transplantation
india

DESTINATION : TOLERANCE IN CLINIC

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Clinical organ transplantation between genetically disparate individuals currently requires the use of immunosuppressive drugs to prevent allograft rejection. Despite of significant advances in immunosuppressive pharmacotherapy allograft loss due to acute and chronic rejection process remains a formidable problem in clinical transplantation. However all these therapeutic strategies have limited success in providing complete and permanent protection against allograft rejection process. Most of the anti-rejection therapeutic approaches are specifically directed towards blockade of allopresentation done by graft resident donor hematopoietic professional antigen presenting cells to CD 4+ lymphocytes through direct and indirect allorecognition pathways. Deleterious side effects of these drugs, and their inability to prevent rejection has led to a continuing search for methods to induce donor-specific transplantation tolerance in allograft recipients. Numerous animal experimental models utilizing different conditioning protocols along with donor bone marrow derived cell transplantation coincident with solid organ transplantation have been proposed to create such donor specific tolerance. However the toxicity of conditioning protocols prevents application of such tolerance induction procedures in clinical transplantation. One of the most significant observations emerging from this data is the association of mixed allogeneic hematopoietic chimerism with establishment of tolerance in clinic.

Since the first report of classical tolerance in experimental model by Billingham, Brent and Medawar in new born mice by injection of donor spleen and marrow cells, there has been a considerable interest in achieving similar results in adult cell and solid organ transplantation. If it was possible to resurrect the same phenomenon of immunological naivity of neonate in an adult, then a similar bone marrow transplantation can be used to tolerize solid organ allograft. The common theme of all these strategies is to initiate the process by infusion of donor antigen followed by use of drugs to delete donor-specific proliferating clones prior to bone marrow transplantation. This strategy will lead to the establishment of mixed hematopoietic chimerism which in turn will sustain the tolerance by supplying the necessary veto cells from the grafted hematopoietic stem cells. Thus the ubiquitous presence of chimeric hematopoietic stem cells will function as a permanent source of vehicles presenting donor antigens for tolerance. More than one mechanisms could be involved.

We have demonstrated hypo-responsiveness to donor alloantigens in our tolerance protocol performed on 500 patients over a period of five years (September 1998 to September 2003). Our principal theme has been high dose donor bone marrow derived cell infusion in live related and cadaver renal allograft recipients. Various conditioning regimens have been used to create tolerance. These include limited irradiation (400 rads of total lymphoid irradiation), use of Sirolimus and inoculation of donor organ specific antigen tissue (kidney tissue) in recipient thymus. The objective of thymic inoculation of donor antigen tissue was to utilize central tolerance mechanism operated by medullary epithelial cells (MEC) of recipient thymus. Since the principal role of MECs is to secrete a tissue specific antigen which will purge high avidity T-cells reactive to that tissue specific antigen, we have proposed that in this set-up ectopic transplantation of donor renal tissue in thymus will provide central tolerance by deletion of donor specific high avidity CD4 /8 + cells. In our experience with this protocol, we have achieved prope' (almost) tolerance demonstrating hyporeactivity to donor alloantigens in the form of very low incidence of acute rejections (less than 5 %), stable and better allograft function, better graft and patient survival with monotherapy immunosuppressant. This concept considered to be ideal at one time, has now already been attained by us in clinic. A considerable progress has been made and is continually being made to make solid organ transplantation without lethal conditioning or long term immunosuppression a clinically achievable goal in near future.

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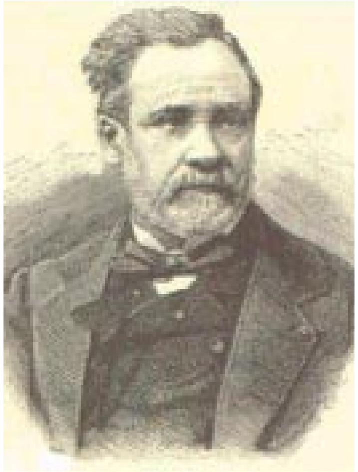

To him who devotes his life to science, nothing can give more happiness than increasing the number of discoveries, but his cup of joy is full when the results of his studies immediately find practical applications.

- LOUIS PASTEUR

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REMINISCENCES OF IMMUNOLOGIC EVENTS IN RENAL TRANSPLANTS: PARTIAL TOLERANCE

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In the early days of maintenance hemodialysis – in the era which bloomed following Belding Scribner's truly memorable presentation, in 1962 to the American Society of Clinical Investigation [1] the process of preparation of either the Rotating Drum or Twin Coil type dialysers involved priming with allogeneic blood. Usually 2 units of blood were used each time a patient was dialysed. This meant that, with twice weekly dialysis (the norm at that time), the patient was exposed to over 100 units of allogeneic blood during the course of every 6 months. Most would conjecture, in our present state of immunologic wisdom, that this would cause widespread sensitization of HLA in all patients. [Of course, at the time of which I write the lymphocytic crossmatch, using recipient patients serum, was still in the future. Nor, indeed, was there any HLA typing, at that time].

Nevertheless in 1963, because of the tragedy of deaths from chronic renal failure and the slow spread of long-term maintenance hemodialysis programs, cadaver kidney transplants were carried out in certain transplant centres—such as the Peter Brent Brigham (Boston)[2] the Cleveland Clinic, and at McGill University (Royal Victoria Hospital, Montreal).

The initial experiences of the McGill group (the first four recipients) were described in 1964 [3]. At the time of that report the world experience in renal transplantation had been reviewed in September 1963, only 23 of 96 live related-donor recipients survived 6 months (excluding identical twin transplants,) and only 4 cadaver kidney recipients survived to 6 months.[4] . It was decided to continue the program, but not to use living donors. Two of the four recipients had prolonged kidney function of 3 months or more. Clearly there were many perplexing aspects to these early experiences and the question was raised that even the cadaver kidney transplant program might be premature and a moratorium should be called—but this was something which desperate patients cannot accept when there are no alternatives available.

The next publication of the McGill series was in 1967 when 58 cadaver kidney transplants were described. When twenty nine (29) of these were further analysed. And unexpected result emerged:

> "Analysis of the 29 patients, ranked by their evidence of rejection activity in the first three post-transplant months show significant difference in renal function, hippuran renogram pathologic changes in glomerul and vessels, but not interstitial cellular infiltration. There was also a significant difference whereby those with **more hemodialysis prior to transplantation showed less evidence of rejection activity**." [5] .

In discussion of this unexpected finding, not only was a) a correlation shown between evidence of acute tubular necrosis and ischaemic time, but also, b) evidence of an inverse relationship between rejection index and the amount of time on hemodialysis (using the Twin Coil dialyser primed with allogeneic blood for each dialysis). This inverse correlation was true for both the group with high rejection index (closed inverted triangles) and those with low rejection index (open upright triangles). There was also correlation between the number of blood transfusions and improved late continuing function in each group (those with evidence of high rejection activity and those with low rejection activity). But it was also acknowledged that the numbers of transplants were low and also that transferring some of the patients in one group to the other would destroy the significance.

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Nevertheless the data convinced us that there was more to HLA sensitization than mere exposure to allogeneic leukocytes. Perhaps it was the route of exposure as well as the overwhelming amount of allogeneic blood exposure that caused decreased evidence of late rejection and improved late functioning.

This conviction that large volumes of allogeneic whole blood, delivered intravascularly, might even be beneficial to subsequent renal transplantation, led us to three lines of investigation:

- in live donor situations, the possible benefit of donor-specific blood transfusion prior to transplantation; especially in situations where the recipient now had the added protection of a negative donor lymphocyte serum cross match. (as described by Kissmeyer et al. in 1966; [6] and was rapidly found to be the principal cause of immediate transplant rejection).
- Some experiences in attempting to lessen allograft rejection using donor antigen, in the rat kidney transplant model; [7] and,
- On one rare occasion, evidence of benefit from actual cross-circulation between a prospective donor and a prospective recipient. [8, 9]. This very unusual circumstance of donor-specific cross-circulation prior to transplantation will be described in further detail, below.

The occasion which justified the cross-circulation experience occurred when woman in chronic renal failure, in her 30s, with five children, was referred from a remote part of Newfoundland. We told the referring physician that long-term dialysis would not be possible and even cadaver kidney follow-up would be very inadequate, in winter, as her home was really only accessible by coastal boat. However, she had arrived and we were on the point of giving her a period of 3 months on the waiting list, on dialysis, before taking the next step—which would probably be to send her home on peritoneal dialysis if she did not receive a cadaveric transplant.

During this time there was another young woman of the same ABO blood group (there was no HLA-typing available that time) who was in critical condition from advanced liver disease, and likely to die. The similarity of the blood groups was noted by the resident in internal medicine who was looking after both patients. She brought this to my attention and, on discussion, we came up with the possibility of putting into practice a plan where each would agree to cross-circulate with the other, using the function of the organ in the other which each uniquely lacked. To this end a consent agreement was drawn up between both patients and both families to continue with cross-circulation for as long as possible. In this agreement each family agreed, also, that if one of them died, the organ which the other lacked would be available as a cadaveric transplant.

Cross circulation had been used baefore, prior to the introduction of the membrane oxygenator [10] and consisted of placing silastic tubes in each partner and connecting them, artery to vein, while both patients lay on bed weigh scales. The volume balance of the two circulations was controlled by adjusting the screw clamps on the linking blood connections in responses to the changes in weight between the patients. (see Figure 1)

Figure 1

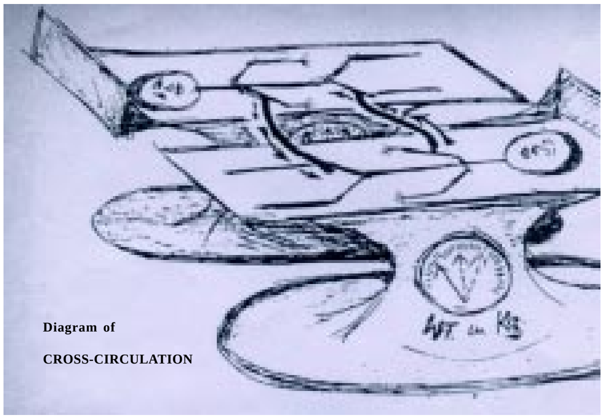
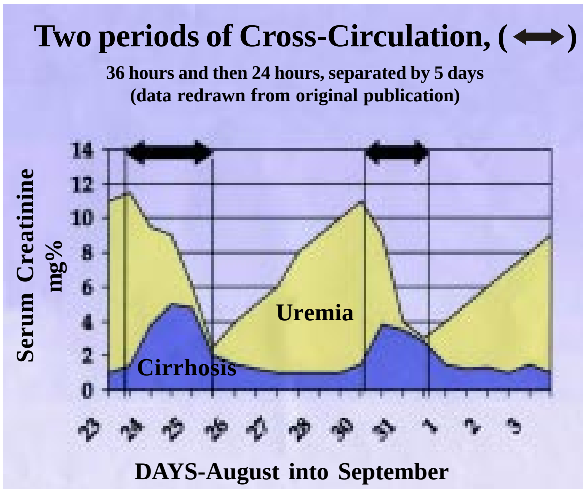


Figure 2 shows the biochemical changes which occurred in serum creatinine during the cross-circulation. Creatinine of the uremic patient fell sharply, and this was accompanied by a temporary rise in creatinine in the cirrhotic patient. Similar mirrored inverse changes were seen in serum bilirubin (not plotted in Figure 2) Both patients became alert, coming out of stupor, as a result of these cross circulations which were undertaken for two periods of 36 hours and 24 hours during the first week.

