goal of discerning immunologic activity against a renal transplant prior to extensive damage may be realized. Fine needle aspiration cytology is performed using a 25 gauge hypodermic needle attached to a syringe with a side arm which permits gentle negative pressure to be achieved. Using this device one can daily obtain safely, without any more than local xylocaine anesthesia, specimens of the inflammatory infiltrate

FINE NEEDLE ASPIRATION CYTOLOGY



	Rejection	ATN	CYA
Lymphoblasts	+ +	–	±
Monocytes	+	–	–
Tubular cells	+	+	+
Tubular cells with granules	–	–	+

Figure 38

from the very transplant itself. Ten to 20 microliters of aspirate are processed onto microscope slides using a cytocentrifuge and stained with the May-Grunewald-Giemsa (MGG) stain. Such a stain allows one to discern the presence of tubular components, to some small extent vascular endothelial components, but in great deal the inflammatory elements that comprise the allograft infiltrate. In order to deal with the variable contamination in the fine needle aspirate by blood obtained during the procedure, one must use differential counts of white blood cells obtained simultaneously from a finger stick. In more recent times, it has been shown that one can apply fluorescent monoclonal antibody techniques to evaluate the cytologic specimens obtained by the fine needle aspirate process.

This cytologic approach allows one to monitor the onset type, size, and duration of inflammatory episodes of rejection as recently reviewed by Häyry and Von Willebrand in transplantation. Biopsies which are described as "good", those with at least 7 tissue cells per biopsy specimen, were shown to have high reproducibility by a double aspiration technique of different poles of the kidney. Moreover, Häyry and Von Willebrand show that these "good biopsies" are highly correlated with the more classic needle biopsies examined by light microscopy. Those who have mastered the technique of inflammatory cell identification have developed a tool which allows them to know fairly precisely what is occurring at the allograft. For example, a sudden catastrophe that occurs to a working allograft may be the result of arterial thrombosis in which the parenchymal cells die prior to the institution of inflammation. Fine needle aspiration cytology reveals normal cellular elements in the face of absolutely no renal function. In acute tubular necrosis one can discern graft tubular cells which appear to be swollen and

pyknotic. The inflammatory infiltrate that one can identify are lymphoblasts, plasmablasts, activated lymphocytes, infiltrating natural killer cells, small lymphocytes, early monoblasts, monocytes, and tissue macrophages. Prior to the rejection episode one does not see the entrance into the graft of appreciable numbers of lymphoblasts. Very precise patterns of lymphoblast analysis by markers may allow investigators to actually predict the nature of background immunosuppressive medications that have been chosen. For the purposes of this review, it is possible to diagnose rejection in the allograft by the increased number of lymphoblasts that are found in the tissue even prior to clinical alterations of renal function. Rejections which will ultimately be unresponsive to present antirejection strategies can often be predicted by the presence of mononuclear cells in high numbers in the biopsy. Thus, the fine needle aspiration technique can define and predict not only the presence of rejection but also the potential and ongoing response to anti-rejection therapy. Lastly, Drs. Häyry and Von Willebrand claim that they have been able to discern unique changes in the tubular cells in the aspirate in patients with cyclosporin A toxicity. More research will be necessary to confirm this very important finding. In addition to the fact that fine needle technique can be used to monitor clinical transplant events, it is an important nontraumatic approach to experimental research in human transplantation, especially as a quantitative tool in randomized clinical trials and as a means to understand the immunologic nature of rejection itself. Early in the coming spring of this year the Parkland transplant team will be learning the fine needle aspiration technique from Dr. Häyry in Helsinki and hope to have it instituted as a clinical and investigational tool in our program within the year.

THE TREATMENT OF REJECTION

In these rounds it is useful to review in detail the Parkland Memorial Hospital clinical protocols that we employ to prevent and to treat transplant rejection. The first portion of this section of these rounds therefore will be a detailed description of our protocols; the remaining sections will deal with experimental approaches to the treatment of rejection that we have studied in our transplant program.

As the University of Texas Health Science Center at Dallas-Parkland Memorial Hospital Transplant Program celebrates its twentieth anniversary of renal transplantation, it shares in the renewed optimism of this modality of therapy for the end-stage renal failure patient. Renal transplantation has entered a new era of clinical success with substantially reduced risk for an ever increasing number of potential beneficiaries. An important contributor to the heightened optimism concerning renal transplantation has been the development of new forms of patient preparation and treatment which has permitted an individualized approach to patients which will be the subject of this review. The overriding philosophy of therapy in our center is to employ a precisely tailored regimen based on our present understanding of tissue matching and effector mechanisms of rejection which is designed to maximize the chances of allograft survival while minimizing potential morbidity or mortality. An important corollary to this precept is the notion that heroic therapeutic attempts to maintain renal allografts are abandoned to reduce the potential of over-immunosuppression. Such an approach might potentially lead to the loss of an allograft which in earlier periods might have been saved but at substantial risk of losing the patient to the complications of therapy.

Following this approach, in fact, cadaver allograft survival at our program in this new optimistic period for renal transplantation is about 70% at one year with patient mortality substantially below 4% per annum, a risk smaller than the reported risk for comparable patients managed by dialytic therapy. Success in the living related arena has been even more spectacular following these precepts with allograft one-year actuarial graft survival for two haplotype matched individuals at 100% for the last several years and survival for 1 haplotype matched individuals following our protocols to be described in this review at 96% with virtually no mortality in the last several years. In structuring this part of the review I would like to consider three topics individually: 1) preparation of potential recipients prior to transplant to maximize allograft survival; 2) our protocols designed at prevention of rejection (background immunosuppression); and 3) our protocols designed to treat allograft rejection episodes.

PRE-TRANSPLANT PREPARATION

In support of our general philosophy that practice should be individualized for each recipient is our approach to the pre-transplant preparation of the one haplotype living related subject. For this category of patients a more precise blood transfusion protocol has been adopted in which donor blood is used and administered in a carefully pre-set time pattern. Following the pioneering observations of the University of California at San Francisco transplant program we have chosen to administer one unit of whole donor blood (which has not been stored) every three weeks for a total of three infusions. Since our protocol has been adopted allograft survival in the one haplotype recipient has improved from 68% actuarial one year survival to 96%, a magnificent improvement which has argued strongly for such preparation of patients with this category of match. Our program, as many other programs using a similar protocol, has found that we sensitize about 30% of potential recipients against their specific donor, a rate which has led some to argue against this form of patient preparation. Several protocols have been designed and are being tested to prepare one haplotype living related recipients with donor blood. All these protocols are intended to reduce the rate of recipient sensitization without loss of the transfusion effect. Our own program has adopted the protocol of using the immunosuppressive medication azathioprine, which will be discussed in greater detail in later sections, at a dose of 1 mg/kg (total dose to be adjusted by peripheral white blood cell count) to potentially reduce the rate of sensitization of the recipient receiving donor specific blood in the temporal sequence already described. Since adoption of such a protocol we have reduced the rate of sensitization in our own program toward the 10% rate reported by others for donor specific blood transfusion under azathioprine umbrella without depreciating graft survival rates.

