Kidney Transplant: Current Practice and New Potential

Because of the use of newer available therapies, 5- and 10-year kidney allograft survival rates have improved. Chronic rejection remains the most common cause of graft failure. Moreover, current immunosuppressive drugs, such as calcineurin inhibitors, azathioprine, Mycophenolate mofetil and prednisone have significant side effects associated with long-term use. While acute allograft rejection can be successfully treated and reversed, there remains no decisive treatment for chronic rejection, and graft tolerance is difficult to achieve in humans. A continued goal is to develop sensitive methods to monitor and predict the host response to the allograft to determine the optimum immunosuppressive regimen and doses required to minimize drug-related toxicities and potentially prevent immunological graft failure and develop new agents with fewer side effects. More work is necessary to understand the basis of immunologic tolerance in humans.

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BARRIER TO GRAFT ACCEPTANCE

Tissue incompatibility at the major histocompatibility complex (MHC) between a donor and recipient of an organ transplant is capable of inducing strong rejection of the donor tissue. The strength of rejecting foreign tissues from the same species (allograft rejection) is more than 100 times harder to treat than eliminating a small foreign protein, such as that found in bacteria or viruses. This attests to the pivotal function of the immune system in discriminating between self and non-self tissues. The rejection process starts by recognizing the foreignness of the graft tissue. (Figure 1 on page 13) The recognition process occurs via direct and indirect pathways. In the indirect pathway, antigen-presenting cells (monocyte and dendritic cells) recognize small determinants disintegrated and shed from the foreign cells. After phagocytosis, the antigen-presenting cells present the modified foreign protein to the recipient T helper (Th) cells. The direct pathways by which we perceive graft foreignness is the direct recognition by the recipient T helper cell of the differences in the MHC exressed on foreign cells (direct recognition). T helper cells are considered the center of the immune response. After receiving the message of tissue foreignness, a cascade of intracellular activation events takes place inside T helper cells, including increase in the intracellular free calcium, thereby activating calcium-dependent enzymes. Calcineurin, a collumedulin-dependent phosphatase, plays a key role in T helper cell activation, which leads to enhanced cytokine production, such as interleukin 2, interleukin 4, interferon gamma, etc. In addition, the cell membrane adhesion molecules increase in number. The cell membrane adhesion molecules are important for direct cell-to-cell interactions. The increased cytokine production and cell membrane adhesion molecules by T helper cells lead to the recruitment of effector cells (cytotoxic T and B lymphocytes) to destroy the graft.

Based on this information, it is clear that an optimal strategy to prevent organ rejection would be to selectively disrupt the immune activation pathway at the point of recognition of the allograft and/or to inhibit the specific T helper cells intended to react against foreign cells, while sparing other helper cells, which must react against other stimulation by other foreign cells or anitgens. Similarly, such a strategy may be used to inhibit the T cytotoxic cells or B lymphocytes, which react against the specific organ, but to spare other T cytotoxic cells and B lymphocytes, which must be prepared to act against other antigenic challenges.

An optimal immunosuppressive drug for use in organ transplantation should have a minimum toxicity profile.

ALLOGRAFT REJECTION

Rejection of an allograft can occur within a few hours (hyperacute), due to the presence of antibodies determined against the foreign allograft. An aggressive immune response can lead to the generation of de novo antibody and cytotoxic cell responses within a few days (accelerated rejection). Acute rejection is usually cytotoxic T cell-dependent and occurs within days to months after transplantation. Late actue rejection may occur due to lack of compliance of patients to receive their immunosuppressive drugs. Chronic rejection occurs within a few months and leads to late graft failure. It is defined as a gradual and unrelenting decline in graft function associated with specific histopathological findings in the allograft, which include inflammatory infiltration of the interstitium, obliteration of the small- and medium-sized blood vessels in the graft and thickening of the glomerular-based membrane. Patients with chronic allograft rejecction present usually with increasing proteinuria in addition to elevation in serum creatinine. Causes of chronic allograft rejection include ongoing low levels of immune activation and non-immune factors such as hypertension, hyperlipidemia, diminished renal reserve due to repeated insults by processes such as previous acute rejection, pyelonephritis and prolonged ischemic time prior to transplantation.