Figure 2



Unfortunately, while preparing for a third episode, on the 10th day, the hepatic patient had an intestinal hemorrhage (presumably from varices) and died. There was considerable delay in implanting her kidney into the uremic patient (it was a holiday long week-end) and there was no diuresis initially. Eventually, renal function returned. No clear cut rejections occurred and she lived for a 9 further years in her remote Newfoundland location, returning for follow-up every year. During this time she successfully gave birth to her sixth (6th) child. Her death (which occurred at her home) was attributed to poorly controlled hypertension, but the transplant had continued to function.

It is difficult not to believe that cross circulation rendered her tolerant in some way to the antigenic constituents of her cross-circulation partner though this cannot be proved, of course.

Evidence later grew to show the hazards of HLA sensitization in transplant recipients and the critical importance of pre-transplant lymphocytotoxic cross- matching. But, also, further evidence accumulated in the literature to show the beneficial effect associated with pre-transplant blood transfusions with definitive confirmatory articles by Opelz and Terasaki [11, 12]. In the second of these papers, 1360 kidney grafts were reviewed and the improved correlation of long term function with numbers of pre-transplant blood transfusions had a statistical significance of $P<0.0001$. Other results came from Festenstein et al. [13] and others. This clinical work was backed up by controlled experiments in the rhesus monkeys by Van Es, Balner et al. [14] which showed the same beneficial effect.

With improved methods of hemodialysis and its frequency (not less than three times, weekly) and improved immunosuppressive regimens, this beneficial effect has faded into the background of clinical transplantation, though the effect was shown again in a more recent series from a large pediatric renal transplant series from Paris. [15]. Here, covering the transfusions with Cyclosporine (short-term) was associated with improved graft survival at 2 and 5 years when compared with those who received blood transfusion without short-term concomitant Cyclosporine coverage. Similar results were reported by Opelz, in 1997, from 14 centres using Cyclosporine as the main immunosuppressive agent— so the topic may still have some validity [16].

The aim of good dialysis care switched to minimal use of blood transfusions— which is a good thing as this era was accompanied by such striking improvements in immunosuppression. But for those who have had the earlier experiences such as I have described above— they were generously referred to by Dr. Trivedi on page 3 of his editorial in the first issue of Transplantation India— the dream of inducing partial immunologic tolerance in a donor-specific context never disappeared. It was always there in the background. This is why it was so exciting to hear of the remarkable series of clinical outcomes from Ahmedabad in the last few years.

The mechanisms underlying these remarkable improvements are very complex, indeed and include the concepts of donor chimerism, donor-specific stem cell transplantation, and basic immunologic processes which become increasingly unraveled as the science progresses. But increasing knowledge at the molecular level still does not render unnecessary those bold leaps at the clinical level by which we remember such pioneers as Tom Starzl, Joe Murray, Roy Calne, Christian Barnard, and of course, Willem J. Kolff (who pioneered with hemodialysis) and Peter Medawar (who pioneered with the whole concept of immune tolerance).

(I will make no attempt to give a comprehensive list of these pioneers— it would be too difficult and might even be perilous!)

I, too left the field of possible induction of partial immunologic tolerance at the clinical level and took up research opportunities in:

a) histocompatibility, (with studies in two populations who have very restricted HLA antigen profiles— as a result of in-breeding— the Hutterites in Alberta and the Inuit population of the Canadian Arctic;

b) post-transplant immunological monitoring, using recipient serums and lymphocytes with donor spleen cells as targets (preserved frozen donor spleen cells), leading to

c) an increasing commitment to study of the ethical issues in the whole field of organ and tissue transplantation.

However, these researches will not be reminisced in this paper as it is dedicated to those who have reopened the whole area of induction of partial immunologic tolerance by means of pre-transplant infusion of donor stem cells [17] and more recently, using donor renal tissue into the recipient's thymus as a further adjunct [18].

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IMMUNOSUPPRESSANTS AND ANAESTHETIST

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More than 2000 patients undergo renal transplantation every year in our country obligating them to take lifelong immunosuppressive drugs. The survival rate and quality of life continues to improve after transplantation because of advancement in techniques and availability of newer immunosuppressive drugs. This means that these patients may present for either elective and/or emergency surgery at a variable period after transplantation.

Apart from organ transplantation, immunosuppressants are also used to treat a variety of disorders like collagen vascular diseases, inflammatory bowel diseases etc. The aim of this review is to familiarize the clinician with the potential problems associated with immunosuppressants from the perspective of the anaesthesiologist.

PHARMACOLOGICAL CONSIDERATIONS :

The immunosuppressive drugs in common use are cyclosporine, azathioprine. polyclonal and monoclonal antibodies and steroids. Newer drugs such as tacrolimus or rapamycin may replace cyclosporine and MMF may replace azathioprine in future.

CYCLOSPORINE

It is the most commonly used immunosuppressant which prevents the activation of T lymphocytes by interrupting signal transduction through inhibition of calcineurin. Because of its narrow therapeutic window, unwanted end-organ complications occur during cyclosporine therapy.

Nephrotoxicity is the major complication [1] due to renal arteriolar vasoconstriction leading to reduction of GFR and creatinine clearance. Over a long term, progressive renal injury may occur if patients are not properly monitored and doses are adjusted or replaced by other drugs. The drugs that depend on kidneys for elimination should be used with caution in patients taking cyclosporine. Neuromuscular blocking agents that rely least on renal elimination e.g. atracurium are optimal choices for muscle relaxation. Non steroidal anti inflammatary drugs may worsen cyclosporin-induced nephrotoxicity [2, 3] and should be used with extreme caution for postoperative pain management.

Hypertension is a common side effect of cyclosporine therapy and is associated with increase in systemic vascular resistance and is relieved by withdrawal of drug [4, 5]. The rise in peripheral vascular resistance is not associated with any evidence of a rise in sympathetic or neural activity [6]. The current treatment of this side effect is calcium channel blockers.

Cyclosporine neurotoxicity can contribute to tremors and seizures especially in the early post transplantation period. Intraoperative management of these patients should include careful attention to serum sodium and magnesium levels and avoidance of hyperventilation [7]. Cyclosporine is also hepatotoxic in some patients and in addition can lead to diabetes mellitus and hyperlipidemia.

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TACROLIMUS (FK- 506)

It is a macrolide like cyclosporine having lower organ-specific toxicity. With its use over last five years, several cardiac complications have been reported as it is known to cause diabetes, hypertension and hyperlipidemia. Hypertrophic cardiomyopathy reported in children appears to be dose related and reversible with drug withdrawal. In addition, torsade de pointes has been reported in few patients so electrocardiogram of patients treated with tacrolimus should be carefully reviewed for Q-T interval prolongation. Tremor is a common complication whereas red cell aplasia is a rare complication of tacrolimus.

RAPAMYCIN

It is a recently introduced macrolide which binds to the same cellular protein as FK-506 but interferes with completely different components of T-cell activation. Hyperlipidemia is a significant side-effect of rapamycin but hypertension is less common. Some patients develop thrombocytopenia on rapamycin, so platelet count is indicated before invasive procedures.

Overall, these macrolide immunosuppressants contribute to relatively high incidence of vascular disease so the anaesthesiologist should approach these patients as having several risk-factors for cardiovascular, cerebrovascular and peripheral vascular disease. The preoperative assessment should be directed at eliciting the presence of these diseases, their severity and optimization before elective surgery.

AZATHIOPRINE

It is an antimetabolite most active against proliferative cells. It is metabolized to 6-mercaptopurine which is responsible for the drug's cytotoxic effect. The major complication of azathioprine is marrow suppression especially leucopenia so complete blood count is necessary before surgery. Drugs that may cause marrow toxicity when given with azathioprine are allopurinol, ACE inhibitors, sulfasalazine and 5-aminosalicylic acid [8]. Pancreatitis and elevation of liver enzymes have also been reported with azathioprine therapy.

GLUCOCORTICOIDS

These nonspecific immunosuppressants increase the transcription of genes for several anti inflammatory cytokines and also suppress other transcription factors that are important in T-cell activation. Because prednisolone is used for a long time, anaesthesiologists are familiar with its side effects [8] : sodium retention, hypertension, diabetes, stress ulcer, gastrointestinal haemorrhage, stunted growth in children, protein wasting, cushinhoid appearance, mood swings, poor skin integrity, osteoporosis, impaired wound healing etc. Care of patients on higher doses of steroids requires careful handling to prevent skin damage, fractures and anti-acid prophylaxis. Acute intravenous administration of methyl-prednisolone has been associated with a few documented cases of circulatory arrest.

MYCOPHENOLATE MOFETIL (MMF)

Mycophenolic acid, the major metabolite of MMF inhibits a key enzyme in denovo purine synthesis, decreasing proliferation of both B and T cells. It is not nephrotoxic. The common side effects are gastrointestinal and bone marrow suppression so complete blood count should be performed before surgery in patients treated with MMF.

ANTIBODY BASED THERAPIES

Polyclonal antibodies – Antilymphocyte globulin & Antithymocyte globulin (ALG and ATG) derived from large animals were used for decades in the treatment of acute rejection that does not respond to other drugs. Their use has declined as other immunosuppressants became available. Patients commonly experience an acute flu-like illness within minutes to hours of infusion of these antibodies. A chronic serum sickness including fever, rash, joint pain and gastrointestinal distress may develop after about one week of therapy. Platelet count and white cell count should be checked if a patient scheduled for surgery has received ATG or ALG recently.

Monoclonal antibodies – OKT-3, a murine-derived monoclonal antibody elicits an immune response when used clinically. Symptoms include photophobia, headache, arthralgia, myalgias, fever with chills and gastrointestinal upset. More severe side effects include swings in blood pressure and/or tachycardia, angina, increased pulmonary shunting leading to hypoxia and renal dysfunction. Small percentage of cases may develop frank pulmonary edema, cardiomyopathy, aseptic meningitis and even cardiac arrest.

The acute syndrome caused by OKT-3 occurs due to massive release of proinflammatory cytokines particularly TNF-a and interferon-b. Before specific therapy is available, it should be treated with diuretics, dopamine, antipyretics and steroids.

Anti- IL2R a

Anti-interleukin 2 receptor (Daclizumab) is a relatively new therapy directed against an epitope that is expressed on almost all activated T- cells. It is a humanized monoclonal antibody in which only 10 % of antibody sequence is derived form mouse so acute flu-like syndromes are not seen with this drug.

Future immunosuppressants

Other drugs deserve mention because they may become more commonly prescribed in future. Brequinar is an inhibitor of de novo pyrimidine synthesis. Antibodies are also being developed that target molecules important in the interaction between endothelial cells and lymphocytes e.g. vascular cell adhesion molecule. Other drug targets include molecules involved in antigen presentation. Non drug therapies include total lymphoid radiation, manipulation of thymic environment, haemopoietic stem cell transplantation etc. As these newer therapies are under trial, potential side effects may not be yet reported in the literature.

ANAESTHETIC CONSIDERATIONS :

IMMUNOSUPPRESSANTS IN THE PERIOPERATIVE PERIOD

A major problem in immunosuppressive therapy has been the marked individual variation in pharmacological profiles. In the initial period after transplantation, drug level monitoring (particularly for cyclosporin and tacrolimus) is used to decide individual dosage but in long term, majority of patients do not require very fine tuning of their dose regimen. All patients preoperatively should be asked about their drug regimen and drug compliance. Younger patients are often reluctant to adhere to their drug schedules because many of side effects of immunosuppressants are linked to appearance e.g. gingival hyperplasia, weight gain, acne, hirsutism etc. It is also important to know whether underlying surgical condition has disrupted their regime. Obviously oral regimens are not appropriate in the presence of vomiting, abdominal surgery, gastrointestinal dysfunction or intravascular fluid shifts. For these reasons, immunosuppressive drug levels must be monitored aggressively and these levels alongwith graft organ function studies can be used to guide perioperative administration of immunosuppressive drugs in consultation with the transplant physician. To maintain therapeutic blood levels, oral cyclosporin is administered 4-7 hrs. before surgery [9]. Supplemental “stress coverage’’ steroids are probably not necessary except in patients recently withdrawn from them [10].