THERAPIES DESIGNED TO PREVENT ALLOGRAFT REJECTION

I have envisioned the history of renal transplantation as one of three distinct eras. In the first era, the era of experimentation, a wide range of therapeutic modalities were studied and accepted or discarded. As surgical and medical practice were sharpened, transplant results improved and a conventional approach to renal transplantation was developed. This conventional approach was uniformly applied in the second era of transplantation in the 70s and early 80s in which renal transplantation, no

longer being a completely experimental approach to end-stage renal failure, was more widely offered to potential recipients. During this middle period, in fact, transplant results declined while patients were managed with what has been defined as the "conventional immunosuppressive regimen". More recently, our program and others have entered a third, optimistic era in which therapeutic approaches have been individualized along the philosophical lines described in the introduction. In the use of therapeutic modalities to prevent renal allograft rejection one can see good examples of this individual approach.

Many centers, ours included, have found an important allograft survival advantage in matching completely for or avoiding complete mismatches of class II HLA antigens. In our hands complete DR matching for cadaveric transplantation alone has conferred about a 20% advantage in transfused patients over mismatched patients receiving conventional immunosuppression. Allograft survival using the conventional immunosuppressive regimen in DR matched individuals has been similar to that using newer drugs such as cyclosporin A. In my discussion of this latter agent it will be clear that the new drug presents difficulties in its use that, in our opinion, can be obviated by excellent DR matching. Our general approach is to employ the conventional immunosuppressive regimen in the two DR matched or zero DR mismatched, first allograft recipients reserving cyclosporin A for other patients. Because patients receiving their second or more transplant (having lost the first allograft to rejection) have a reduced chance of allograft acceptance whether prepared by pretransplant blood transfusions or whether receiving perfect DR match kidneys, we have abandoned conventional immunosuppressive drugs for these patients. Thus, our approach is to use the new combination of cyclosporine plus corticosteroids for all patients receiving second or more transplants, and all patients receiving poorly DR matched kidneys.

Corticosteroids

From the earliest experiences with renal transplantation, it has been clear that cytotoxic drugs alone are inadequate to prevent renal allograft rejection in the majority of the patients. Corticosteroids, drugs which commonly are used to treat rejections, confer major advantage when used in combination with cytotoxic drugs. Our approach is to use a tapering dose of prednisolone beginning at 2 mg/kg/day and tapering to 0.25 mg/kg by 2 months post transplantation to support cytotoxic drugs in prevention of allograft rejection episodes. We have chosen prednisolone, the active moiety of the synthetic corticosteroid prednisone, because this drug avoids the requirement for activation by the liver in that liver problems in the transplant recipients are so frequent. Our present understanding of the generation of activated lymphocytes directed against specific allograft target structures and of recruitment of sensitized effector lymphocytes has allowed the characterization of the mechanism by which corticosteroids may function in the prevention of rejection. In this formulation, recognition of class II disparate antigens by specific clones of lymphocytes initiates the lymphocyte activation cascade. Release of important lymphokines activates monocytes which release interleukin I, a glycopeptide which amplifies the activation of antigen-stimulated lymphocytes, permitting the lymphocyte to develop interleukin II receptors and to synthesize and release the lymphokine interleukin II for further recruitment of sensitized lymphocytes and

effectors. It is understood that corticosteroids inhibit the release of monocyte produced interleukin I which could abort the development of sensitized and activated lymphocytes and thus potentially prevent allograft rejection episodes.

Azathioprine. Azathioprine inhibits a number of enzymatic pathways in purine metabolism, leading to abortion of cellular growth in rapidly growing cells or clones of activated lymphocytes. It is used to prevent allograft rejection by destroying the clone of sensitized lymphocytes which are undergoing rapid expansion. The mechanism by which azathioprine functions allows one to predict that it is effective in inhibiting primary immune responses but would have little capacity to alter the function of committed effector cells. The pharmacobiology of the drug teaches that it is most effective when used at least three days prior to the presentation of antigen. In the circumstance of the living related donor, which can be construed as elective surgery, our practice is to begin azathioprine in a dose of 2.5 mg/kg body weight on admission which is four days prior to allograft placement. Such a luxury is not available to us in performing cadaveric renal transplantation. To overcome partly the time course problem, we administer 5 mg/kg body weight of azathiaprine on the day of surgery followed by 2.5 mg/kg/day body weight maintenance thereafter.

The major route of metabolism of azathioprine is via the liver with only a trivial amount being excreted into the urine. Because of these pharmacokinetics it has often been stated that the dose of azathioprine does not have to be adjusted for renal function. On the other hand there is excellent experimental evidence to suggest that the combination of uremia and therapeutic blood levels of azathioprine increases myelosuppression especially with respect to reduced colony forming units. Our present approach is to empirically reduce by 1/3 the azathioprine dose in the face of azotemia which has reduced greatly the number of instances in which severe leukopenia is encountered.

Two important complications of azathioprine need be discussed. As an agent which reduces purine precursors for DNA and RNA metabolism, all rapidly growing cells are susceptible to increased cell death culminating in potential myelosuppression, the most important side effect of azathioprine therapy. An advantage of azathioprine over other cytotoxic agents in this regard is the fairly wide therapeutic to risk ratio and the differential susceptibility of different subsets of myeloid elements to the drug with activated lymphocytes more susceptible than polymorphonuclear leukocytes with platelets being least susceptible. Careful attention to myelosuppression with rapid dosage adjustment can prevent an aplastic crisis. Our practice is to reduce by half the azathioprine dose in patients whose peripheral white blood cell count falls below $5000/mm^3$ and to discontinue the dosage altogether for several days when the white blood cell count falls below $3000/mm^3$. If the observed leukopenia is the result exclusively of azathioprine toxicity then cessation of the use of the drug rarely may precipitate an allograft rejection, a risk that must be accepted to reduce potentially lethal aplasia. In other instances leukopenia may be the result not only of azathioprine usage but also of infections which themselves myelosuppress such as those caused by cytomegalovirus (CMV). In our experience these infections are greatly immunosuppressive so that discontinuance of the cytotoxic drug does not offer increased risk to the allograft. A rare patient exhibits profound marrow

sensitivity to azathioprine. Some of these patients may actually exhibit an idiosyncratic reaction in that switching to a different cytotoxic agent when the marrow elements have recovered does not lead to a second episode of near lethal marrow suppression. In other patients who have marked marrow sensitivity the risk of morbid or mortal complications in the face of severe leukopenia argues for discontinuation of all cytotoxic agents even at the risk of allograft loss.

The second major complication of azathioprine is a dose-related cholestatic jaundice unrelated to hepatic infections. One must carefully discern the diagnostic difference between hepatocellular destruction accompanied by jaundice in the face of post transplant hepatitis, most often the result of infection with non A, non B virus, from the pure cholestatic drug reaction related to azathioprine. In our experience sharp reduction in azathioprine dosage most often is accompanied by amelioration and even complete resolution of the drug-related cholestatic picture. In occasional patients increased sensitivity to azathioprine with respect to cholestasis precludes its continued use and necessitates a switch to a different cytotoxic agent. In the face of severe chronic active hepatitis, the presumed result of a viral assault on the liver, we have found that complete cessation of all immunosuppressive drugs including corticosteroids even at the risk of allograft loss stabilizes the destructive liver disease.