CURRENT CHEMICAL IMMUNOSUPPRESSIVE FUNCTIONS

I. Calcineurin Inhibitors

Cyclosporin A (CsA - Neoral) is central to many immunosuppressive regimens. The clinical efficacy of any new agent must be compared to that of CsA before it is approved for general use in organ transplantation. Many of the immunosuppressive effects of CsA are thought to be due to its inhibition of the early events of T lymphocyte activation, such as lymphokine gente transcription in response to signals initiated at the antigen receptor. CsA specifically inhibits the deoxyribonucleic acid (DNA) binding activity of several cytoplasmic and nuclear proteins, which are important in transcription activation of the genes for IL-2 and its receptor, as well as several other lymphokines.

The principal receptor for CsA is the 18-kD protein, cyclophilin. The binding of CsA to cyclophilin is the first step in its immunosuppressive action within the cell. The terminal phase of intracellular signal transduction requires activation/expression of DNA-binding proteins (transcription factors) that trigger gene transcription. Calcineurin, a calcium-dependent phosphatase, plays a key role in activation of factors required for IL-2-gene transcription. The molecular target of calcineurin is a component of the trancription complex that must be assembled and/or activated for the coordinated expression of early T-cell activation genes. One extensively studied candidate is the nuclear factor of activated T cells (NF-AT). The dephosphorylated NF-AT form enters the nucleus, where it combines with another protein to form an active nuclear factor, which binds to the IL-2 promoter of the IL-2 gene. Cyclosporin causes dose-dependent inhibition of calcineurin activity. Cyclosporin-immunofillin complex binds to calcineurin and prevents its availability for dephosphorylation (activation).

Although CsA improves one-year graft survival and decreases acute cellular rejection by about 30%, its impact on long-term graft survival and chronic rejection has not been demonstrated. Side effects associated with long-term use of CsA include nephrotoxicity (50%), hepatotoxicity, neurotoxicity, hyperlipidemia, hypertension, hypertrichosis, gingival hypertrophy and lymphoid cancers. Monitoring CsA levels 2 hours after the does gives a better indicator for the dose required to maintain a rejection-free state and decreases dose-related side effects.

FK506, along with rapamycin (discussed later), has structures similar to the macrolide compounds. The biologic activity of FK506 closely parallels that of CsA, while the spectrum of action of rapamycin is distinct.

FK506 binds to the cytosolic receptor (immunofilin) designated as the FK-binding protein (FKBP). Four such isoforms have been described in this cytosolic compound; however, FK506 binds mainly to the isoform FKBP 12, which mediates its immunosuppression. The immunofilin-ligand complex interacts with the catalytic subunit A of the calmodulin-dependent phosphate calcineurin, resulting in inhibition of calcineurin activity. Calcineurin activity is highly linked to IL-2 production (see above). FK506 inihibition of cell activation requires a rise in intracellular-free calcium concentration. Although rapamycin binds similarly to intracellular immunophilins, it inhibits cell activation-signaling pathways in a calcium-independent pathway.

FK506 has been utilized as the primary immunosuppressor in at least two series. Both graft and patient survival were similar to that seen with CsA therapy. Toxicity consisted of renal impairment, gastrointestinal complaints, hyperkalemia, tremor and hyperglycemia.

Corticosteroids (14), used for clinical immunosuppression, are synthetic glucocorticoids, which interfere with macrophage function, thus inhibiting antigen processing from macrophages and presentation. They inhibit the synthesis and release of IL-1, which enhances the T helper cell's recognition of the antigen presented on the antigen-presenting cell. IL-1 also has many other functions, including promoting B lymphocyte action and activating many of the proinflammatory homeostatic mechanisms.

Glucocorticoids also inhibit the production of IL-2 and its receptor, decrease immunoglobulin serum levels through increased catabolism and diminished synthesis and may slow the generation of cycotoxic T lymphocytes. The cumulative toxicity of long-term steroid therapy can lead to hypertension, diabetes mellitus, osteoporosis, aseptic necrosis of bones, increased incidence of infection, fluid retention and increased capillary fragility.