INFECTION CONTROL :

The most important feature of anaesthetic care in immunosuppressed patients is attention to infection control. Patients walk on a tight rope between inadequate immunosuppression with danger of graft rejection and threat of overimmuno-suppression with risk of tumor development and/or fatal infection. It is absolute mandatory to take aseptic precautions during placement of central lines, performance of central or peripheral neural blockade and other invasive procedures. Use of disposable anaesthesia accessories is advisable. During general anaesthesia, oral intubation is preferable to nasal intubation because of risk of transient bacteremia [11]. The use of LMA is acceptable [12]. Severe perioperative airway obstruction has been reported by underlying post transplant lymphoproliferative disease [13]. It is also prudent to continue antibiotics and antiviral drug schedules during surgical procedures. Amongst antivirals, gancyclovir is repeatedly used in these patients which is not reported to cause specific interaction with anaesthetics.

INTERACTION BETWEEN IMMUNOSUPPRESSANTS AND ANAESTHETIC DRUGS

A considerable number of studies [14,15] have discussed the anaesthetic management of transplant patients undergoing non-transplant procedures, but there has been little reference to the interaction of routine anaesthetic drugs with immunosuppressive protocols. However, there are some areas worthy of comment.

Both cyclosporine and tacrolimus are metabolized by the P 450 system, so many drugs administered perioperatively may affect cyclosporine blood levels (Table I).

Table I : Drugs that affect cyclosporine & tacrolimus blood levels

Increase blood levels	Decrease blood levels
Bromocryptine	Carbamazepine
Chloroquine [a]	Octreotide
Cimetidine [b]	Phenobarbital
Clarithromycin	Phenytoin
Co-trimoxazole	Rifampycin
Danazole	Ticlopidine
Diltiazem	
Erythromycin	
Fluconazole	
Ketoconazole	
Metoclopramide	
Nicardipine	
Verapamil	

a Reported with cyclosporine; may not interact with tacrolimus

b May not interact with cyclosporine

Fortunately, most of these drugs are not often administered by anaesthesiologists. Cirella et al [17] in animal studies have suggested that a single dose of cyclosporine may result in increased duration of action of both barbiturates and narcotics but these observations were not confirmed by Molendez and colleagues [17] recently, diltiazem commonly used to increase the blood levels of cyclosporin has been shown to be associated with slow elimination of midazolam and alfentanil.

Animal studies [18,19] have suggested that isoflurane may lower blood levels of cyclosporine but no parallel human studies have been performed. Propofol infusion does not modify the cyclosporine blood levels in humans [20]. Neuromuscular blocking drugs used as a part of balanced anaesthetic technique have been reported to have prolonged effect with cyclosporine [21] but this association is probably in the context of renal failure. The solubilizing agent of cyclosporine (Cremophor) has been shown to augment the action of neuromuscular blocking agents like vecuronium and atracurium in both humans and cats as demonstrated by the studies of Gramstad et al [22]. Although an elegant study by Sharp and Gelb [23] showed that depth and duration of action of neuromuscular blockade with vecuronium was prolonged by concomitant administration of cyclosporine, these observations were obscured by reports from other centers [24]. Therefore, other potential causes for prolonged neuromuscular blockade in this patient population must be considered.

Bone marrow suppression associated with use of azathioprine or ATG may produce thrombocytopenia, so clotting studies and platelet count should be normal if an epidural or spinal anaesthesia is planned. Bupivacaine, a commonly used local anaesthetic, is safe when used in clinical doses [25]. Some transplant recipients who have undergone repeated surgery do seem to develop tolerance to opioids requiring dose titration to clinical effect and potential side effects. Although the excretion of morphine is not affected by renal impairment, its metabolites can accumulate leading to prolonged sedation postoperatively [26].

Administration of antibody based therapies require vigilance due to side-effects which are common within minutes to hours of its administration. Hemodynamic consequences of intraoperative use of OKT-3 in 23 cardiac transplant recipients were reviewed by Stein [27] in 1989. There was a biphasic response even after prior administration of antihistaminics, steroids and paracetamol. Within the first hour, patients develop fever, hypertension and tachycardia followed by a secondary response 5 to 7 hours later characterized by mild hypotension, hypoxia and decreased indices of vascular resistance. Out of 23, 18 patients required treatment with oxygen and/or vasopressor support. They concluded that there was a complex hemodynamic response to OKT-3 exhibited by a delayed non-coincident hemodynamic instability, fever and hypoxia requiring close observation during the initial period. In contrast to these, Robinson et al [28] and Robinson [29] reviewed the effects of administration of OKT-3 in 12 patients prior to cadaveric renal transplant. They showed no significant changes in heart rate, mean arterial pressure, pulmonary arterial pressure, central venous pressure, pulmonary capilllary wedge pressure and pulmonary vascular resistance. There was 22 % increase in cardiac index and 21 % decrease in systemic vascular resistance about 2 hours after OKT –3 but these were within acceptable limits. There were no cases of non-cardiogenic pulmonary edema as described by Cosimi [30]. This may have been due to the fact that all patients were euvolemic who received prophylactic steroids and diphenhydramine. This conclusion may not apply to cardiac transplant who represent different population with different pathophysiology.

SUMMARY

Although direct drug interactions between immunosuppressants and anaesthetic drugs are relatively insignificant, the side effects of immunosuppressants have direct implications for anaesthetic management. Preoperative assessment should aim to evaluate cardiac, renal , hepatic, hematological and neurological function so that proper selection of anaesthetic agents can be planned. Because of high incidence of cardiovascular disease in these patients, thorough assessment of cardiac symptoms, functional cardiac reserve and serial electrocardiograms should be performed. All invasive procedures should be performed under strict asepsis. Monitoring drug levels during the perioperative period and maintenance of adequate immunosuppression during surgery and postoperative recovery should be part of the anaesthetic plan. It is important to remember that drug levels may be disrupted not only by drug interactions but also by fluid shifts, changes in absorption related to gastrointestinal dysfunction, changes in elimination related to renal and hepatic dysfunction and underlying disease process that brought them to operating room. In conclusion, optimal care of immunosuppressed patients implies knowledge of particular problems associated with their drug therapy.

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IMMUNOBIOLOGY OF REJECTION : THREE BRIDGES TO CROSS!

Aruna V. Vanikar

ABBREVIATIONS

APC-	Antigen presenting cell
CsA-	Cyclosporine A
HLA-	Human leucocyte antigen
Ig-	Immunoglobulin
IL-	Interleukin
MHC-	Major histocompatibility complex
TCR-	T cell receptor
miH-	Minor histocompatibility
VAL-	Very late activation

Transplantation has been an effective remedy for failing organs. However a balance between prevention of acute rejection and drug-related toxicity still remains elusive. Organ transplantation from a genetically disparate donor induces immune responses by both donor and the recipient. An uncontrolled, cumulative effect of these responses can eventually destroy the grafted tissue. The immunological nature of tissue rejection was suggested for the first time by Gorer in 1938 and proved later by Medawar whose group subsequently showed profound, specific and sustained graft acceptance by inducing tolerance in newborn cattle[1-6]. The cascade of acute rejection process begins with presentation of donor alloantigens to the recipient T- cells through antigen presenting cells (APCs) or directly.

Role of human leucocyte antigen (HLA) in rejection

The alloantigens are encoded by major histocompatibility complex (MHC) called HLA in human beings. The HLA complex encompasses more than 3.5 x 10^6 base pairs of DNA on short arm of chromosome 6. It encodes polymorphic cell-surface glycoproteins, class I molecules (HLA- A, B, C) and class II molecules (HLA- DP, DQ, DR) which determine the recognition of antigens by T lymphocytes. HLA class I molecules are constitutively expressed by most cells and tissues whereas class II molecules are expressed only by B lymphocytes, macrophages, monocytes and follicular dendritic cells. However agents like interferon can stimulate certain other cells like T lymphocytes, renal tubular cells, endothelial cells and pancreatic beta cells to express HLA class II molecules[7]. The focus of immunology research was shifted from major groups in class I molecules to class II molecules in seventies. Zinkernagel and Doherty in 1974 brought about a conceptual breakthrough with their discovery that antigen receptor of T lymphocyte interacts specifically with a composite ligand made up of peptide fragment of foreign protein bound to the peptide-binding groove of an MHC molecule[8]. The physiologic function of an MHC molecule is to present antigen to T- cell receptor (TCR). In strong alloreactive T- cell responses leading to rejection of the transplanted tissue, the foreign MHC molecules act as both the presenting molecule and the foreign antigen; although the role of peptides bound to MHC molecules is unclear. The wide range of peptides that can potentially be presented to the peptide site of MHC molecules is the basic reason responsible for HLA polymorphism. The number of HLA molecules expressed may represent an evolutionary compromise between maximizing the recognition of foreign antigens and minimizing the recognition of self. Individuals with different HLA types respond in different ways to pathogenic microorganisms. Thus each person will mount strong responses to some organisms and weaker responses to others. Similarly, the phenomenon of transplantation rejection is a direct consequence of the advantage to the species of having more HLA types.

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Antigen presentation to recipient T lymphocytes

The specific immune response to a graft occurs in two main stages. In the first, the afferent arm, donor antigens are presented to the recipient T lymphocytes, which become activated, proliferate and differentiate further while sending signals for growth and differentiation to a variety of other cells. In the second stage, the efferent arm; effector leucocytes are recruited into the organ where they can wreak havoc to bring out tissue destruction.

Antigens that stimulate graft rejection are single MHC system and numerous minor histocompatibility (miH) system. MHC is mainly responsible for vigorous rejection. The miH antigens are a composite of peptides from low polymorphic proteins, presented in the MHC groove. It is difficult to raise antibodies against miH antigens since antibodies most frequently recognize conformational determinants on proteins and peptides bound within the MHC group, and may not be accessible for recognition by the antibody producing B lymphocytes. The miH antigens can lead to smouldering rejection episodes in case of MHC compatible grafts of presensitized host but rarely are responsible for graft loss. A significant host immune response can only be generated in presence of MHC class II antigens and not I antigens [9-11].

The non-lymphoid passenger leucocytes, which clearly are not the macrophages, migrate rapidly out of a tissue after transplantation, to the recipient lymphoid organs where they are able to interact with and stimulate host immune response [12, 13]. These passenger leucocytes have the characteristics of immature dendritic cell which on migration, mature rapidly into APCs, particularly potent to stimulate T lymphocytes[14]. Mature dendritic cells express high levels of MHC class I and II antigens and are able to stimulate CD 4 + and CD 8 + T lymphocytes. They also have the capacity to stimulate naïve (previously unactivated) T cells, hence are also known as “professional APCs”. T-cells are central to the rejection process hence animals deprived of T-cells through genetic or experimental manipulation are unable to reject grafts.

Activation of recipient T-cells

Helper/ inducer T-cells expressing CD 4 bind to MHC class II molecules and cytotoxic/ suppressor T-cells expressing CD 8 bind to MHC class I molecules. These receptor- ligand pairs increase overall avidity of interaction between T-cells and target cells and play a part in signal transduction. The passenger leucocytes including dendritic cells are released from the grafted tissue in to the recipient circulation. Recipient T-cells recognize cell surface molecules on APCs at this stage through their specific TCRs. The relatively quiescent T-cells are activated and undergo clonal expansion and differentiation of antigen- specific T-cells. This process evolves major intracellular biochemical changes [15-17].

Direct pathway of allorecognition

The immune response to a nominal antigen is initiated when antigen specific T lymphocytes recognize foreign peptide presented in context of self-MHC molecules. T- cells specific to peptides derived from a nominal antigen constitute a very small fraction (less than 0.1%) of the total T-cell repertoire. In contrast, a much higher frequency (about 10 %) of T- cells react to a transplanted organ[18, 19]. There are two pathways of allorecognition responsible for this frequency.

