2050

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22. Takaya S, Iwaki Y, Starzl TE: Liver transplantation in positive cytotoxic crossmatch cases using FK506, high dose steroids, and prostaglandin E_1. Transplantation 1991; 54:927-933.
23. Starzl TE, Demetris AJ, Rao As, et al: Spontaneous and iatrogenically augmented leukocyte chimerism in organ transplant recipients. Transplant Proc 1994; 26:3071-3076.
24. Rao AS, Fontes P, Zeevi A, et al: Augmentation of chimerism in whole organ recipients by simultaneous infusion of donor bone marrow cells. Transplant Proc 1995; 27:210-212.
25. Fontes P, Rao A, Demetris AJ, et al: Augmentation with bone marrow of donor leukocyte migration for kidney, liver, heart, and pancreas islet transplantation. Lancet 1994; 344:151-155.
26. Karavias D, Jabbour N, Felekouras E, et al: Right diaphragmatic paralysis following orthotopic liver transplantation. Submitted.
27. Reyes J, Abu-Elmagd K, Tzakis A, et al: Infectious complications after human small bowel transplantation. Transplant Proc 1992; 24:1249-1250.
28. Sigurdsson L, Green M, Putnam P, et al: Bacteremia frequently accompanies rejection following pediatric small bowel transplantation (Abstract). J Pediatr Gastroenterol Nutr 1995; 21:356.
29. Bueno J, Green M, Kocoshis S, et al: Cytomegalovirus infection after intestinal transplantation in children. Clin Infect Dis 1997; 25:1078-1083.
30. Reyes J, Bueno J, Kocoshis S, et al: Current status of intestinal transplantation in children. J Pediatr Surg 1998; 33:243-254.
31. Garau P, Orenstien SR, Neigut DA, et al: Role of endoscopy following small intestinal transplantation in children. Transplant Proc 1994; 26:136-137.
32. Reyes J, Tzakis AG, Todo S, et al: Nutritional management of intestinal transplant recipients. Transplant Proc 1993; 25:1200-1201.
33. Abu-Elmagd K, Tzakis A, Todo S, et al: Monitoring and treatment of intestinal allograft rejection in humans. Transplant Proc 1993; 25:1202-1203.
34. Lee RG, Nakamura K, Tsamauda ACm, et al: Pathology of human intestinal transplantation. Gastroenterology 1996; 1820-1834.
35. White FV, Reyes J, Jaffe R, et al: Pathology of intestinal transplantation in children. Am J Surg Pathol 1995; 19:687-698.
36. Abu-Elmagd K, Reyes J, Todo S, et al: Clinical intestinal transplantation: New perspectives and immunologic considerations. J Am Coll Surg 1998; 186:512-527.
37. Kang YG, Martin DJ, Marquez JM, et al: Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. Anesth Analg 1985; 64:888-896.
38. Stahl RL, Duncan A, Hooks MA, et al: A hypercoagulable state follows orthotopic liver transplantation. Hepatology 1990; 12:553.
39. Green M, Reyes J, Nour B, et al: Early infectious complications of liver-intestinal transplantation in children: Preliminary analysis. Transplant Proc 1994; 26:1420-1421.
40. Manez R, Kusne S, Green M, et al: Incidence and risk factors associated with the development of cytomegalovirus disease after intestinal transplantation. Transplantation 1995; 59:1110-1114.
41. Reyes J, Tzakis A, Bonet H, et al: Lymphoproliferative disease after intestinal transplantation under FK506 immunosuppression. Transplant Proc 1994; 26:1426-1427.
42. Reyes J, Todo S, Green M, et al: Graft-versus-host disease after liver and small bowel transplantation in a child. Clin Transpl 1997; 11:345-348.

185

Future of Transplantation (Including Xenografting)

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In Chapter 175, a detailed history of organ transplantation is provided. In order to foster the appreciation and understanding of the forces that will drive advances in transplantation into the next century, this chapter focuses on a few of the past developments in transplantation that have helped to shape current transplant practices (Fig. 185-1). The beginning of solid-organ transplantation can be traced back to the technical achievement of Alexis Carrel[1]; in 1902, he described the techniques of vascular anastomosis, thus ushering in accounts of autologous and homologous transplantation. Although a number of animal-to-human kidney transplants were reported in the ensuing three decades, a human donor organ was not used until 1933, by the Russian surgeon Voronoy.[2] This and other attempts at using human kidneys for transplantation failed owing to acute tubular necrosis and rejection. The first successful human transplant was performed on December 23, 1954, by the Boston team of Moore, Murray, Merrill, and Harrison.[3] The transplantation of an identical twin kidney from one brother to another was the immunologic advantage that distinguished the early successes in kidney transplantation from those that otherwise were doomed to fail.