Azathioprine (15) was the most widely used immunosuppressive drug until the clinical introduction of CsA in 1979. Azathioprine combined with prednisone and CsA as triple therapy is a widely used regimen in clinical organ transplantation. After absorption, azathioprine is cleaved to mercaptopurine (6-MP), principally by red blood cell glutathione. 6-MP is then converted to a series of mercaptopurine-containing nucleotides, among them thioguanylic acid, which interferes with the synthesis of DNA, and polyadenylate-containing ribonucleic acid (RNA). By interfering with lymphoid cell mitosis, azathioprine affects the division of activated B and T lymphocytes. Long-term side effects of azathioprine therapy include bone marrow suppression, hair loss, hepatotoxicity, megaloblastic anemia, skin fragility and increased incidence of lymphoid cancer.

Mycophenolate mofetil (CellCept) (16, 17) is the 2-(4-morpholino) ethyl ester of mycophenolic acid (MPA). It is rapidly absorbed following oral administration and selectively and reversibly inhibits inosine 5'-monophosphate dehydrogenase and, therefore, inhibits the de novo pathway of purnie synthesis in T and B cells. Unlike most cells, lymphocytes rely on the de novo pathway more than the salvage pathway (hypoxanthine guanine phosphoribosyl transferase) for purine byosynthesis. The most important side effect is G.I. toxicity. This agent's efficacy has been shown in recent pivotal studies.

Rapamycin (18) also has a structure similar to macrolide compounds. Animal studies deomonstrate rapamycin to be 50 times more potent, and synergistic with, CsA. It has a demonstrated beneficial effect on patient and graft survival in animal models undergoing a variety of organ transplants. The toxicity of rapamycin includes is affecting the gastrointestinal tract with anorexia, vomiting, diarrhea and hyperlipidemia as the main presentation. Diabetes mellitus, myocardial necrosis, testicular atrophy and significant reduction in weight gain are reported with the use of the drug. It has no detrimental effect on renal or liver function. Rapamycin is produced by Wyeth-Ayerst and is approved for clinical use in transplantation.

NEW INVESTIGATIONAL CHEMICAL AGENTS (19-21)

Mizoribine's chemical structural analysis was determined to be 4-carbamoyl-1-b-ribo-furanosl imidazole-5-olate. It exerts its immunosuppressive function by inhibition of guanosine 5'-monophosphate synthesis. Its immunosuppressive effect is observed only in cells in which purine is synthesized significantly. It suppresses both cell-mediated and humoral-mediated immunity. It is expected that mizoribine may have clinical applications similar to azathioprine; however, it is a much more gentle drug, causing less inhibition of bone marrow cells.

Deoxyspergualin (DSG) and deoxymethylspergualin (DMESG) are antibiotics, which were purified from culture filtrates of the cells' laterosporus in 1981. Their immunosuppressive action may involve the specific inhibition of the proliferation and differentiation of cell-mediated lymphotoxicity. They also inhibit primary and secondary antibody formation. In a clinical study of renal allograft cellular rejection, DSG reversed acute rejection and early chronic rejection.

Leflunomide, an isoxazole derivative, originated from a series of compounds designed as agricultural herbicides. Based on experimental evidence in murine models, the compound has been found to be equal or superior to CsA in its ability to inhibit B cell-mediated autoimmune diseases. Leflunomide is under investigation in patients with rheumatoid arthritis, and it may have a promising role in immunosuppression of organ transplantation. Its mode of action includes inhibition of tyrosine kinase, leading to disruption of messenger RNA IL-2 receptor signaling.

FTY720 is a metabolite of the ascomycete isaria sinclairii. It acts by preventing infiltration of donor lymphocytes into the graft by sequestration of circulating lymphocytes into the spleen and lymph nodes, but not into the foreign graft. It is produced by Novartis and is expected to be available for clinical use within one to two years.