In the direct pathway, the donor antigen is presented to the recipient T lymphocyte by donor APC. Here the allogeneic MHC and non-polymorphic peptide are recognized as non-self by TCR. Donor peptide could be foreign or self- peptide (e.g. a cellular protein common to both the donor and the recipient). The T-cells here predominantly try to eliminate the invading APC (recognized as foreign). In the direct pathway an unusually high number of T-cells react to the allogeneic MHC, since many different self- peptides are derived from the graft, and the combination of these with the allogeneic MHC stimulates many different T-cell clones in the recipient[20]. (fig.1)

The indirect pathway of allorecognition

The second route of indirect pathway was found out when elimination from graft of passenger leucocytes did not abrogate rejection completely. Here the graft-derived antigens (of MHC/ non-MHC origin) are presented to the recipient immune system by the recipient’s own dendritic cells[21-26]. This is the process by which normal antigens are displayed to the host on APC and only a small number of T-cells are activated here. Most allogeneic peptides are presented in context of MHC II antigens, because this pathway deals with proteins exogenous to the cell. Some cross–over between MHC class I and II antigens does occur, in such a way that allogeneic MHC peptides also may be presented in context of self-class I antigens. Indirect presentation is believed to play the dominant role in acute rejection and also can provide continuing antigenic stimulus required for chronic graft rejection[27-30]. (fig. 1)

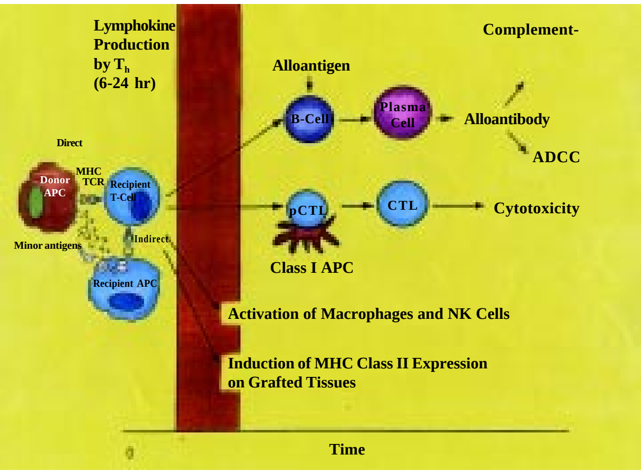

Figure 1 :- Pathways of allorecognition

T-Cell recognition - Presence of alloreactive memory T-cells

Immune response to a transplanted organ results from stimulus of naïve and memory alloreactive T-cells. Unlike the naïve T-cells, memory T-cells survive in absence of antigen and are maintained by factors distinct from those of naïve T-cells. Their activation is independent of costimulatory molecules essential for survival of naïve T-cell activation. Although both these types are susceptible to apoptosis, memory T- cells are suspected to be resistant to the same and hence it is difficult to delete memory T-cells to prevent acute rejection.

Antigen specificity in transplantation rejection by T-cells is provided by clonally restricted TCR-cell surface molecules comprised of a and b polypeptide chains linked by disulfide bonds. Rearrangement of TCR genes takes place in thymus. Through a poorly understood mechanism, cells with appropriate antigen receptors are selected in thymus for their capacity to interact with body's MHC (positive selection). Cells bearing self reactive TCR are deleted through the process of negative selection[30]. It is likely that all TCRs have an intrinsic affinity for HLA and that conserved portions of HLA and TCR are involved in this reaction.

Activation of dendritic cells –Signal 1

T cell– dendritic cell interactions are reciprocal and T-cells control dendritic cell maturation and phenotype. Ligation of CD40 on dendritic cell by CD154 (CD40 ligand, gp39) on T-cell results in the upregulation of B-7 proteins, which in turn may affect T-cell further. (fig. 2)

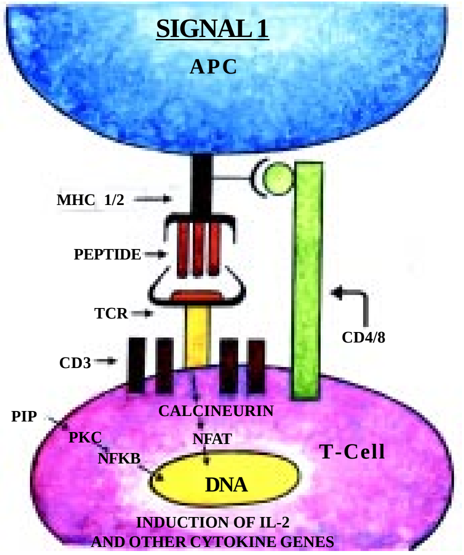

Figure 2 : Activation of dendritic cells –Signal 1

Dendritic cells and macrophages do not have the vast range of antigen receptors with fine specificity like T and B cells; but they have pattern recognition receptors that recognize common features on pathogens[31]. There are functional subsets of dendritic cells, some of which are critical for tolerance.

Second or co-stimulatory signals

If a CD4+ T cell receives the first signal through CD3 complex only and does not receive the second or co-stimulatory signals, it becomes anergic (unresponsive)[32-34]. This will also result into prevention of activation of the neighboring T-cells [35, 36]. There are many cell surface proteins on T-cell that potentially contribute to its activation. (fig. 3)

CD4 and CD8 are linked to intracellular proteins involved in transducing further signals to the T- cells. Series of additional proteins on the T- cell surface like CD 54, CD2, CTLA4 and CD28, act largely to increase the affinity of interaction between T-cell and APC, although they also may transduce further signals to T-cells.

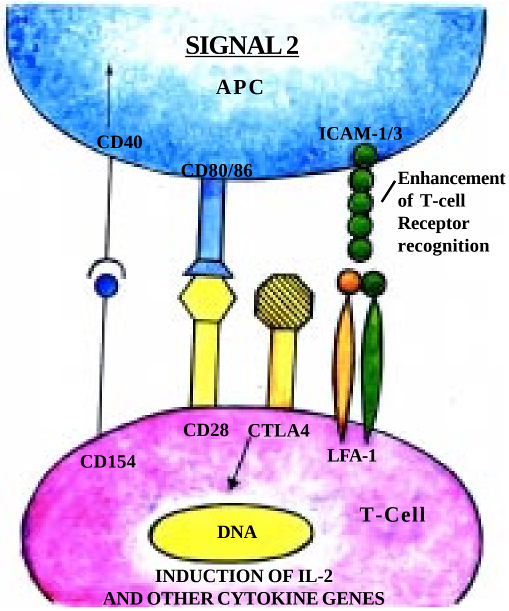

Figure 3 : Second or co-stimulatory signals

T cell co-stimulation begins with interaction of CD28 pathway. CD28 homodimeric glycoprotein, present on surface of T cell interacts with its two counter receptors CD80 and CD86 (the B 7 proteins) expressed on surface of APCs. Both have similar structure yet react in different ways with CD28. CD86 is constitutively expressed at low levels by professional APCs and is rapidly up-regulated after its interaction with T- cells. It has ten- fold greater binding capacity with CD28 than does CD80. CD28 ligation with CD86/CD80 results into increased cytokine synthesis and proliferation after signaling through incompletely understood intracellular pathways. CTLA4, a homologous protein of CD-28 is expressed by T- cell for resolution of immunity. CTLA4 has much higher affinity for binding to CD80 than to CD86; and also it has higher affinity for binding to both these receptors than CD28. It is believed that this co-stimulatory pathway is essential for activation of memory CD4+ T -cells. Blocking of CD28 pathway has shown prolonged graft survival[37-39].

Activated T- cells express another cell surface protein, CD154 (CD40L gp39). CD40L interaction with CD40 located on APC leads to activation of B cells, dendritic cells and monocytes. Larsen et al showed that blocking this pathway could lead to prolonged cardiac graft survival in a mouse model. Combined blocking of CD28 and CD40 interactions can induce permanent survival, as noted in allogeneic skin grafts in mice[40, 41].

Generation of different types of effector immunity

APC and T- cell interaction leads to either humoral or cell mediated immunity, which is determined by cytokines produced by APCs in the process. Cell mediated immunity is driven by Th1 cells and related cytokines where as humoral immunity is driven by Th2 cells and their related cytokines.

The third signal (autocrine arc)

Activated T- cells express various lymphokines to coordinate immune responses. The early immune responses are noted by wide range of cytokine production by T- cells, however continued antigenic stimulus leads to divergence of T- cells and thereby cytokine production[42]. Genes encoding interleukin 2 (IL-2) and IL2- receptor are the first to be activated and lead to formation of products essential for T- cell proliferation. Subsequently IL- 3 is formed which stimulates stem cell proliferation, which in turn differentiates in to granulocytes and macrophages. Interferon (IFN)–g is prototypic cytokine of T1 cells; their predominance generates cell mediated immunity and subsequently cytotoxic T lymphocytes and macrophages. T2 cells make cytokines like IL4, 5, 6 which are critical for induction of humoral immunity, isotype immunoglobulin switching and eosinophilia. (fig. 4)

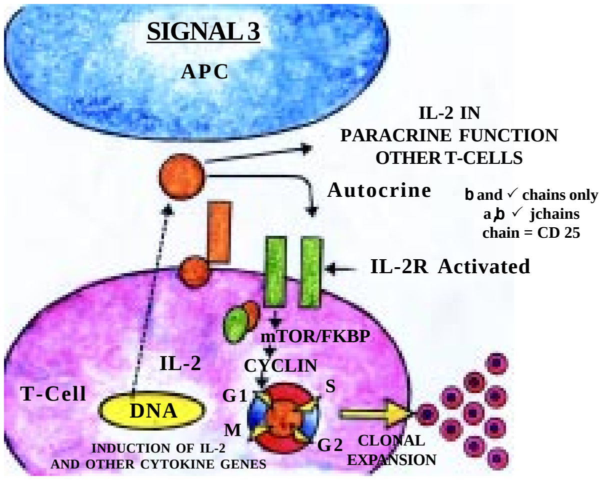

Figure 4 : The third signal (autocrine arc)

T1 driven responses may result into graft rejection whereas T2 driven responses induce tolerance. However, it has been observed that clones of T- cells with T2 like properties are capable of initiating chronic graft rejection like T1 clones and in fact for induction of true tolerance, a rapid shut down of cytokine production may be observed[38, 43].

Finally, a group of very late activation (VAL) molecules is produced, one to two weeks after stimulation[44]. T-cell proliferation and differentiation is rather autonomous. The triggering antigen of TCR commits about 50% cells to activation within 30 -120 minutes. IL-2 is then produced and stimulates T- cell proliferation (autocrine effect) as well as proliferation of nearby cells (paracrine effect).