Cyclophosphamide

The alternative cytotoxic agent is cyclophosphamide (1 mg/kg body weight/day). This agent is an extremely effective immunosuppressive agent which by virtue of crosslinkage to the DNA skeleton leads to gene misreading and cell death of cells undergoing rapid growth such as activated lymphocytes. The drug thus attacks the immune cascade at the same point as does azathioprine. This agent is generally not the first choice of cytotoxic drug because its therapeutic to risk ratio is much narrower than azathioprine. Additionally, since cyclophosphamide is eliminated by the kidney large fluctuations in plasma level may occur. Its most important side effect is myelosuppression with all formed elements equally. Marrow recovery takes more than two-fold longer than with azathioprine. Other problems are alopecia, sterility, and hemorrhagic cystitis.

Cyclosporin A. Cyclosporin A is a cyclic undecapeptide metabolite of the fungi Cylindrocarpon lucidium and Trichoderma polysporum first isolated by Dreyfuss and colleagues and shown to be an effective immunosuppressive in vitro by Borel and associates. Although there are multiple mechanisms by which cyclosporin A may be immunosuppressive, that which is important for prevention of allograft rejection involves blockade of the release of the lymphokine interleukin II from antigen activated lymphocytes. Initial contact by susceptible clones of lymphocytes with class II HLA antigens initiates the activation process which requires substantial amplification to recruit the necessary activated lymphocytes to complete the sensitization process and initiate effector cell development. Such amplification and recruitment requires the elaboration and release of interleukin II which is specifically inhibited by cyclosporin A. Thus, one can see that cyclosporin A is effective in preventing but not treating rejection in that the agent has no effect in abbrogation of effector cells already committed against specific targets. We have chosen to use Cyclosporin A in combination with daily low dose

prednisone. One can construct an immunologic hypothesis to support the synergy between cyclosporin A and steroids in that steroids affect the release of monocyte generated interleukin I which permits antigen activation of lymphocytes, while cyclosporin inhibits the release of activated lymphocyte synthesized interleukin II, the glycopeptide which expands and recruits antigen activated sensitized lymphocyte clones. The two agents, therefore, working together attack the immune system response to antigen in two different but connected points in the recognition and response cascade.

Cyclosporin is a difficult agent to use at this time in that its most important drug related toxicity is directed at the very organ we are attempting to replace. There appears to be a dose related nephrotoxicity which even in the successful allograft event has led to lower stable glomerular filtration rates reflected in higher average serum creatinine values. Nephrotoxocity also makes the diagnosis of subtle allograft rejection very difficult indeed. Dosing schedules for cyclosporin therefore have varied widely in various clinics. We administer cyclosporin in a dose of 14 mg/kg body weight on the day of transplantation, a dose which is continued daily for the first week postoperatively. Cyclosporin then is tapered as tolerated at 2 mg/kg every 2 weeks until either 5 mg/kg per day is reached or unless an episode of nephrotoxicity is encountered. If reduced transplant function is thought to be related to cyclosporin dosage the total dose is reduced by 100 mg per day orally and halved if toxicity continues. To assist in the differential diagnosis of rejection versus drug toxicity, renal transplant biopsy is performed more often than with conventional immunosuppressive medications looking for the classic cellular hallmarks of allograft rejection. Interstitial fibrosis and even inflammation may occur with the cyclosporin toxicity so we have found it important to pay attention to the pathologic site of infiltrating inflammatory cells. Cells that are found in the perivascular region are felt to be indicative of allograft rejection; cyclosporin dosage would not be lessened and protocols to treat rejection would be employed. For the most effective use of cyclosporin A the precise pharmacokinetics of the drug will have to be learned in order to maximize immunosuppression and minimize nephrotoxicity. Toward that end serum levels of the drug have been attempted to be monitored by various techniques including a radioimmunoassay and by high pressure liquid chromatography (HPLC). Our experience to date has been that the blood levels are only guideposts that may be supportive of a clinical impression bolstered by biopsy material the latter of which should dictate practice.

Two major advantages of cyclosporin A need be discussed. The first important advantage over conventional immunosuppressive regimen is the fact that cyclosporin A is not myelosuppressive and lessens the potential for anemia, thrombocytopenia, and leukopenia with resultant infection. In addition, cyclosporin A may be steroid sparing for two reasons. Firstly, the combination therapy reduces the number of rejection episodes substantially in our clinic from about 2 out of 3 cases on conventional immunosuppression to about one out of two of the patients thus diminishing the potential for anti-rejection therapy with high dose corticosteroid medications. Secondly, in order to reduce the total immunosuppressive burden of the patient we have more rapidly tapered the corticosteroids used in the combination regimen to be at 20 mg of prednisolone per day by the end of the first week.

In addition to nephrotoxicity and the difficulty in the use of the agent several other problems have been defined. There is a mild dose related hepatotoxicity manifested mostly by slight elevations in blood levels of hepatocellular enzymes. This problem is rarely important clinically and is completely ameliorated upon dosage adjustment. A dose-related neurotoxicity which is heralded by a fine motor tremor but which can culminate in frank seizures may be encountered. Post-transplant hypertension occurs more often and is more difficult to treat. We have found that calcium channel blockade is the most effective management for the severe hypertension during cyclosporin administration. Lastly, early reports of lymphoproliferative disorders including neoplasms were worrisome. With wider use of the agent, the incidence of such neoplasms appears no greater than that experienced with azathioprine alone. The several cases of aggressive, rapidly growing lymphoproliferative neoplasms seem to be associated with excessive immunosuppression.

For some patients cyclosporin toxicity is enough of a problem to cause conversion to the conventional immunosuppressive regimen of corticosteroids and azathioprine. Because of the initial fear of the use of Cyclosporin A in combination with azathioprine, conversions were first affected by stopping Cyclosporin A abruptly and initiating the standard maintenance dose of prednisolone and azathioprine. The experience in our center and in other centers was that such abrupt conversion was a substantial risk to initiate an acute rejection episode. Our present approach is to taper cyclosporin A over a four day period while increasing azathioprine from 0.25 mg/kg to 2.0 mg/kg in that same time period.

TREATMENT OF ACUTE ALLOGRAFT REJECTION

Corticosteroids

Although corticosteroids have been important as adjunctive medications to prevent allograft rejection, they are the primary treatment of first rejections in our clinic. The precise mechanism by which the corticosteroids are effective in over 2/3 of the cases of first allograft rejections is unknown. The corticosteroids may be effective in treating rejection in that they alter lymphocyte trafficking patterns, stabilize membranes and reduce the release of phlogistic mediators which reduces the specific and nonspecific inflammatory infiltrate of the allograft, reduces edema formation in the allograft, and abbrogates the release of gamma interferon by sensitized lymphocytes, the latter mechanism contributing to the fever development of acute rejection. Our clinic's protocol for the use of high dose steroids to treat first allograft rejections employs one gram of methylprednisolone administered intravenously on the first, second, fourth, and sixth days following diagnosis of rejection. This approach to rejection is used whether patients are receiving the conventional immunosuppressive regimen of corticosteroids and azathioprine or cyclosporin A and corticosteroids for the prevention of allograft rejection. If this approach is successful in reversing allograft rejection as evidenced by a fall in the serum creatinine by at least 1/2 or an increase in the glomerular filtration rate, then the patient is allowed to continue his background immunosuppressive regime. In that setting if a second renal allograft rejection is experienced bolus intravenous methylprednisolone in a similar format may be again employed. On the other hand, if there has been no response to this initial approach to rejection by the third bolus injection we turn to use antithymocyte globulin.