POLYCLONAL AND MONOCLONAL ANTIBODIES (MOB) (22-25)

Cell membranes are studded with many constitutive receptors that are necessary for cell activation. Further, after the initial activation phase, many of these constitutive molecules, as well as other adhesion molecules, are exposed in large numbers on cell membranes. The increased expression of these molecules is necessary for optimum cell activation and cellular interactions. Targeting these cell surface molecules by bioreagents has been used as a therapeutic option to block cell activation. Of the constitutive molecules important for T cell reaction to foreign cells, the CD3, CD4, CD8, CD45, HLA and the IL-2 receptors are important targets. Of the adhesion molecules important for efficient T cell response to foreign allografts, the lymphocyte function-associated antigens (LFA family), CD7, CD28, intracellular adhesion molecule (ICAM) family are important. Several monoclonal and polyclonal antibodies have been used to target these relevant antigens in order to block allograft rejection.

When used early, the nephrotoxic properties of Cyclosporin may prolong early renal allograft dysfunction, increasing the requirement for dialysis and resulting in a poorer long-term graft function. As a sequence, many centers have elected to use polyclonal or monoclonal antibodies to prevent early rejection and allow renal function to become established before starting Cyclosporin. The other clinical therapeutic indication for antilymphocyte bioregents, in the field of transplantation, is to treat steroid-resistant graft rejection.

Monoclonal Antibodies against T-Cell Receptor (26-31)

OKT3 is the only monoclonal antibody presently approved for routine use in clinical transplantation. It is a mouse monoclonal antibody that binds to the å chain of the T-cell receptor complex. Its clinical efficacy in induction therapy

and treatment of rejection is well established. The two major problems associated with OKT3 include the development of antibodies against OKT3 as a murine protein. The human antimurine OKT2 antibodies generally peak one to two weeks after the first course of therapy and may reduce the efficacy of a second course. Much of the human antibody against OKT3 is directed against the constant region of mouse monoclonal antibodies. By the use of genetic engineering, the immunogenicity of murine monoclonal antibodies, such as OKT3, can be decreased by the construction of a chimeric molecule, which has a human constant region, Fc, and a mouse variable region. This region is essential to its immunosuppressive function. Humanized monoclonal antibodies constructed by genetic engineering consist of human amino acid sequences, except at the hypervariable regions where the original murine amino acid sequences are present. Such humanized monoclonal antibodies are not immunogenic for humans. Humanized monoclonal antibodies are expected to have a very long half-life in the human circulation. Although humanized OKT3 has been constructed, clinical trials have not yet been reported.

The second problem with OKT3 is the potentially dangerous "capillary leak," or "first-dash dose" sydrome. When OKT3 cross-links monocytes to T lymphocytes, T lymphocytes will be initially and transiently activated, producing IL-2, interferon ã and the human tumor necrosis factor among other cytokines. The first-dose effect is the result of this initial transit activation of T lymphocytes. The first-dose reaction is expected to be alleviated if the Fc region of the OKT3 is altered such that transient T cell activation is prevented. This can be achieved by usage of the new engineered form that has constant fraction (Fc) regions that do not bind to moncytes.

Several other monoclonal antibodies directed against CD3 or T-cell receptor monomorphic epitopes have been tested in human renal transplantation. The efficacy of these antibodies remains to be seen.

Monoclonal Antibodies against Interleukin-2 Receptors (32,33)

Interleukin-2 has been well documented as a major pivotal growth factor for T-lymphocytes. After it binds to its receptor, IL-2 triggers the activation of protein and lipid kinases, translocation into the cytosol of serine and threonine kinases. These events lead to the expression of several DNA-binding proteins and to the progression of the cell cycle. Antibodies that block IL-2 binding to its receptor are potent inhibitors or IL-2-driven lymphofilteration. Simulect and Zinopax are available in clinical practice.