The third pathway

Endothelial cells of donor origin are uniquely located at the interface between recipient's blood and the allograft; and have been implicated in graft rejection. Endothelial cells may promote indirect allorecognition by cross-talk mechanism involving recruitment and transformation of recipient monocytes by endothelial cells in to highly efficient antigen presenting dendritic cells[45, 46]. The later may recirculate to peripheral lymphoid organs for maturation, and in turn present alloantigen via the indirect pathway to memory T-cells in periphery or in transplanted tissue. (fig. 5)

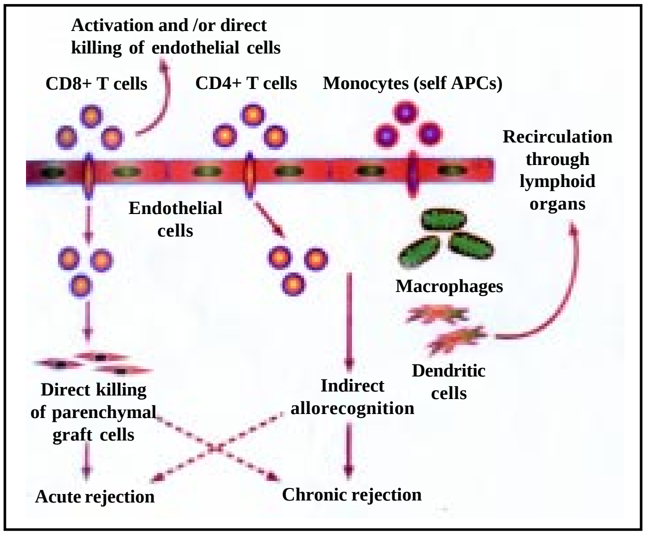

Figure 5 : The third pathway

The graft endothelial cells also express donor- MHC class I and II molecules suspected to directly stimulate T- cells. They can also induce proliferation and production of cytokines by allogeneic T- cells in vitro; and induce and regulate cytolytic T- cell differentiation. Graft endothelial cells could promote direct allorecognition pathway by serving as APCs and/ or as targets for T-cell mediated cytotoxicity. Lakkis et al believe that peripheral or secondary lymphoid organs are necessary to induce allograft rejection. According to their concept of immunologic ignorance, unprimed T-cells must encounter antigens first outside the graft, in the guise of donor APCs that have migrated to host lymph nodes or processed allopeptides presented by self APCs. It is postulated that T- cells after recognizing antigens on graft endothelium, migrate to peripheral lymphoid organs for further maturation and differentiation before being recruited to the graft to effect tissue destruction. Kriesel et al observed in transgeneic animal model that CD 8+ T- cells can recognize alloantigen on graft endothelial cells and react with them to induce rejection in absence of CD 4+ T- cell help and professional hematopoietic APCs.

Migration of activated leucocytes in to the graft

The leucocytes must migrate across the vascular endothelium to enter the site of immune/ inflammatory response. This process is controlled by elaboration of cell attractants or chemokines and cell–interactions between leucocytes and endothelium. The activated and memory cells bear adhesion proteins, chemokine receptors and addressins, which allow homing to and migration into peripheral tissues[47, 48]. (fig. 6)

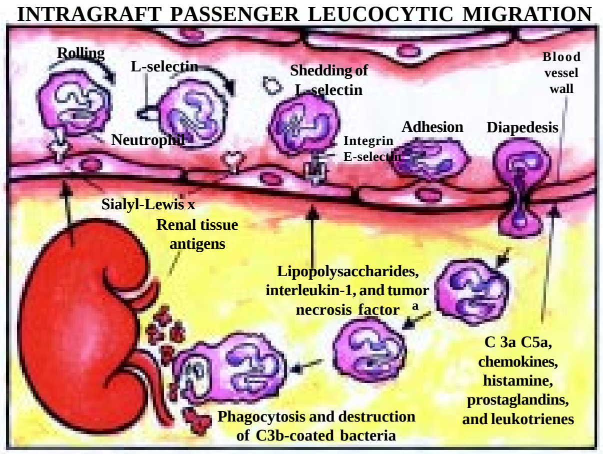

Figure 6 : Migration of activated leucocytes in to the graft

The leucocytic rolling across the endothelium continues for the initial period and is controlled by proteins called selectins. Later on integrins and adhesion protein molecules of immunoglobulin (Ig) superfamilies come to action to arrest their rolling and allow extravasation. The expression of many adhesion proteins is upregulated by pronflammatory cytokines. These proteins include ICAM-1 and VCAM-1 of Ig super family and E–selectin (endothelial specific selectin). The graft becomes attractive to circulating leucocytes even before immune response is generated. Antigen-activated lymphocytes now migrate to extra lymphoid sites and may show their preference for sites where they are most likely to reencounter their specific antigen. Vascular endothelium, rich in MHC class II/ peptide complexes further facilitates this process.

Rejection of histo-incompatible alloantigens is an immunological evolutionary phenomenon essential for humans to coexist with microbial world in this universe. However the same is also a major biological obstacle for transplantation of allografts. It would be naïve to assume that a group of general immunosuppressive drugs will safely block host versus graft immune reactivity without significant morbidity or mortality. The understanding of phenomenon of rejection has taken us closer to designing of strategies to induce donor-specific immune tolerance to allograft antigens and appears to be the only and the final hope for transplantation biologists of this millennium.

ACKNOWLEDGEMENTS:

I am indebted to Ms. Nalini Patel, Ms. Jyotsana Suthar and Mr. Yazdi Wadia for typing and preparation of the manuscript and Mr. Bharat Pandya for illustrations.

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ROLE OF LAPAROSCOPIC SURGERY IN RENAL TRANSPLANTATION

A. K. Hemal , M. Kumar

INTRODUCTION :

The morbidity of surgical procedures has been reduced with the advent of minimally invasive surgery. The first laparoscopic nephrectomy for a renal mass was performed in1990 by Clayman et al [1]. In a relatively short period of time the efficacy and minimally invasive nature of this surgery was evident. Since then laparoscopy has been used to undertake a wide variety of urologic surgeries.

Renal transplantation is the only treatment of end stage renal disease that gives the patient a near normal quality of life. The graft and patient survival is better with living than with cadaveric donor renal recipients. The one and five year graft survival for cadaveric allografts is 81% and 59% respectively, compared to 92% and 76% for live donor allografts [2]. In addition the waiting period for the recipients of live donor kidneys is dramatically shorter than the patients waiting for a cadaveric renal transplant.

In India the cadaveric program is still in its infancy and live donors form the bulk of all renal transplants.

The major disadvantage of living donors is that a healthy person must undergo a major surgical procedure. It is also of paramount importance that surgery poses minimum risk to the healthy patient. The disincentives associated with donation include factors such as prolonged hospitalization, post-operative pain and the cosmetic results of major abdominal surgery. The other major indication for laparoscopic surgery in renal transplant patients is in the management of post transplant lymphoceles.

The technique of laparoscopic live donor nephrectomy in humans was first developed by Ratner et al in 1995 [3]. Since then several investigators have reported their experience with this procedure[4,5,6]. Laparoscopic live donor nephrectomy has resulted in decreased hospital stay, less post-operative analgesic requirements and an earlier return to normal activities.

PATIENT SELECTION :

The donor is carefully evaluated for emotional stability, motivation and undergoes a battery of investigations including radiological and histocompatibility tests. The preoperative evaluation can vary somewhat among the different transplant centres. It is done to ensure that the patient has both normally functioning kidneys and will have normal renal function after unilateral nephrectomy.

During the preoperative evaluation delineation of the vascular anatomy is of paramount importance for laparoscopic surgery. As compared to open surgery, laparoscopic live donor nephrectomy requires a higher degree of resolution of the venous anatomy [7]. Of late, dual phase spiral CT with three-dimensional angiography is being used for pre-operative evaluation. Smith et al [8]. have shown that CT angiography adequately depicts the renal vascular anatomy.

OPERATIVE PROCEDURE:

Both the transperitoneal and retroperitoneal approaches can be used to do laparoscopic live donor nephrectomies.

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Transperitoneal approach:

This approach is similar to the one described by Fabrizio et al [7]. The patients do not undergo any preoperative bowel preparation. The procedure is done under general anesthesia under broad-spectrum antibiotic cover. The bladder is catheterized preoperatively and the patient placed in modified flank position with 45-degree lateral decubitus tilt. Veress needle is used to create the pneumoperitoneum, and initially three transperitoneal laparoscopic ports are placed. The first port is placed laterally at the level of the umbilicus, the second in the midline between the umbilicus and the xiphoid while the third is placed at the umbilicus. Insufflation of the peritoneal cavity is upto 15 cm of water. A thirty-degree laparoscope is introduced through the umbilical port.

The patient is kept volume expanded during the procedure in order to improve renal blood flow and reduce the effects of increased intraperitoneal pressure. Using the two operating ports the ipsilateral colon is reflected medially beginning at the splenic flexure going down to the sigmoid colon, incising along the line of Toldt. The phreno-colic ligament must be divided to allow the colon to be completely reflected medially. The linorenal and splenocolic ligaments at the inferior border of the spleen are divided, allowing the spleen to be retracted superiorly as needed. The Gerota's fascia is exposed and the upper pole of the kidney is mobilized within the Gerota'sfascia. This is done by blunt dissection using the suction tip. During this part of the procedure there is risk of injury to the kidney, spleen and renal hilum,. The hilar vessels come into view once the upper pole of the kidney is freed. Gerota's fascia is incised on the medial aspect of the kidney and the renal vein is dissected. The adrenal, gonadal and lumber veins are identified and ligated. The renal artery is now identified posterior to the vein and freed upto it's proximal origin at the aorta. Topical papavarine may help prevent renal arterial spasm during the dissection.

The lateral, posterior and inferior attachments of the kidney are left intact in order to prevent torsion of the vascular pedicle. After dissection of the renal vein and artery the ureter is dissected from the level of the iliac vessels upwards within the periureteral sheath along with the gonadal vessels, in an attempt to preserve its vascularity. The kidney is then finally mobilized all around and the ureter transected. A midline peri-umbilical, extraperitoneal incision is made (to prevent the loss of pneumoperitoneum) prior to the ligation of the vessels. This helps in rapid retrieval of the organ after ligation of the vascular pedicle.

The vascular pedicle is ligated using a linear stapler. The kidney is removed through the short midline incision after incising peritoneum, immersed in iced saline solution and perfused with cold perfusion fluid.

As advocated by Sasaki et al the risk of complications can be minimized by

1. Identifying the correct plane between the mesocolon and retroperitoneal structures by tracing the gonadal vein as it crosses the iliac vein to the renal vein.
2. Keeping the ureter-gonadal vessels complex intact throughout the length of the graft ureter in order to prevent the risk of ureteral ischemia.
3. Confine renal vein dissection medial to the gonadal and adrenal vein origin so as to prevent injury to the renal pelvis.
4. Transect the lateral attachments and the ureter after the pedicle is free. This minimizes the likelihood of torsion of the renal pedicle and urine is kept out of the field.

Conversion to open surgery may be indicated if there is uncontrolled bleeding, trauma to adjacent organs, difficult anatomy, renal ischemia during the procedure and prolonged dissection time.

RETROPERITONEAL PROCEDURE:

The preparation for the patient is similar to the transperitoneal procedure and the patient is positioned in the standard kidney position. A 2cm incision is made a little below and posterior to the tip of the 12th rib down the thoracolumbar fascia into the retroperitoneal space and the retroperitoneal space created using blunt finger dissection. As with the transperitoneal approach the patient is kept volume expanded and the retroperitoneal space is insufflated to a pressure of 15cm of water, this is in order to ensure good renal blood flow. The second 10-mm cannula is introduced in line with the first port, a little above the iliac crest in order to avoid hindrance to the maneuverability of the cannula by the bone. A third 10-mm cannula is inserted under vision in the midaxillary line two centimeters below the costal margin. During insertion of this third port, special care needs to be taken to prevent the trocar from traversing the peritoneum. A fourth port is inserted posteriorly later in the procedure. Initially the kidney is mobilized within the Gerota's fascia, which is then incised posteriorly, and the renal pedicle is dissected starting posteriorly. The renal artery and vein are freed from their adventitial attachments. The ureter is mobilized within the perureteral sheath along with the gonadal vessels. The rest of the kidney is dissected free from within the Gerota's fascia. Prior to transection of the ureter and the ligation of the renal vascular pedicle the primary port site incision is enlarged as for a flank incision down to the thoraco-lumbar fascia, so that following ligation the kidney can be delivered with minimum delay. Once the kidney is freed all around and the ureter transected the pedicle is ligated and the kidney delivered. Mannitol, fursemide and heparin are given as in all cases of donor nephrectomy. It is important to dissect the kidney completely without undue traction on the renal pedicle. On the delivery of the kidney subsequent management is similar to that described for the transperitoneal route.