For a separate group of patients in a research protocol we have attempted to reverse steroid resistant cellular allograft rejections (confirmed by tissue biopsy) by a switch from the conventional immunosuppressive regimen to cyclosporin A in a technique we have called "Cy A rescue". If the kidney biopsy reveals predominant humoral rejection in a steroid resistant patient, we have attempted to reverse allograft rejection in a research protocol fashion using plasma exchange.

Corticosteroids have a wide range of important and serious complications from which has been generated a general search for therapeutic approaches to acute allograft rejection which can be steroid sparing. Such high doses of steroids are associated with potentially psychologically crippling changes in body image and in body habitus. Cumulative doses of 1000 mg or more have been associated statistically with increased incidences of gastrointestinal bleeding which in the setting of the transplant recipient has increased mortality. Such doses of steroids are also associated with increased risk of infections particularly by viral or intracellular obligate organisms, which may constitute the chief risk of mortality for the transplant recipient. Aseptic necrosis of skeletal elements, especially the head of the femur, requiring extensive surgical repair has also been associated with steroid use. Increased patient mortality can be shown to be directly related to the number of times one has had recourse to courses of pulse steroid therapy. To reduce our patient mortality so that it has been under 5%, we have constricted sharply the use of high dose steroids making a judgment early in the course of therapy if a patient will be a potential steroid resistant rejector and moving to other modes of therapy. Additionally, we have made the judgment that no patient will receive more than 3 courses of high dose intravenous steroids for three separate rejections. Indeed, we think very carefully before administering even the third course of therapy and may use in most patients an alternative therapy.

Anti-Thymocyte Globulin (ATG)

Polyclonal antibodies directed against lymphocytes have been prepared and used in renal transplantation experimentally and clinically for many years. In animals, anti-lymphocyte serum (ALS) has been a very potent agent to prevent acute allograft rejection. In susceptible strains of certain species, ALS can induce tolerance and lead to unlimited allograft survival. Unfortunately, the experience with these forms of polyclonal antibodies to prevent rejection in man has not been equally successfully. Some of the preparations have been more successful than others in this regard but at the completion of over 18 years of clinical experience with these agents, one can conclude that it is not the universal panacea for human vascularized organ transplantation once envisioned. On the other hand, the pioneering work of Cosimi, Hardy, and Filo has demonstrated that more than 90% of all first rejections will be reversed using polyclonal antibody preparations raised against thymus (ATG). Because of expense, morbidity, and prolonged requirement for hospitalization, we have reserved ATG for patients who are experiencing a steroid resistant- or steroid requiring-rejection episode. In our hands, more than 80% of steroid resistant first rejections can be reversed by the use of the "ATG rescue" approach. Our protocol uses 15 mg/kg of horse anti-thymocyte globulin administered intravenously as a daily bolus injection for 14 days. At present, we are administering all 14 doses during hospitalization since almost all patients experience some constitutional

symptoms related to the infusion of this biologic, including fever, chills, muscle aches and pains. We avoid all patients with a history of horse protein allergy and administer a skin test prior to the first dose. Even when such precautions are taken a rare patient may experience a more significant allergic reaction including wheezing and shortness of breath. We and others have not seen frank anaphylaxis after the use of this material although the possibility of its occurrence exists. Premedication of patients with antihistamines and a slightly increased steroid dose in many cases may reduce but not completely ameliorate these constitutional symptoms. In most patients, the symptomatology recedes after several doses which can allow for more convenient outpatient dosing in the future. The effective dose of the drug is monitored by assessing the white blood cell count and differential one hour after the dose has been administered early in therapy to insure that the peripheral lymphocyte count has been reduced to zero.

Other important complications of this course of therapy must be mentioned. Because the polyclonal antibodies are raised from a pool of animals there is important lot-to-lot variation in efficacy and side effects. The antisera also contain a number of antibodies some of which are directed not only to the T-lymphocyte population but also to B-lymphocytes, macrophages, neutrophils, and even to platelets. Some lots have had the propensity to cause thrombocytopenia and/or leukopenia and must be monitored carefully. This course of therapy in combination with background immunosuppression offers increased risk for infectious complications, particularly the DNA virus family and intracellular obligates. Patients must be watched carefully for the induction of an infection so that therapy may be abandoned, the infection treated directly, and morbidity and mortality reduced. Lastly, the rate of serum sickness in treated patients is small but real and may reduce the ability to use this drug for all patients or to complete a course of medication in many patients. A protocol of high dose antihistamines administered on an every sixth hour basis together with corticosteroids is being tested in our clinic.

Cyclosporin A Rescue

This review has already discussed in great detail the use of cyclosporin A for preventing allograft rejection in patients in our clinic who receive their second or greater renal allograft or are receiving their first transplant without a good DR match. The therapeutic approach to steroid-resistant rejection in patients on conventional immunosuppression approach must be considered carefully. In our experience, a second high dose corticosteroid bolus course has salvaged a number of these kidneys but at high risk of complications. In general, we have abandoned giving second courses of high dose steroids to patients who are steroid failures and have used ATG to "rescue" these kidneys. With the advent of cyclosporin A we have recently embarked upon a protocol in which patients on conventional immunosuppressive regimen may be switched to cyclosporin A in an attempt to abort an acute rejection episode. It has already been discussed when converting a patient from cyclosporin A to conventional regimen our practice is to slowly introduce the new drug while tapering the old regimen. In going in this other direction, we more abruptly stop the conventional regimen and introduce cyclosporin A at 14 mg/kg PO immediately. Our preliminary experience with cyclosporin A rescue has been favorable.

Monoclonal Anti-T-Cell Antibody Treatment

Based on the proven success of polyclonal antibodies directed against lymphocytes in reversing acute allograft rejection, one could surmise that a more precise reagent, a mouse hybridoma-derived monoclonal antibody directed against lymphocyte surface antigens, might be equally successful while obviating the problems of administering large amounts of foreign protein. The more precise monoclonal antibody accomplishes the same goals as the polyclonal antibodies in ridding the circulation of target lymphocytes which are thought to have an important role in allograft rejection with as little as 1/100,000 of the amount of protein normally administered when the polyclonal antibodies are employed. Several monoclonal antibodies directed against different markers on mature peripheral lymphocytes have been prepared and tested in limited circumstances in the clinic. Our clinic has tested the efficacy of OKT3 which is directed against the peripheral, mature pan T-cell marker, T3. Although some concern was raised about monoclonal antibodies directed against the T3 marker which may have a mitogen potential in vitro, our laboratory has clearly shown that OKT3 does not enhance spontaneous blastogenesis of lymphocytes from patients receiving the monoclonal demonstrating the absence in vivo of mitogen activity. We have further shown that OKT3 is cytotoxic, reduces patient allospecific response and response of recipient lymphocytes to lectins. Importantly, our center in particular and an ongoing multicenter trial in general has shown that more than 95% of all first rejections that occur on the fifth or later post-operative day are reversed by such therapy. Careful analysis of the rare failures reveals predominant antibody directed rejection by biopsy, a mode of rejection which one could predict a priori would be less amenable to reversal by antibodies directed against lymphocytes. During our trial, OKT3 has been administered following an appropriate negative skin test as a 5 mg IV bolus after a patient has been evaluated and found not to be volume overloaded and under corticosteroid pretreatment. Even in the face of such pretreatment almost all patients experience fever and chills after the first one or two doses which subsides in almost all patients for the remainder of a 14 day course. Anaphylactoid reactions have been experienced in a rare patient with clinically important consequences only when patients were concomitantly volume overloaded. Anti-mouse antibodies have developed in many patients which does not seem to lessen the efficacy of the 5 mg dose in the first course of therapy. Active investigation is proceeding to determine whether second courses of these monoclonal antibodies in the face of anti-mouse antibodies are equally effective. Other mouse hybridoma-derived monoclonal antibodies directed against different surface structures have been tested in a few clinics for which advantage has been claimed. Comparison of these very preliminary results are premature. Suffice it to say that the monoclonal OKT3 reagent, which is closest to being clinically available, is efficacious and may have its own role in the transplant armamentarium.