Polyclonal Antibodies (34, 35)

The current polyclonal antibodies in clinical practice include the equine (horse) and rabbit antilymphocyte preparations. The immunogens are used to produce these antibodies include thymocytes, unactivated lymphocytes and T and B lymphoblasts. In Canada, Connaught Laboratory Ltd. and Senstat Canada Ltd., Mississauga, Ontario, produce both the rabbit and horse anti-human thymocyte immunoglobulins. Upjohn Ltd. produces the rabbit antithymocyte immunoglobulin. Fresenius, in Oberursel, Germany, produces rabbit anti-human T lymphoblast globulin. These bioreagents act primarily by interfering with functional lymphocyte surface molecules and by increasing the susceptibility of the targeted lymphocytes for destruction by macrophages. Prophylactic administration of polyclonal antibodies is a longstanding technique for suppressing acute rejection in organ transplantation. In clinical studies, anti-human polyclonal antibodies have reduced the frequency and intensity of graft rejection and reverse steroid-resistant rejection.

Contraindications to their use includes known allergies to equine or rabbit serum, bacterial, viral or mycotic infections, significant thrombocyopenia or leukopenia and pregnancy. Side effects include anaphylactic reaction, hypotension, serum sickness, fever, urticaria, leukopenia and thrombocytopenia. These antibodies bind to several cell surface molecules necessary for cell activation. Recently, rabbit antilymphocyte globulin (RAT) has been shown to bind to CD6, CD16, CD18, CD28, CD38, CD40 and CD58 surface antigens with a titre above 1:4000, most of which are not T cell-specific antibodies. RAT was found also to bind to at least another 15 other service epitopes that are present on T and B lymphocytes, as well as moncyte, thymocytes, natural killer cells, leukocytes and dendritic cells. Therefore, inhibition of cellular function other than those important in allograft rejection is unavoidable.

Compared to horse preparation, rabbit antithymocyte may have a few advantages. It spares B lymphocytes to a greater extent. Hence, antibody production against infective agents is relatively spared. It predominantly affects CD4-positive T lymphocytes. It produces lymphocytopenia for a much longer time and spares the other leukocytes. Prospective controlled studies have shown that polyclonal antibodies are as effective or superior to OKT3 in prevention and treatment of allograft rejection. Difficulties in lot standardization of the anti-lymphocyte globulin preparations are associated with variations in potencies and side effects from contaminating antibodies. The limited availability of these reagents, the non-specific immunosuppressive action and the advent of monoclonal anti-T cell antibody as a modern alternative have reduced their clinical use. These issues are among the concerns that led to the development in the mid-1980s of OKT3.

IMMUNOSUPPRESSIVE STRATEGIES

Induction Phase

Potent immunosuppression is initially required to prevent acute rejection. There is no universal regimen that suits all patients.

A combination of different drugs is usually used to minimize their side effects. A combination of high dose steroids, Mycophenolte mofetil and Cyclosporin, or FK506, is a common regimen given to patients at average risk to reject an allograft. Patients who are known to have higher risk to reject an allograft usually receive the above combination in addition to a polyclonal or monoclonal antibody. Newer regimens to spare immunosuppressive-relat-

ed toxicities are currently under investigation. These regimens include sparing corticosteroids altogether, or rapid tapering of it and sparing or rapid tapering of the calcineurin inhibitors.

Maintenance Phase

Once patients are stable on the induction regimen, tapering of the immunosuppressive drugs can be initiated a few moths after transplantation. It is a common practice to maintain patients on at least two agents. A classical maintenance immunosuppressive regimen includes Cyclosporin 3 to 5 mg/kg b.i.d. to maintain a trough level of 100 ug/L and two hour levels of 600 to 800 umol/L in addition to mycophenolate 1/2 g b.i.d.

TREATMENT OF ACUTE REJECTION

High dose intravenous Solu-Medrol (5 to 15 mg/kg body weight for three to five days) is usually used to treat acute rejection. If failed, then monoclonal or polyclonal antibodies are usually used.

COMMON COMPLICATIONS AFTER KIDNEY TRANSPLANTATION (36-48)

Nephrotoxicity

Caused by calcineurin inhibitors, it is one of the complications of chronic allograft dysfunction.

Hypertension

It occurs in 60 to 80% of patients. Causes of post-transplant hypertension are multifactorial and include decreased renal function, calcineurin inhibitor, acute and chronic rejection and the presence of old, scarred kidneys.