POSTOPERATIVE MANAGEMENT:

The patient is transfered to the standard post-operative floor unless otherwise indicated. They are allowed a soft diet on the first post-operative day and are usually on a normal diet by the next day. The Foley catheter removed on the first post-operative day. Most patients are fit for discharge by the third postoperative day.

RESULTS:

Several authors have published their series of the results of laparoscopic live donor nephrectomies. A review of experience with 201 live donor nephrectomies by Shaffer et al showed 4.5% major and 16.5% minor complication rate [9]. Most of the major complications occurred during the early period of development of this procedure learning curve of most surgeons and included bowel injury, ureteral devascularization, retroperitoneal hematoma and epigastric arterial bleeding [10]. Conversion to open nephrectomy often secondary to uncontrollable bleeding has been necessary in 5.7-8.3% of cases [11].

The overall performance of the allograft, measured by post-transplant serum creatinine, urine output, incidences of acute tubular necrosis, rejection episodes and ultimate graft survival appear to be similar to the kidneys obtained by open surgery [10].

POST-TRANSPLANT LYMPHOCELE:

The other major indication of laparoscopic surgery is in the management of transplant patients is in the treatment of persistent lymphoceles. The reported incidences of lymphocele formation in recipients of renal allografts is of the order of 0.5% to 18.1% [12]. Large and symptomatic lymphoceles may cause hydronephrosis, impaired renal function, ipsilateral leg swelling, edema overlying the graft, venous and arterial obstruction and infection [13]. Routine postoperative ultrasound is the best method of detection of the lymphocele. The first line of management of symptomatic lymphoceles is percutaneous drainage. It is however associated with prolonged catheter drainage, risk of infection and protein loss from the lymph and a high recurrence rate (50-80%). Laparoscopic internal drainage of the lymphocele was first reported by McCullough et al in 1991. Since then the overall success rate of the procedure is around 88%.

The procedure of laparoscopic internal drainage of the lympohocele is done via the transperitoneal approach after the placement of a Foley catheter and nasogastric tube. Preoperatively the sac may be filled with methylene blue in order to delineate the lymphocele better at surgery. The ports are inserted at the umbilicus, right midclavicular line just below the umbilicus and the third port in the hypogastrium. After the lymphocele is identified as a bulge any adhesions over it are removed, its wall incised and the fluid aspirated. A part of the wall is then removed and the omentum fixed around the edge of the cavity. This procedure should be avoided if the lymphocele is infected. Injury to the transplanted ureter can occur during the procedure especially if the lymophocele is located posteriorly and inferiorly [14].

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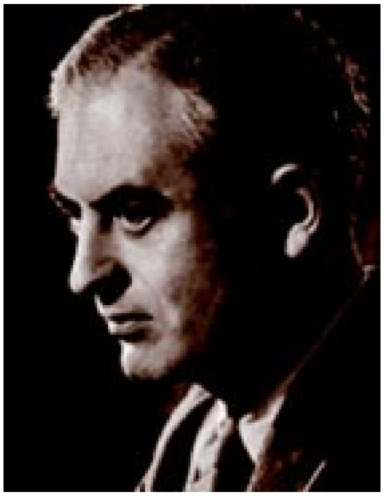

"The human mind treats a new idea the same way the body treats a strange protein; it rejects it!"

The Noble Prize for physiology and Medicine in 1960. (Discovery of acquired immunological tolerance)

- SIR PETER BRIAN MEDAWAR

transplantation
india

CONDITIONING (PREPARATORY) REGIMENS IN BONE MARROW TRANSPLANTATION

Ashwin P. Patel

Bone marrow transplantation (BMT) is now used as a treatment of choice in hemoglobinopathy, chronic myeloid leukaemia, myeloma, immunodeficiency disorders, high risk acute leukemia, relapsed leukaemia and lymphoma. It is now also used for autoimmune disorders and as a part of immune tolerance in solid organ transplantation.

The first unsuccessful bone marrow transplantation was attempted in 1939 in a patient of gold – induced aplasia [1]. First successful marrow was done in 1965 in a patient of acute lymphoblastic leukaemia [2]. Bach FH et al stressed the need of conditioning of the patient with immunosuppressive therapy prior to Bone marrow transplantation [3]. Preparatory regimen was not used in the past for BMT in severe combined immunodeficiency disease (SCID) but these patients may need multiple transplants or intravenous immunoglobulin (IV IgG) therapy. Patients who receive preparatory regimen before BMT don't need IV IgG or further transplants [4, 5].

Purpose of conditioning regimen in allogeneic transplantations (Allo BMT) is twofold: (1) to eradicate underlying disease (2) to provide sufficient immunosuppression to allow grafting of donor cell. Conditioning regimen in autologous transplantation (ABMT) allows maximum dose delivery to the patient. There are various regimens use for the conditioning.

(1) Radiation based regimens:

Initially radiation was given to the whole body (TBI) in a day but it had unacceptably high toxicity. Fractionated radiation delivered over days has reduced morbidity and mortality [6]. 1200 – 1320 cGy is used routinely, though 1575 cGy dose showed decreased relapse rate, it increased toxic deaths and so overall survival remained same [7]. Radiation is combined with cyclophosphamide 120 mg /kg [8]. or etoposide 60 mg/kg [9]. or etoposide + cyclophosphamide [10,11].

Radiation dose to the diseased organ can be increased, without increasing the toxicity, by using monoclonal antibodies tagged with radiation isotopes. Anti-CD 33 and anti- CD 45 conjugated to ^{131}. I were used along with standard preparatory regimen of total body irradiation + cyclophosphamide [12, 13]. Studies are going on to determine whether addition of these targeted monoclonal antibodies increase disease survival or not.

Major toxicity of irradiation is mucositis, lung toxicity and infertility. Table I list some of the commonly used radiation based conditioning regimens.

(2) Non-radiation based regimens:

Most commonly used regimen is oral busulfan, 4 mg/kg/day x 4 days with cyclophosphamide 60 mg/kg/day x 2 days [14].

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This regimen is as good as radiation-based regimen in leukaemia [15, 16]. Busulfan can cause liver damage, pulmonary fibrosis and veno-occlusive disease. Treosulfan is less myelotoxic and hepatotoxic and can be used instead of busulfan [17, 18].

Busulfan + etoposide, BEAM, carmustine + cyclophosphamide + etoposide regimens are used more frequently in autologous setting [19, 20].

Melphalan 200 mg/ M^2 is used frequently in ABMT of Myeloma patients [21].

In aplastic anaemia, cyclophosphamide alone is used in the dose of 50 mg/kg/day for 4 days. Anti-thymocyte globulin (ATG) 30 mg/kg/day for 3 days or TBI is used if patient has received multiple transfusions to avoid graft failure. In Fanconi anaemia, lower dose of cyclophosphamide is used, as stem cells of these patients are very sensitive to chemotherapy.

Table II list commonly used non-radiation-based regimens.

(3) Nonmyeloablative regimens:

Preparative regimens with less intensity are devised with following observations using standard preparative regimens:

- Standard preparative regimens are very toxic and are not suitable for old patients.
- Usefulness of donor lymphocyte infusion (DLI) in relapsed disease after transplantation has shown importance of immune mechanism in eliminating host tumour cells.
- Low intensity regimens may be sufficient for genetic and autoimmune diseases requiring bone marrow transplantation.
- Graft versus tumour (GVT) effect reduces relapse rate as evident by low relapse rate in allogeneic BMT compared to autologous BMT, and high relapse rate in T cell depleted allograft and syngeneic transplants.

Nonmyeloablative conditioning regimens fall in to two categories:

1. **Reduced intensity regimens:** Here aim is to deliver minimum possible dose of chemotherapy/radiation to eliminate Host versus graft (HVG) and obtain mixed chimerism. Here aim of conditioning is not to eliminate all tumour cells. Additional donor lymphocytes are used to eradicate underlying malignancy by immunological mechanisms. Low dose of conditioning reduces toxicity and hence it reduces mortality, morbidity, hospital stay, cost of therapy and can be given in old patients also. This approach is useful in rapidly progressive diseases like leukaemia and lymphoma.

   Fludarabine, busulfan, Anti-T-lymphocyte globulin, micofenolate mofetil, cyclosporin and low dose radiation are used successfully with demonstrable chimerism. Long-term follow-up is needed to comment about disease free survival and efficacy compared to standard regimens [22, 23, 24]. Nonmyeloablative regimens are not useful in the treatment of haemoglobinopathies due to proliferative marrow and sensitization to minor histocompatible antigen from transfusion exposures [25].

2. **Minimally myelosuppressive regimens:** These regimens rely on pre and post transplant immunosuppression to over come HVG reaction. This approach is useful in slowly progressive diseases like CML, CLL and low-grade lymphoma.

   Table III list some of the commonly used regimens.

TOXICITY OF THE REGIMENS

(1) Mucositis:

This is more prominent in radiation-based regimens. Mucositis is treated with painkillers, good oral hygiene and parenteral nutrition in patients who can't maintain caloric intake. Keratinocyte growth factor has shown promising results in the animals [26].

(2) Infection:

Bone marrow transplant patients are more prone to infection due to neutropenia following chemotherapy /radiation, graft-versus-host-disease (GVHD), indwelling catheters and mucositis.

Isolation, hand washing, masking, gowning, sterile diet, gut decontamination, intravenous immunoglobulins (IV IgG) is used to reduce infective episodes. (IV IgG) decreases incidence of interstitial pneumonitis, septicemia and GVHD but does not improve survival [27]. Prophylactic low dose amphotericin and fluconazole helps in reducing fungal infection [28, 29].

(3) Veno – Occlusive disease:

It is characterized by upper abdominal pain, weight gain, ascites and jaundice. Busulfan containing regimen predisposes the patient for veno – occlusive disease if busulfan level reaches an area under the curve of more than 1500 mol/min per litre [16, 30].

(4) Lung toxicity.

BCNU or Carmustine and irradiation to lung are the predisposing factors among preparatory regimens [31, 32]. 20% of patients show restrictive ventilation dysfunction following TBI.

(5) Cardiac toxicity:

1-2 % of BMT patients has cardiac toxicity. Cyclophosphamide predisposes patients for cardiac toxicity [33].

(6) Cystitis

Cyclophosphamide can cause cystitis and bladder fibrosis. Mesna is now used routinely to avoid cyclophosphamide-induced cystitis. Acrolein, a cyclophosphamide metabolite in the body, is responsible for the bladder toxicity.

(7) Growth failure :

Radiation based regimens in children can cause stunted growth in most of the patients [34].

(8) Cataracts :

Patients who receive total body irradiation develop this complication. 80% of patients developed cataracts after unfractionated TBI. Fractionated dose reduces this complication rate to 20% [35].

(9) Hypothyroidism :

50% of patients developed hypothyroidism following radiation-based regimens. It is easily correctable by thyroid hormone replacement [36].

(10) Second malignancies :

The cumulative incidence is 2.2 % at 10 years after transplantation. Patients have 8.3 times more risk of malignancy at 10 years in comparison to general population [37]. Occult cytogenetic abnormalities in the bone marrow prior to bone marrow/stem cell collection can identify patients at higher risk of developing second malignancies [38].

(11) CNS toxicity :

Radiation based regimen can cause cognitive dysfunction [39]. Busulfan can cause convulsions and so anticonvulsant is given along with busulfan.

(12) Sterility :

Almost all females develop sterility following TBI. Oligo-azoospermia is the rule in male patient after conditioning that can improve spontaneously in some patients. Pregnancies are reported in females after conditioning with cyclophosphamide.

(13) Endocrine dysfunction :

30% of children develop growth hormone and adrenal steroid deficiency.