Plasma Exchange

It must be clear by now that not all allograft rejections can be easily reversed by assaulting cellular effector mechanisms. A finite failure rate for all modalities discussed above exists. Morphologically, the bulk of these failures can be ascribed to what has been called humoral rejection in which infiltrating cellular effectors appear to play only a minor role in tissue

damage with the major role assumed by cytotoxic antibodies. It is reasonable to hypothesize that treatment techniques directed at removing the cytotoxic antibodies may be effective in reversing this less common but devastating variety of renal transplant rejection. Taking the example of the Hammersmith Postgraduate Hospital of England in the therapy of anti-glomerular basement membrane glomerulonephritis, we have tested the use of plasma exchange therapy for this category of patients. In practice patients undergoing acute allograft rejection are generally treated with a course of high dose corticosteroids. In the face of corticosteroid failure patients undergo when clinically feasible, a renal transplant biopsy. If the biopsy material reveals a severe and almost exclusive humoral rejection we attempt to reverse this rejection with plasma exchange therapy in which patients undergo five body volume exchanges daily for five to seven days under the umbrella of their background immunosuppressive regime. Unfortunately, our controlled series, and the controlled series of at least two other groups, have not demonstrated with confidence that such an approach can alter the dismal outcome for allografts undergoing this variety of rejection. It may be that at the time tissue damage becomes pathologically evident, fixation of the antigraft antibodies has already occurred. Removal of antibodies from the plasma space may be too late. It may be possible that vigorous immune monitoring techniques may allow one to characterize rejection as predominantly humoral prior to significant tissue destruction. Institution of plasma exchange at this point theoretically may be more effective.

Cyclosporin A Rescue

As discussed very briefly in the previous section of the protocols used at Parkland Memorial Hospital, we have been studying the attempt to salvage kidneys thought lost to steroid-resistant rejection by the use of cyclosporin A in a rescue mode. This experimental protocol has been adopted at Parkland

PMH-MCH Cy A RESCUE TRIAL

Patients at risk ~ 250
Steroid + ATG failures, n = 20
Cy A rescue = 14 mg/kg PO for these 20
16/20 successes:
Creatinine 1.8 mg/dl (3-12 mo)
4 nonresponders = "humoral" rejection

Memorial Hospital and at our sister program at the Methodist Central Hospital in Dallas where together we have accumulated 15 instances of such steroid-resistant rejections, 11 of which were salvaged by switching to cyclosporin at a dose that would normally be used in a first week post-transplant surgery. Six additional patients who were either antithymocyte globulin dependent or could no longer receive ATG because of important side effects were switched to cyclosporin A with good results. It is predicted that continued investigation into innovative uses for cyclosporin A may improve additionally cadaver allograft survival above the 70-80% per annum that is currently enjoyed.

PMH-MCH Cy A RESCUE TRIAL, 1983-84

Rate of reversal of rejection at PMH with:

IV bolus steroids	63%
Steroids + ATG	87%

Plasmapheresis

Earlier in these rounds I discussed the fact that using antirejection therapies directed at destroying T lymphocytes one can achieve a 90% reversal of a first renal transplant rejection. Additional reversal of rejections will occur with sequential therapy for the 10% that fail a first round. A small but real number of individuals will lose their allograft to rejection despite therapy directed against T lymphocytes. Morphologically, biopsies or nephrectomy specimens of this variety of rejection are characterized by platelet aggregates in capillary lumina, polymorpholeukocytes along vessel walls, microthrombi in vessels, and ultimately organ ischemia. Because the vessels also show the presence of immunoglobulin and complement while cellular infiltrate may be scanty or absent, pathologists have used the description of "humoral rejection" to describe this variety of immunologic rejection, an appelation which suggests that antibodies rather than cells are involved in the process. This assumption concerning the pathogenesis of this variety of rejection may be supported by the failure for therapies directed against lymphocytes to be effective in reversing these rejections. Laboratory and/or clinical evidence cannot a priori permit the determination of the predominant form of immune injury during transplant rejection, but one can clearly demonstrate that patients who have such resistant rejections generally display these pathologic hallmarks of antibody mediated tissue destruction. In these subjects, the treatment strategy which seeks to remove the offending antibodies has recently been constructed, plasmaphoresis therapy. Because of the enthusiasm met with the use of plasma exchange therapy in certain antibody mediated glomerular disorders, clinicians caring for patients exhibiting steroid and ATG resistant rejections have been tempted to unselectively try this modality to reverse the ongoing rejection process.

The premise behind the therapy is that steroid resistant rejection is mediated by circulating antibodies, the removal of which may aid in the prevention of graft failure. Isolated case reports of uncontrolled small series have been generally favorable as shown in Table 12. Cardella and colleagues have been the most enthusiastic supporters of plasma exchange in reversing rejection. Initially, seven rejection episodes in 5 patients receiving standard immunosuppressive regimens were treated for from 2 to 8 days with 3-4 liter plasma exchanges. Replacement consisted of albumin, saline, and fresh frozen plasma. Three of the five patients had a fall in serum creatinine toward pre-rejection values. Difficulties in this pilot include the failure to control the study, the variant protocol per patient, the overoptimistic reading of the data, and the failure to follow the three responders for an adequate period of time. The same group expanded the pilot which led to a more controlled series in the same group.