Hyperlipidemia

LDL and VLDL cholesterol and apo-lipoprotein B increase in 50% of patients post-transplantation. HDL cholesterol may be low, and the lipoprotein A level may be normal or high. The cause of hyperlipidemia is multifactorial. Prednisone and CsA are independent risk factors. Other lipid abnormalities include hypertriglyceridemia. Many patients require HMG-COA reductase inhibitors, such as an atorvastin or Lovastatin. Patients with hypertriglyceridemia may require fibric acid analouges.

Obesity

It occurs in at least 50% of patients. It is the result of removal of the uremic toxins prior to transplantation and secondary to immunosuppressive treatment, especially Prednisone.

Increased cardiovascular accidents

Coronary artery disease is the most common cause of mortality after transplantation. In addition, the prevalence of cerebral vascular disease is increased after transplantation. Therefore, monitoring for symptoms due to underlying coronary or cerebral artery diseases should continue on a regular basis after transplantation. When more than 80% narrowing of the cartoid arteries is documented, the patient should be evaluated by a vascular surgeon.

Recurrence of original renal disease in the graft, such as glomerulonephritis and diabetes

10 to 20% of chronic allograft is due to recurrence of the initial disease.

Malignancy

The risk of malignancy doubles as seen in the general population. Cancer of the skin, lip and non-Hodgkin lymphomas have been closely associated with the cumulative immunosuppressive doses post-transplantation. Non-Hodgkin lymphoma specifically has an increased relative risk of up to 40-fold and can occur from a few months after transplantation up to several years after.

Increased risk of infection

Most common is the reactivation of viruses, such as CMV infection. Therefore, prophylaxis against reactivation of CMV is routinely practiced. Symptomatic CMV infection develops in 50% of patients with primary infection from their respective donors. Reactivation of hepatitis C may produce serious hepatitis. Patients are also at higher risk for bacterial infection.

Diabetes mellitus

It occurs in 4 to 20% of patients. Patients on FK506 have higher risks of developing diabetes as compared to patients on CsA.

Gastrointestinal complications

After transplantation, the risk of gastrointestinal toxicity increases due to complex issues, such as the toxic effect of immunosuppressive therapy, increased risk of infection and toxicity of liver.

Osteoporosis

It occurs in at least 60% of patients. Major fractures may occur up to 10%. Therefore, it is recommended that all patients receive biphosphanate therapy as a prophylaxis against osteoporosis. Bone densimetric measurements are performed annually in some centers.

Other medical complications

These include complications secondary to immunosuppression, such as increased risk of infection. Vaccination against the common pathogens is usually recommended, such as pneumovax and the flu vaccine. Others include gum hyperplasia, secondary to Cyclosporin therapy, neurotoxicity due to calcineurin inhibitors, cataract formation and cushingoin changes secondary to corticosteroids.

Surgical complications

They include stenosis of the transplant renal artery or vein, obstruction secondary to ureteral fibrosis, serous or lymphatic fluid collected around the kidney or uretric fistula.

FUTURE STRATEGIES (49-52)

Immunological monitoring of graft function

Various efforts have been made for early diagnosis of rejection by checking the host immune response. Enumeration of activated cytotoxic T cell products in the peripheral blood or in the urine have proved useful as a non-ivasive method to monitor graft rejection. Similarly, monitoring of surrogate markers present on activated T helper cells in the peripheral blood have been shown to correlate with acute and chronic rejection.

Induction of transplant organ tolerance

Tolerance means that the immune system is anergic to a particular graft but able to reject any other organ. There is good evidence that T cell energy may result after incomplete signaling of the T cell by the antigen-presenting cells. This goal can be achieved by blocking specific cell markers on T cells, such as the CD28 molecule, IL-2 receptor, LFA-1 and ICAM-1 adhesion molecules. Monoclonal antibodies to these molecules are expected to be tested in the near future.

Intrathymic injection of donor cells has been shown to induce a specific tolerance in some animal models. Injection of donor cells may have a future application in organ transplantation.