ACKNOWLEGDEMENT:

I sincerely thank all my staff members; Dr. Dinesh Rathod, Harish Kaithwar, Vipul Patel, Sanjay Prajapati, Zuber Sheikh and Vaishali Kapadia, for their help in preparing this manuscript.

TABLE – I

	REGIMEN	DRUGS	DOSE	ROUTE	DAYS
1	**Cy/TBI**	Cyclophosphamide	60 mg/kg	IV in 1 hour	-6, -5
		TBI	2-2.4 Gy, BD		-3 to –1
2	**TBI/VP**	TBI	2-2.25 Gy, BD/TID		-7 to
		Etoposide	60 mg/kg	IV in 2 hours	–4 -3
3	**Cy/TAI**	cyclophosphamide	5-60 mg/kg	IV in 1 hour	-4, -3
		Thoraco-abdominal RT	4-7.5 Gy		-1
4	**Cy/VP/TBI**	cyclophosphamide	60 mg/kg	IV in 1 hour	-6, -5
		Etoposide	30-60 mg/kg	IV in 2 hours	-4
		TBI	2-2.5 Gy, BD		-3 to -1

TABLE II

	REGIMEN	DRUGS	DOSE	ROUTE	DAYS
1	**Bu/Cy**	Busulfan	4 mg/kg	P.O.	-9 to –6
	(Santos)	Cyclophosphamide	50 mg/kg	IV over 1 hr.	-5 to –2
2	**Bu/Cy**	Busulfan	4 mg/kg	P.O.	-7 to -4
	(Tukschka)	Cyclophosphamide	60 mg/kg	IV over 1 hr.	-3, -2
3	**Cy**	Cyclophosphamide	50 mg/kg	IV in 1 hr.	-5 to –2
4	**Cy/ATG**	Cyclophosphamide	50 mg/kg	IV in 1 hr.	-5 to –2
		ATG	30 mg/kg	IV in 8-10 hrs.	-5 to –3
5	**Melphalan**	Melphalan	100-200 mg/m^2	IV in 1 hr.	-1
6	**BEAM**	BCNU	300 mg/m^2	IV in 2 hrs.	-6
		Etoposide	150-200 mg/m^2	IV in 2 hrs.	-5 to –2
		ARA-C	200-400 mg/m^2	IV in 2 hrs.	-5 to –2
		Melphalan	140 mg/m^2	IV in 1 hr.	-1
7	**CBV**	Carmustine	100-200 mg/m^2	IV in 2 hrs.	-8 to –6
		Etoposide	250-800 mg/m^2	IV in 2 hrs.	-8 to –6
		Cyclophosphamide	1.2-1.8 g/m^2	IV in 1 hr.	-5 to –2
8	**ICE**	Ifosphamide	4 g/m^2	IV in 2 hrs.	-6 to –3
		Carboplatin	600 mg/m^2	IV in 24 hrs.	-6 to –4
		etoposide	250 mg/m^2, BD	IV in 2 hrs.	-6 to –4

TABLE - III

	DRUGS	DOSE	ROUTE	DAYS
1	Fludarabine	30 mg/m^2	IV	-10 to –5
	Busulfan	4 mg/kg	P.O.	-6, -5
	ATG	10 mg/kg	IV	-4 to –1
2	TBI	2 Gy (single fraction)		0
	MMF(micofenolate mefotil)	15 mg/kg, BD/TID	P.O.	0 to 27 *
	CSA (cyclosporin A)	6.2 mg/kg, BD	P.O.	-1 to 35 *
	Fludarabine	30 mg/m^2	IV	2 to 4

* MMF and CSA are given for a longer period in unrelated transplant.

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DONOR HEMATOPOIETIC STEM CELL INFUSION IN THYMUS AND PERIPHERY : AN INTEGRATED APPROACH TO ACHIEVE TOLERANCE IN CADAVER RENAL ALLOGRAFT RECIPIENTS

HSC INFUSION IN CADAVER RENAL TRANSPLANTATION

H L Trivedi[1], A V Vanikar[2], V R Shah[3], P R Modi[4], D M Viroja[1], V B Trivedi[2]

ABSTRACT

Introduction- We designed a prospective, randomized clinical trial to evaluate immune response of donor-derived hematopoietic stem cell (HSC) infusion in thymus and periphery to create tolerance in cadaver renal allograft recipients. **Method:** We studied 24 patients divided into two equal groups. In treated group (A), 350 mL of un-fractionated bone marrow (BM) was aspirated from anterior iliac crest of cadaver donors. Two ml of concentrated marrow was infused into thymus, 100 ml into BM before surgery; remaining 250 ml was infused peripherally post-transplantation. Recipients were lymphocytotoxicity cross-match negative in both groups. Group B (controls) received kidney and no HSC. Group A received low dose Prednisolone and Cyclosporine; Azathioprine was added in controls. Average mean cell count of thymic inoculum was 3.3×10^4 cells / cmm and in periphery, 8.6×10^7 cells/ kg body wt. Rejection was treated with standard anti-rejection treatment, tacrolimus was added as a rescue therapy. **Results:** Over a mean follow-up of 703 days of both groups, group A had significantly better graft function with one acute rejection(AR) episode and no CMV infection; mean serum creatinine(SCr) was 1.23 mg.% and no graft/ patient loss. Group B with mean SCr of 2.19 mg % had 3 patients with single AR episode out of which 2 patients were lost following progressive uncontrolled rejection- associated infection. Third patient is maintaining SCr of 2.5 mg %. Actuarial graft survival 2 years post-transplantation was 87.5 % in controls and 100% in group A. **Conclusion:** This novel integrated un-fractionated HSC infusion approach into thymus and periphery to create tolerance is safe, efficacious, gives significantly better graft function with minimal AR/CMV disease, with minimum immunosuppression as compared to controls on standard triple drug therapy.

ABBREVIATIONS

ATN	:	Acute tubular necrosis	GvHD	:	Graft versus Host Disease
AR	:	Acute rejection	HSC	:	Hematopoietic Stem Cells
BM	:	Bone marrow	MP	:	Methylprednisolone
CsA	:	Cyclosporine A	SCr	:	Serum creatinine
ESRD	:	End stage renal disease			

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KEY WORDS

Donor derived hematopoietic stem cells, cadaver renal transplantation, tolerance

INTRODUCTION

Tolerance has been the ultimate dream of transplant scientists for the last three decades. A reasonable success has been achieved in laboratory animals and it is time to take this concept into the clinic. Induction of immune tolerance will reduce the requirement of immunosuppressive drugs and thereby their side-effects, infection and malignancy–related morbidity and mortality. Transplantation will thereby be rendered more cost-effective for the common man with limited financial resources. Immunological mechanisms involved in allograft rejection being quite complex, require a combination of different protocols addressing different pathways of rejection to achieve tolerance. We designed a tailor-made integrated clinical protocol to address the central and peripheral mechanisms of tolerance.

PATIENTS AND METHODS

Study design

We carried out a prospective, randomized, parallel, open-labeled, clinical trial between June 2001 to September 2003 at the Institute of Transplantation Sciences and Institute of Kidney Diseases and Research Centre, to evaluate the effects of donor-derived haematopoietic stem cell(HSC) infusion into thymus and peripheral circulation of our cadaver renal allograft recipients (treated group-A). Controls (group B) were transplanted without HSC infusion. Every time two recipients with end-stage renal disease (ESRD) were selected from cadaver transplantation waiting list with ABO blood group and negative lymphocytotoxicity cross-match. At this point, through randomization process, one patient was selected for HSC infusion and the other was subjected to transplantation directly. All patients were approached with an intention to treat and their informed consent was obtained. This clinical trial was conducted in accordance with the revised Declaration of Helsinki. Institutional Ethics Committee approved the study protocol and consent forms of the clinical trial.

Selection of patients

Twenty four consecutive patients with ESRD from cadaver transplantation list were divided into two equal groups of 12 patients each (table 1). Treated group (group A) received HSC infusion in to thymus, bone marrow and peripheral circulation and controls (group B) underwent renal transplantation without any HSC infusion. Both groups were fairly balanced with respect to clinical profile. They were followed up at the same out patient clinic at weekly intervals for the first three months, fortnightly for the next three months and at monthly intervals thereafter. In every visit, their renal and liver function status was monitored, blood counts were performed and cyclosporine A (CsA) levels were measured. They were monitored at monthly intervals for HIV, HBsAg, HCV and CMV (IgG, IgM antibodies) infection status by using ELISA technique.

Table:-1 PATIENT CHARACTERISTICS

GROUP (n=12)	A (C+P)	B (Control)
Mean follow up (days)	703	703
Age in years (Mean)	32.1 (21-45)	38.3 (20-60)
Gender – M:F	4:8	10:2
Average 3rd party infusions	24	23
Etiology of ESRD		
Chronic Glomerulonephritis	07	06
Diabetic Nephropathy	01	02
Reflux Nephropathy	01	00
Autosomal Dominant Poly-		
Cystic Kidney Disease	02	03
Systemic lupus erythematosus	01	01

Protocol design

Our aim was to inoculate concentrated bone marrow (BM) aspirate in thymus and optimum HSCs in BM and periphery. We wanted to integrate central and peripheral arms of tolerance in clinic. Hence we included thymic infusion in this protocol. Recipients were monitored for development of skin rashes, fever and gastrointestinal symptoms of graft versus host disease (GvHD).

Stem cell collection, inoculation and infusion techniques

BM aspiration was performed from the anterior superior iliac crest of the cadaver donors. First 6 mL of the aspirate was concentrated and 2 mL was kept for thymic infusion. Subsequently, out of 350 ml of the aspirated un-fractionated BM, 100 mL was inoculated into sternal BM of the recipient. Remaining 250 mL aspirate was administered by intravenous infusion set in the peripheral circulation of the recipient at the end of transplantation surgery. Cell counts (including CD34 + cells) of marrow aspirate for thymic inoculum and for periphery/ BM were separately performed.

Thymic inoculation

Under general anesthesia, following transplantation surgery 4 cm. long incision was made into the right second intercostal space. After cutting all muscles, mediastinal fascia was opened and thymus was identified in the retrosternal space. Then 2 mL of concentrated marrow was inoculated with 20 gauze needle. Hemostasis was checked and wound closed.

Recipient immunosuppression

Both groups were administered Prednisolone and Cyclosporine (CsA). Azathioprine was to be added in event of acute rejection (AR). CsA was the principal immunosuppressant for both groups. The doses were adjusted with an intention to maintain trough blood levels around 100 ng /ml. CsA levels were measured by employing EMIT 2000 CsA assay (Syva Co., Dade Behring, USA, intended trough levels: 50-176 ng / mL). Both groups were administered Prednisolone, 0.5 mg/ kg body wt./day for the first month post-transplantation followed by 0.2 mg /kg body wt /day subsequently. Azathioprine, 2.5 mg /kg body wt /day was added following AR episodes and subsequently continued.

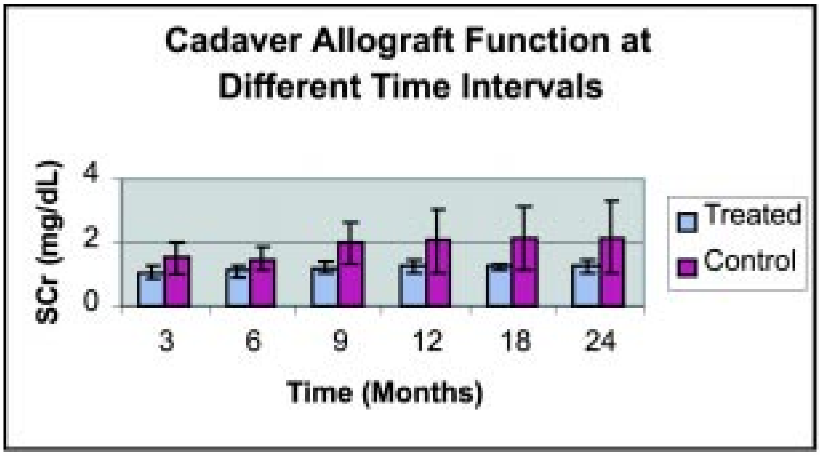
Diagnosis of rejection and its treatment

All recipients were biopsied whenever clinically suspected for AR. Rejection was diagnosed according to the modified Banff criteria[1] and treated with intravenous methylprednisolone (MP), 250 mg/day for three consecutive days. Tacrolimus was used as rescue therapy in MP-resistant rejections.