Table 12

PUBLISHED EXPERIENCE OF UNCONTROLLED SERIES USING PLASMAPHERESIS TO TREAT TRANSPLANT REJECTION

STUDY (REF)	REJECTION	EXCHANGE PROTOCOL	*RESPONSE	N
11	SR	3-4L, 2-8 x	3	5
12	SR	4L, 3-10 x	2	8
13	SR	2.5-3.5L 4-5d 2-3 x/wk 1-4 wk	2 full, 3 part,	8
14	1ST	4L x 6R x in 7d	1	1
15	PO	2.52 6 alt. d	0	6
	1ST		2	5

SR--Steroid resistant
1ST-1st rejection
PO--Post-operative
*Maintenance of allograft

On this background of control studies listed in Table 13, Cardella et al performed and reported a prospective controlled study in which first rejection episodes were treated with local radiation and three days of bolus steroids (n=22) or with a standard regimen followed by 5 consecutive daily four liter exchanges (n=15). If no response in terms of rejection reversal in either group was noted, patients in either group received antilymphocyte globulin for six more days. In this study, there was no statistical difference in the

Table 13

PUBLISHED EXPERIENCE OF CONTROLLED SERIES USING PLASMAPHERESIS TO TREAT TRANSPLANT REJECTION

STUDY (REF)	REJECTION	EXCHANGE PROTOCOL	*RESPONSE	N
16	1ST	bolus + 4Lx5d	no difference	15P 22C
17	1ST V	bolus, 4L day 1 2L on days 2,3,4,7,6,11,13	dismal	12P 12C

1ST-1st rejection
V--Vascular rejection
P--Plasmapheresis
C--Control
* Maintenance of allograft

PMH TRIAL OF INTENSIVE PLASMA EXCHANGE - 1981

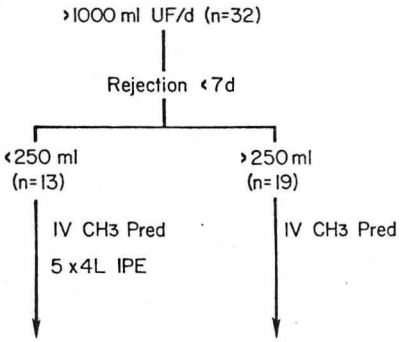


Figure 39

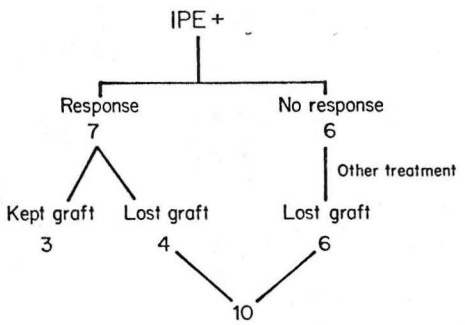


Figure 40

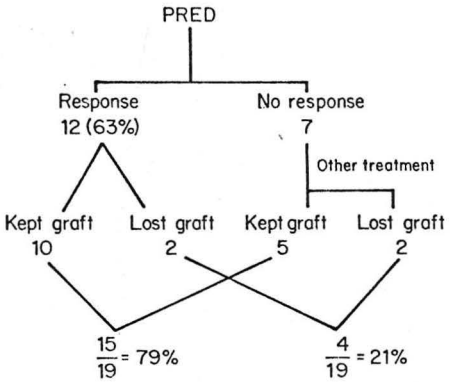


Figure 41

number of grafts lost to rejection in either group, though a trend toward a favorable outcome in the plasmapheresis treated group was found. A control study of plasma exchange in early, severe vascular rejection proven by biopsy was recently reported from Australia. Twelve randomly selected allograft rejecting recipients were conventionally treated with bolus steroids and twelve received an additional four liter exchange on the first day and two liter exchanges on days 2, 3, 4, 7, 9, 10, 11, and 13. Improvement was noted in 3 of 12 recipients in both treatment groups but 8 of 12 in each group returned to dialysis.

Our own published experience from the Parkland Memorial Hospital-Southwestern Medical School renal transplant program during an ongoing prospective trial has been equally dismal. Our protocol also sought to test plasmaphoresis in the patients with rejection that generally presages graft failure in most such recipients, oliguric rejection with "humoral" biopsies. We employed five consecutive two body volume exchanges in addition to 1 g intravenous boluses of methylprednisolone daily for four days. Initially, 8 of 9 exchanged patients regained urine flow and clearance within a week of treatment, perhaps accounting for the original optimism generated by the individual case reports. Unfortunately, by three months after treatment only 2 of 9 (25%) of treated patients retained graft function which was similar to the experience of a control on nonphoresed group. Graft loss was pathologically related to humoral rejection in each case.

Transplant rejection is a complex phenomenon, as discussed earlier in these rounds, with a variable intermix of immune effector mechanisms at work. The ideal antirejection therapy has not yet been designed. Strategies aimed at removing effector cells have only been partially successful. When these strategies fail, graft loss occurs often the result of humoral mechanisms. Plasmaphoresis theoretically should play a role in the amelioration of this form of rejection but has to date been less than successful. Published series do not permit a firm view with respect to ultimate utility of this therapy. There is no uniformity of the type of rejection studied, treatment regimen employed, the degree of control followed. As presently used one might conclude that failure of therapy as we have shown is perhaps a consequence of the employment of plasmaphoresis at a time when antirenal antibodies have already been tissue fixed and initiated injury. It is possible that for carefully characterized patients with severe antibody mediated rejection, plasma exchange can reverse a universally poor outcome if used quite early in rejection. The goal of therapy, therefore, is to diagnose the presence of humoral rejection at this early stage and then to attempt plasmaphoresis. Such a use of this modality will have to await the means to diagnose early rejection that has been previously discussed in the last section.

Monoclonal Anti-T-Cell Therapy for Rejection

The use of polyclonal anti-T-cell antibodies to prevent or treat rejection has been standard practice in many transplant centers. As discussed in the section on the Parkland Memorial Hospital treatment protols, these anti-lymphocyte or thymocyte sera have a multiplicity of side effects and complications which have narrowed their utility. They have a lot to lot variation which makes standardization of their use impossible. They have a multiplicity of specificities against which the antibody is directed which leads to pathologic alterations in other formed elements in addition to the

T-lymphocyte. This is especially troublesome when red blood cells and platelets are involved and have often precluded the continuance of an effective therapy in the middle of an acute allograft rejection. Because of the polyclonal nature of the antibody one generally must administer large amounts of immunoglobulin protein to obtain the desired destruction of the peripheral mature T-lymphocyte population. Administration of such large amounts of protein lead to immediate infusion related constitutional symptoms which are troublesome to patients and even dangerous. Fever, chills, myalgias and arthralgias, and even anaphylactoid symptoms are encountered. Furthermore, administration of this amount of protein over time leads to a substantial fraction of treated individuals experiencing the serum sickness syndrome. Based on the excellent results from the Filo Laboratory in reversal of allograft rejection in over 90% of the patients treated with polyclonal antibodies, one can make the a priori assumption that more precise reagents, monoclonal antibodies to mature peripheral lymphocytes, may be equally efficacious as their polyclonal relatives with the administration of "minute" amounts of protein obviating many of the difficulties of the use of these more nonspecific reagents. Advantage has been taken of the fact that clones of lymphocytes can be functionally identified by virtue of specific peptide structures on their membranes which have been called marker substances. One set of such markers can identify the T-lymphocyte subset with the T3 marker relevant for mature peripheral lymphocytes. A monoclonal antibody, then, directed against the T3 antigen on the lymphocyte cell surface which is cytotoxic has the capacity to destroy the so marked cells ridding the circulation of the mature peripheral lymphocyte.

The Parkland Memorial Hospital-Southwestern Medical School transplant program has been involved in a multicenter trial of one such monoclonal antibody directed against T3, PAN OKT3 antibody, to determine its clinical efficacy. The first table details the clinical characteristics of the patients entered in the randomized OKT3 trial. Notice the important features

Table 14

Clinical Characteristics of Patients Randomized to OKT3 or Steroid Treatment for Acute Renal Allograft Rejection.