Immunotoxins as alternative therapy

Attachment of a toxin to the monoclonal antibody results in the delivery of a toxic molecule to the target cell, which is killed by intoxication rather than by complement-mediated lysis.

Generation of human monoclonal antibodies

Many human antibodies have been developed, and some have been applied clinically. Human antibodies display important advantages over their murine counterparts. They are immunologically tolerated, while mouse immunoglobulin G elicits an immune response to prolonged therapeutic use. They are endowed with antigen-specific properties more representative of those that occur physiologically in vivo, and they display effective in vivo biologic functions, including enhanced antibody-dependent cellular cytotoxicity due to a higher affinity for their receptors on effector cells of the immune system.

Xenotransplantation

It is obvious that we should aim at strategies that increase the donor pool. We should act together to raise awareness to the need for the importance of transplantation and encourage living-related transplantation. Even with increased Living Related Donors (LRD), we still need other innovative strategies, such as xenotransplant as an alternative strategy to meet the demand for organ transplants.

Xenotransplantation is the transplantation of living organ cells or tissues from one species to another, such as from a pig to a human. Pig heart valves and insulin have been used in humans for medical purposes for many years. Attempts at transplanting live animal organs to humans, however, have been unsuccessful because they are rejected by the human immune system.

Xenotransplantation is not a recognized medical practice in Canada or in other industrialized countries. However, some countries have allowed limited clinical trials because the shortage of organs and tissues is so severe. To date, Health Canada has not received any requests for clinical trials or xenotransplantation.

The reemergence of xenotransplantation as a therapeutic option for patients with end-stage organ failure has raised difficult social and scientific questions. Xenografts have been able to support human life for an extended period. It is this fact that investigators wish to exploit in clinical bridging studies. If one views bridging strategies as a first feasibility test, then cross-species transplantation does offer the possibility of eventual long-term organ replacement.

Recently, there has been increased interest in xenotransplantation because of advances in anti-rejection drugs, progress in the field of biology and a severe shortage of human organs. Investigators at the University of Pittsburgh reported two cases in which they transplanted the pig liver into a human recipient, obtaining a 70-day survival in their first reported case and a 26-day survival in the second. Other investigators also showed a limited but encouraging success.

One of the long-term opportunities offered by xenotransplantation, besides a solution to the problem of organ shortage, is the chance to develop truly graft-specific ways to enchance graft survival, thereby reducing the need for systematic immunosuppression.

Challenges in xenotransplantation include aggressive antibody mediated rejection of the xenograft, intravascular thrombosis and implications of cross-species infection in the transplant setting. Overcoming the immune barrier requires diverse research efforts for developing novel approaches to prevent the production of anti-xenograft rejection. Antibodies against pig antigens mediate much of the pig xenograft rejection process in humans and in evolutionary-related primates. Studies have established the feasibility of several potential approaches to inhibit thrombosis by manipulation of porcine endothelial cell (AC) antigens. Genetic manipulation of porcine AC has been used to inhibit the expression of pro-coagulant genes present in porcine AC. Critical questions that need to be addressed for assessing the risk of infection after xenotransplantation (zoonotic infection) include: What is the capability of viruses, such as retroviruses of gross species infection to human? Will infection of the recipient lead to virus replication and spread through the host? Will infection lead to pathology and transmission to other individuals, thus causing a public health risk? Some known viruses cannot be eliminated. All pigs, for example, carry the virus called porcine endogenous retrovirus, which can affect human cells in the laboratory. It isnot known if this virus can be transmitted through the xenotransplant or if it would cause disease in human beings. Research on infectivity experiments on primate and human felines is in progress to answer these vital questions.

We believe that we have the tools to determine

the suitability and risk assessment of xenotransplantation. Improved public awareness and a strong research support are a necessary logical step to remove the hurdles against cross-species transplantation that leads eventually to a better treatment strategy for patients with end-stage organ failure.