RESULTS

Average BM aspirate cell count of thymic inoculum was 3.3 x 10^4 cells / cmm, CD34 + cell count was 1.1 %; and of the remaining aspirate was 1.9 x 10^4 cells / cmm and CD34 + cell count was 0.7 %. One patient had procedure related pneumothorax and required under-water intercostal drainage for 24 hours following thymic inoculation.

Over a mean follow-up of 703 days, three (25 %) patients in group B and one (8.3 %) patient in group A had single AR episode each. One patient in both groups had borderline acute tubulo-interstitial rejection along with acute tubular necrosis (ATN) and 2 patients of group B had moderate acute tubulo-interstitial rejection, type 1B with ATN and required tacrolimus for rescue. Average time interval for these biopsies was 10 days post-transplantation. All patients in group A achieved early graft function and required no dialysis support. Three (25 %) patients in group B were supported with dialysis three times a week till they achieved stable graft function. Serum creatinine (SCr) values were used as indicators of allograft function. Comparative study of SCr values of both groups were undertaken at 3, 6, 9, 12, 18 and 24 months post-transplantation (fig. 1).



Time interval (months)	p Value
3	0.005
6	0.002
9	0.0004
12	0.004
18	0.004
24	0.02

Figure: 1 Comparison of graft function of treated group Vs controls in terms of SCr values over 2 years post-transplantation.

It was observed that mean SCr was significantly better in group A than group B. Statistical analysis was performed using student's paired t test. Eight patients in each group have completed 2 years of transplantation. Two patients with their grafts were lost in group B within first 6 months of transplantation. Kaplan Meier analysis was performed to evaluate the actuarial survival of eight patients of each group who had completed 24 months of the study period and qualified for this test (fig. 2).

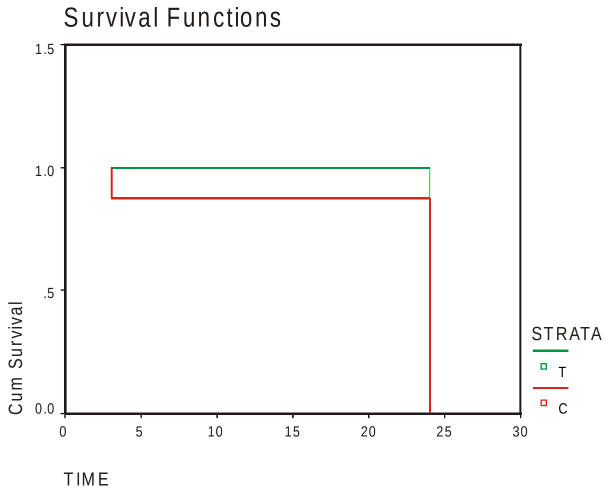


Figure 2: Kaplan Meier analysis of graft survival of treated group Vs controls over 2 years post- transplantation.

It was observed that 100 % patients of group A and 87.5 % from group B were surviving with stable allograft function at the end of two years. None of the patients in group A had GvHD/ CMV or other infections.

DISCUSSION

We had developed a preoperative, non-myeloablative, mega dose, un-fractionated HSC infusion protocol to create tolerance in renal allograft recipients wherein we observed absence of GvHD, hepatic dysfunction, almost AR free, stable allograft function and very low incidence of CMV disease with minimum immunosuppression[2,3]. This experience encouraged us to improvise upon our protocol by adding stem cell inoculation in to thymus and we implemented it in our cadaver renal transplantation program. Engrafted donor-derived HSCs give rise to a chimeric immune system resulting in to tolerance to donor MHC antigens. Based on this hypothesis, we fortified our protocol with intrathymic inoculation of donor derived HSC to achieve donor-specific central tolerance along with peripheral infusion thus addressing both arms of tolerance.

Remuzzi et al had found that intrathymic inoculation of donor antigen and purging of donor-specific allo-reactive T-cells from the peripheral T-cell repertoire was necessary to create classical central tolerance[4]. Instead of adopting this classical technique of peripheral T-cell clonal depletion we had used megadose DBMC infusion to achieve peripheral T-cell clonal depletion and augment lympho-hematopoietic chimerism.

R.D. Owen had observed that paternal bovine twin that shared a common placental circulation - 'a mixture of two distinct types of erythrocytes' can be found long time after birth…describing a naturally occurring state of mixed chimerism[5]. It was further observed by Anderson et al that majority of cattle twins at birth and long after, were fully tolerant of each-other's skin grafts[6]. This observation inspired Billingham, Brent and Medawar to create the first experimental tolerance model in neonatal mice. In this experiment, splenic and HSCs from adult mice were injected in neonatal mice with undeveloped immune system. The infused cells when engrafted, produced mixed chimerism, and skin graft from adult mice to neonatal mice survived indefinitely. This seminal work of cell transplantation in a defenseless host became the base for bone marrow transplantation of the future[7].

Experimental studies suggested that donor-specific unresponsiveness could be achieved by intrathymic inoculation of donor allo-antigens however it was not until the published work of Posselt et al that led to a dramatic renewal of interest in this direction. The intrathymic route is now well-established for inducing a robust donor-specific tolerance and now the interest is turned towards elucidating the mechanisms involved in it; chiefly clonal deletion followed by clonal anergy and generation of regulatory T-cells [8-11].

There are no reliable laboratory markers to measure the degree of tolerance in clinic. However we have demonstrated direct relationship between the dose of stem cells infused and the degree of tolerance measured by absence of rejections and quality of allograft function[12]. Monaco has observed that dose of stem cell infusion directly affects the development of level and duration of chimerism[13]. Zinkernagel has envisioned the use of donor hemato-lymphopoietic cells as the key therapeutic maneuver to achieve stable drug-free antigen-dependent T-cell exhaustion and chimerism[14]. It will therefore be logical to infer that chimerism is strongly predictive of successful tolerance induction.

CONCLUSION

This innovative approach of thymic plus peripheral infusion of unfractionated donor-derived HSC to achieve tolerance in cadaver transplantation is safe, efficacious, without GvHD and gives stable and significantly better graft function with minimum rejection and no CMV infections, as compared to controls.

ACKNOWLEDGEMENTS:

We are indebted to Ms. Priyadarshini Chaudhary, Ms. Nalini Patel, Mr. Bharat Pandya and Mr. Yazdi Wadia for updating patient charts, statistical analysis and preparation of the manuscript.

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SAFETY AND EFFICACY OF SHORT TERM SIROLIMUS THERAPY IN RENAL ALLOGRAFT RECIPIENT WITH ACUTE REJECTION AND WITH DELAYED GRAFT FUNCTION – A SINGLE CENTRE EXPERIENCE

B B Mehtalia, A R Mehta, N H Shah, J Vakil, H L Trivedi

INTRODUCTION AND OBJECTIVE: To study safety of sirolimus as cyclosporine (CsA) sparing agent in renal allograft recipients with delayed graft function.

To study efficacy of sirolimus as rescue therapy in steroid resistant acute rejection during early post transplant period.

METHODS: In a retrospective analysis 34 patients who received sirolimus therapy during March 2001 to April 2003 were included. All patients were started on standard triple drug immunosuppression including Pred. + CsA + MMF / Aza.

Sirolimus was used to treat steroid resistant acute rejections as well as in patients having delayed graft function due to ATN. Graft Biopsy was done in all patients. Average duration of therapy was 3 – 4 weeks in patients with rejection and 2-3 weeks in patients with ATN. A loading dose of 5 ml was given to all patients and then continued on 1.5 – 3 ml per day.

RESULTS: 23 patients had steroid resistant acute rejection including 18 with vascular and 5 with cellular rejection. All patients responded to therapy with partial response in 5 patients. Mean Scr decreased from 2.89 to 1.76.

CsA was rapidly tapered off in 11 patients receiving sirolimus for delayed graft function and was restarted once Scr improved. None of the patients had acute rejection during CsA free period.

Adverse events recorded were CMV infection in 2 patients, CMV and fungal coinfection in 1 patient and interstitial pneumonia in 1 patient. We lost 1 patient due to CNS infection. None of our patients had leucopenia or thrombocytopenia.

CONCLUSION: Sirolimus was effective in reversing steroid resistant acute rejection in majority of patients. CsA could be safely withdrawn in patients with ATN treated with sirolimus.

This abstract was presented in the 14th Annual Conference of Indian Society of Organ Transplantation in August, 2003.

Institute of Transplantation Sciences and Institute of Kidney Diseases and Research Centre, Civil Hospital Campus, Ahmedabad, Gujarat, India.

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POST TRANSPLANT DIABETES MELLITUS IN RENAL ALLOGRAFT RECIPIENTS – A SINGLE CENTRE EXPERIENCE

M R Gumber, P A Thaker, B B Mehatalia, N H Shah, A R Mehta, H L Trivedi

INTRODUCTION: Post-transplant Diabetes Mellitus (PTDM) has emerged as one of the major risk factors of immunosuppression in renal allograft recipients, by adversely affecting allograft and patient survival. Early diagnosis and aggressive management may help in preventing long term complications. The incidence of PTDM has dropped to less than 10% as compared to 4 to 46% in pre-Cyclosporine era. Conclusive risk factors are use of steroids and calcineurin inhibitors, higher recipient age, family history of Diabetes Mellitus (DM) and pre-transplant glucose intolerance. Role of obesity, cadaveric donor and HLA – A 28, A 30, B 18, Bw15, Bw18 and Bw42 are controversial.

In early post-transplant period, steroids predominantly induce insulin resistance whereas CsA and Tacrolimus are toxic to b cell activity demonstrated by decrease in DNA and mRNA synthesis resulting into depleted insulin secretion.

PATIENTS AND METHODS: In retrospective analysis performed on renal allograft recipients between January 1998 to April 2003, we evaluated 793 patients who received steroids and CsA as immunosuppressants. Diagnosis of PTDM was made if a patient required anti-diabetic drugs after transplantation. Various risk factors including age, gender, hepatitis C virus infection, immunosuppressive drugs, etc. and their relationship vis a vis PTDM was evaluated.

RESULTS: Thirty five (4.41 %) patients were diagnosed as having PTDM with predominance in males (4.84 % versus 2.25 % in females), in older patients (9.92 % above 45 yrs v/s 3.22 % in patients below 45 yrs of age) and in HCV positive patients as compared to non-HCV patients (7.63 % v/s 4.41%). Fifteen (44.11 %) patients received anti-rejection therapy with methylprednisolone. Twenty two (62.84 %) patients developed PTDM within 6 months of transplantation.

CONCLUSION: Close and regular blood sugar monitoring is recommended in renal allograft recipients especially those on triple drug immunosuppression and who received anti-rejection therapy. More frequent monitoring is recommended in first six months post-transplantation. Hepatitis C virus positive patients were more prone to develop PTDM.

ABBREVIATION: DM – Diabetes Mellitus, PTDM – Post-transplant Diabetes Mellitus,

This abstract was presented in the 14th Annual Conference of Indian Society of Organ Transplantation in August, 2003.

Institute of Transplantation Sciences and Institute of Kidney Diseases and Research Centre, Civil Hospital Campus, Ahmedabad, Gujarat, India.

TRANSPLANTATION INDIA

Editor-in-chief:
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