	Treatment Group*	
	OKT3	Steroids
Sex (male/female)	43/20	39/21
Age (years)-median (range)	38 (17-65)	36 (16-64)
Weight (kg)-median (range)	67 (42-103)	66 (30-111)
Race (caucasian/non-caucasian)	50/13	49/11
Diabetics (number)	20	14
Number of prior transplants (0/1/2)	55/7/1	52/6/2
Number of pretransplant transfusions (0/1-4/>4)	1/12/44	3/10/40
Acute tubular necrosis and dialysis at entry	12	9
Renal disease - glomerulonephritis	23	18
Crossmatch (-/+)	63/0	59/1
Preformed antibodies (0-24 percent/26-100 percent positive)	44/10	39/18
Time from transplant to rejection (days)-median (range)	10 (6-74)	11 (6-91)

*There were no statistically significant differences between the two groups in any of these parameters.

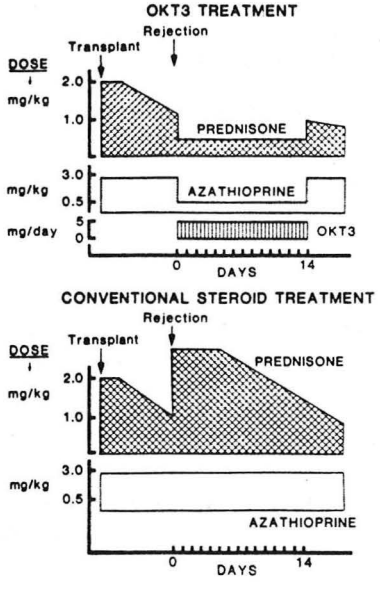


Figure 42

of the table include the fact that most of the patients were receiving their first allograft, must have been transfused and had a negative crossmatch at the time of the transplant. The treatment protocol of the OKT3 wing and the conventional steroid treatment wing are depicted in Figure 41. In the control arm, the time of diagnosis of rejection, the prednisone or prednisolone dose was raised to 3 mg/kg orally or 1 g intravenously for 4 days followed by a taper with no change in the azathioprine dose. The OKT3 arm actually reduced the background immunosuppression of prednisone and azathioprine in order to prevent oversuppression and added 14 daily doses of anti-OKT3 at 5 mg per day as an intravenous bolus injection. Parkland contributed 12 patients to the 123 patients in the multicenter trial,

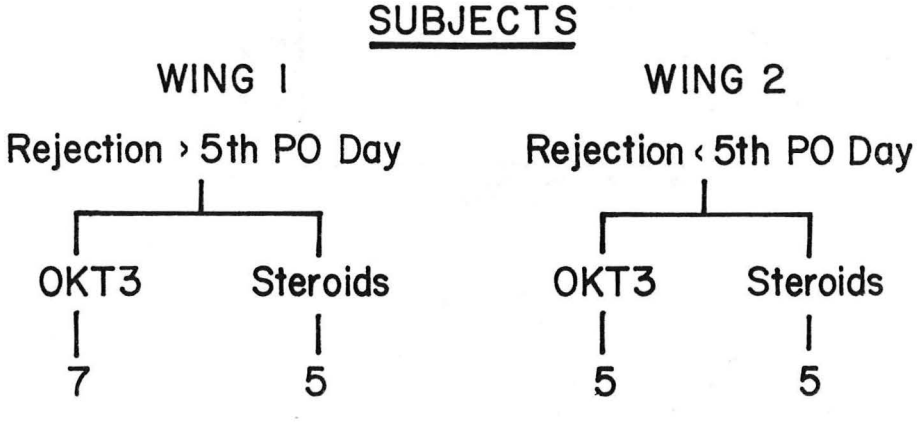


Figure 43

those patients whose rejection occurred on or after the fifth postoperative day. In addition, we also studied the efficacy of OKT3 treatment in individuals with early rejections depicted in this figure as wing 2. The local experience was similar in wing 1 to that of the general study. All seven patients receiving OKT3 experienced reversal of rejection while only 2

REVERSAL OF ACUTE REJECTION

	Wing 1		Wing 2	
	OKT3	Steroids	OKT3	Steroids
Reversal of Acute Rejection	7/7	2/5	1/5	2/5
Re-Rejection	4/7	5/5	0/1	1/2

of 5 steroid treated patients reversed their rejection. The excellent results with OKT3 in rejections that occur in the fifth or later day was not experienced in the early rejectors, all who had a dismal outcome regardless of

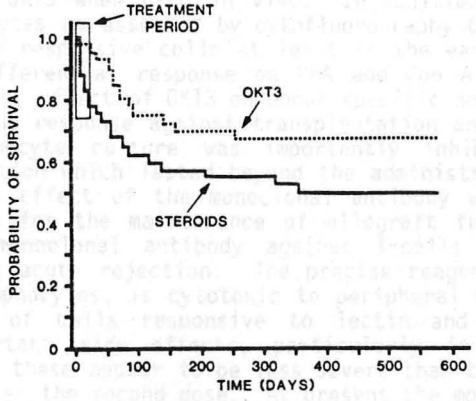


Figure 44

therapy. Graft survival in the OKT3 treated group was 71% at one year with excellent serum creatinine statistically greater than the steroid result on the local series which is nearly identical to that of the larger multicenter trial.

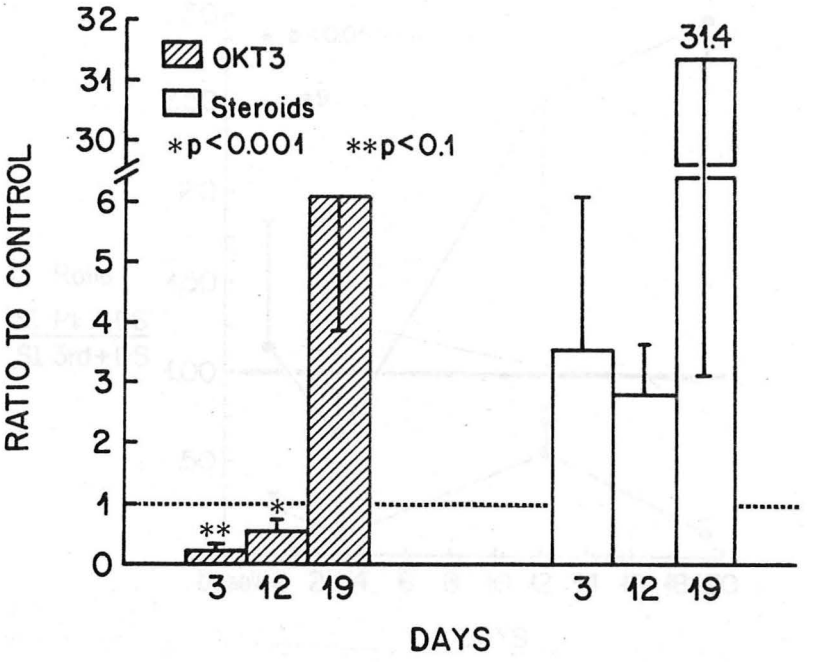
Dr. Alan Bowen in my laboratory asked several important immunologic questions of the patients treated with this particular monoclonal antibody against T cells. There is in vitro evidence that the anti-OKT3 antibody may even be mitogenic for sensitized lymphocytes rather than immunoinhibitory. Moreover, there is again in vitro evidence that attachment of the antibody to its surface antigen, rather than leading to cytotoxicity to the assaulted cells, was met with shedding of the antigen-antibody complex so that cells could not be defined by fluorescent marker studies but were still present and functionally intact. The clinical study provided source material to assess whether these real worries were present in in vivo circumstances when doses of OKT3 employed in the study group were given. Bowen asked four questions:

1. Is OKT3 mitogenic in vivo?
2. Are T-cells present and functional?
3. Is there an effect to reduce donor specific alloresponse?
4. Is there an effect to reduce nondonor specific alloresponse?