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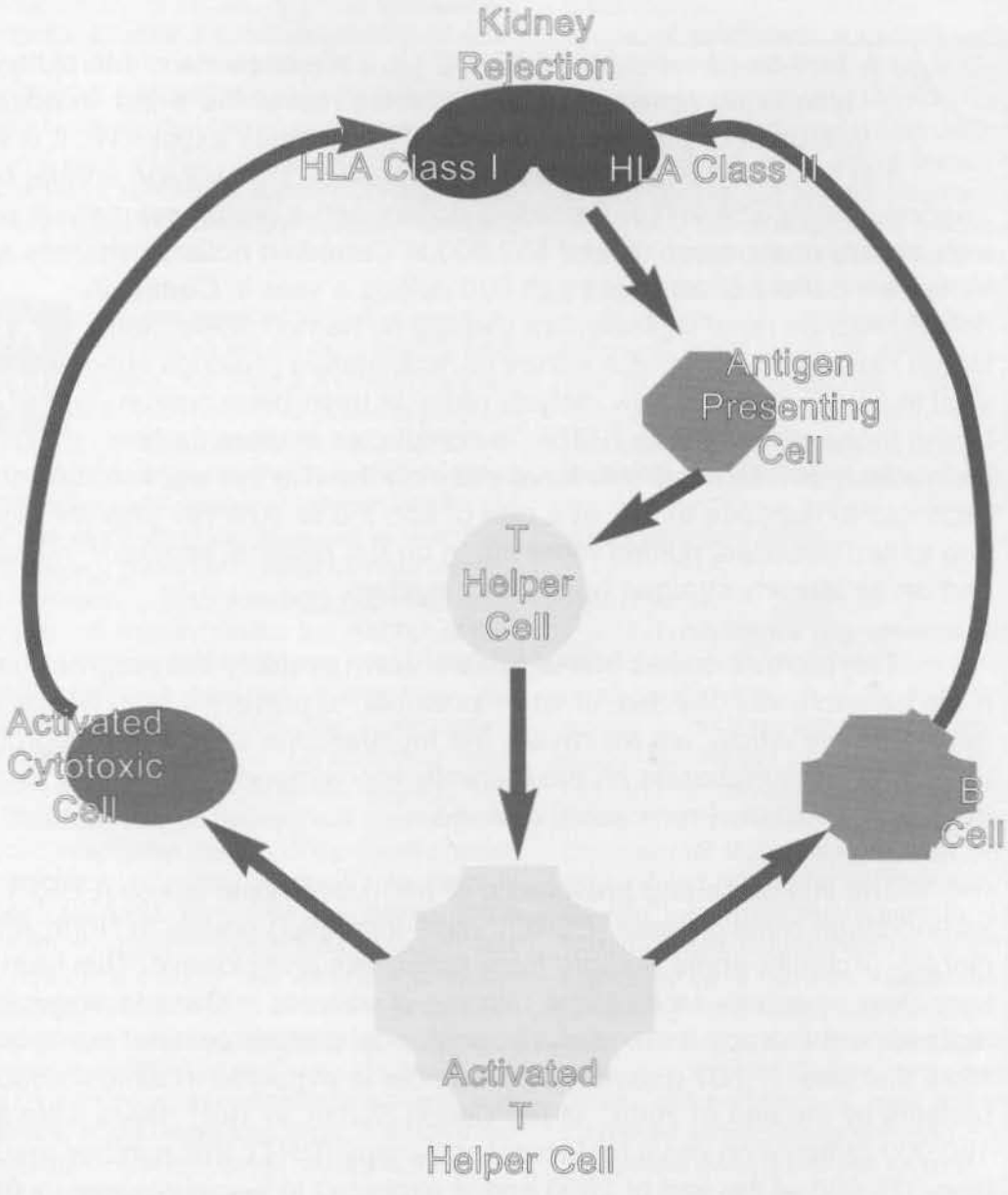

FIGURE 1

Mechanisms of Allograft Rejection. T-helper cell perceives directly the foreignness of the graft and also indirectly through presentation of some foreign proteins (HLA antigens presented on the graft) after processing by antigen-presenting cells. Activated T-helper cells produce a variety of small polypeptides that activate other cells of the immune system (cytokines). Both cytokine-dependent and direct activation of cytotoxic T cells and B lymphocytes lead to attack of the foreign graft and induce rejection.