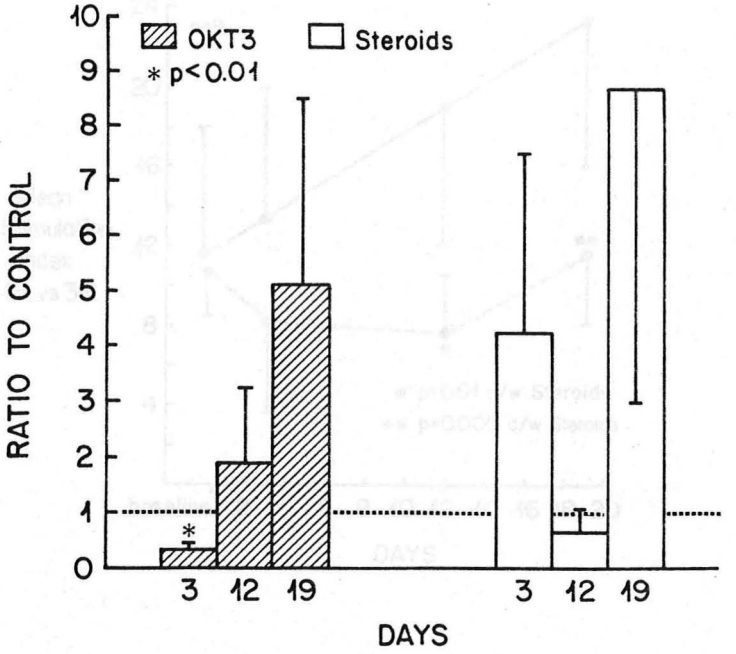
To answer these questions four different sets of immunologic studies were performed: 1) spontaneous blastogenesis, 2) responses to various lectins, 3) one-way mixed lymphocyte cultures against donor spleen, and 4) one-way mixed cultures against third party. Some of the results from Dr. Bowen's studies are presented in Figures 45-48. Taken together, Dr. Bowen demonstrated that there is no enhancement of spontaneous blastogenesis which argues against a mitogenic effect of OKT3 when used in vivo. In addition to the absence of OKT3 bearing lymphocytes as assessed by cytofluorography there appeared to be a deletion of lectin responsive cells at least in the early portion of OKT3 treatment with a differential response on PHA and Con A responsive clones. Most important was the effect of OKT3 on donor specific and donor nonspecific alloresponses. Immune response against transplantation antigens as discerned in the mixed lymphocyte culture was importantly inhibited by the OKT3 treatment, an inhibition which lasted beyond the administration of the drug. It may be this very effect of the monoclonal antibody which ceases active rejection and allows for the maintenance of allograft function in the long run. In summary, monoclonal antibody against T-cells is an efficacious therapy at reversing acute rejection. The precise reagent tested, OKT3, is not mitogenic to lymphocytes, is cytotoxic to peripheral mature lymphocytes, and deletes clones of cells responsive to lectin and to donor specific alloantigens. Important side effects, particularly in volume overloaded patients, exist, but these appear to be less severe than the polyclonal agent and not to occur after the second dose. At present the monoclonal antibodies are limited to a single course since patients make substantial anti-mouse antibody (as these proteins are mouse immunoglobulins). Research protocols are now in process to assess means to prevent the formation of murine antibodies so that this efficacious agent may be used a second time.

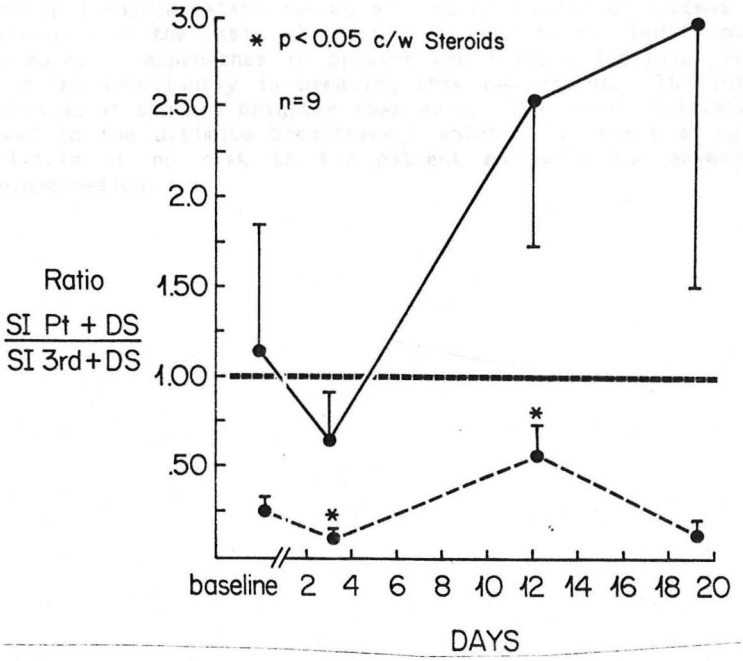
Summary

As the Parkland Memorial Hospital-Southwestern Medical School transplant program looks to its twenthieth anniversary of kidney transplantation, several

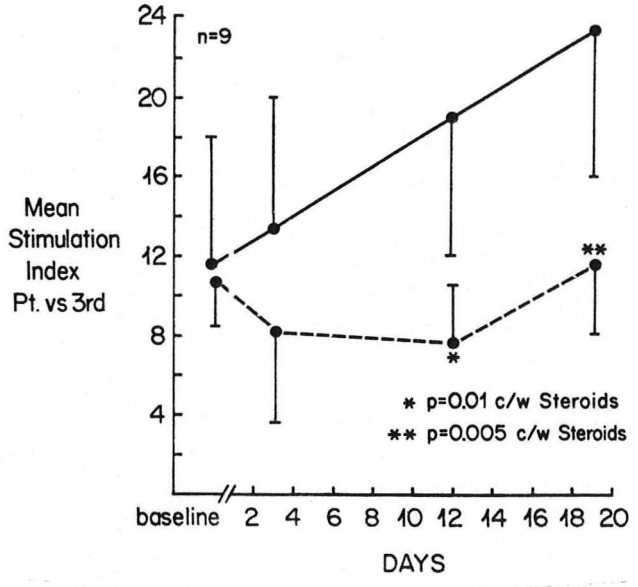


Figures 45 - 46





Figures 47 - 48



trends are discerned. Our program with many other good centers has entered a new era in transplantation marked by wonderful clinical success. A broadened understanding of the basis of transplant immunity has led to multiplicity of new therapeutic approaches to prevent and treat rejection. Our center has participated importantly in breaking this new ground. The future of renal transplantation appears brighter than ever. The trends initiated in the 80s may lead to the ultimate breakthrough which will permit allograft survival with little or no risk to the patient and with the absence of further immunosuppression.

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