Gibson and Medawar[4] ascribed an immunologic basis to the rejection of tissues between genetically nonidentical individuals. In 1960, Calne and Murray[5] used azathioprine, developed several years earlier by Burroughs-Wellcome, in attempts to gain success in unrelated kidney transplantation using immunosuppressive agents. Starzl and colleagues[6] then modified the immunosuppressive regimen by adding corticosteroids for rejection and began routinely to achieve success. This success led to growing attempts at human kidney transplantation, aggravating the shortage of organs to use for transplantation. A number of animal-to-human transplantations were attempted. The longest survivor was a 23-year-old woman who lived for 9 months after receiving kidneys from a chimpanzee.[7]

In 1968, the Ad Hoc Committee of the Harvard School of Medicine proposed the concept of "irreversible coma."[8] Further clarification of the pathophysiology of irreversible brain stem injury and subsequent somatic death followed, as did objective criteria to document irreversible brain injury. The brain death concept has eventually been accepted throughout the United States (see Chapter 174). The details of brain death evaluation and certification vary from state to state but require a clinical picture of (1) coma not due to drug overdose (e.g., alcohol) or to physical reasons (e.g., hypothermia) and (2) lack of cranial nerve reflexes. Confirmatory tests are used to document the absence of blood flow to the brain and the lack of cerebral and brain stem electric activity. The use of brain-dead donors, with optimal hemodynamic parameters, offers the possibility of better-quality organs with minimum damage from warm ischemia. It has also allowed procurement of extrarenal organs in a systematic manner.[9] Another improvement in the area of donor management was the development of preservation solutions, first Collins solution[10] and currently

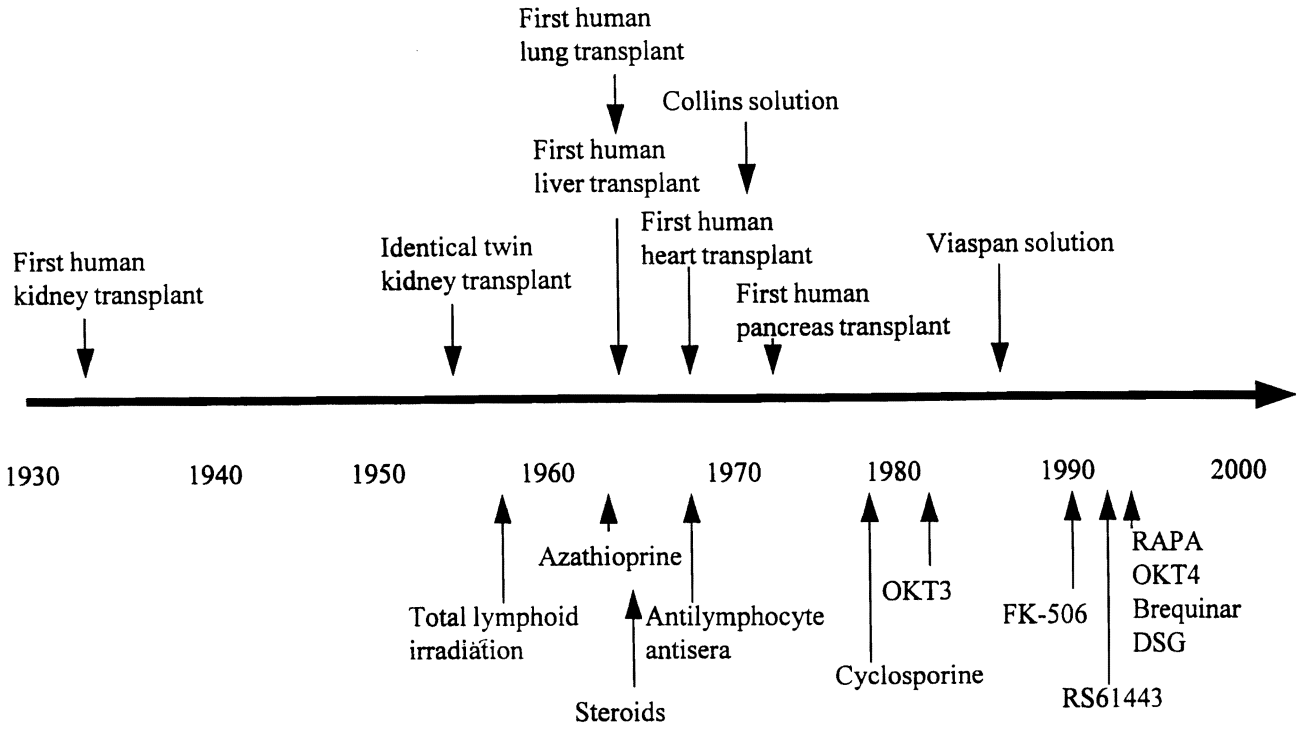

Figure 185–1. Abbreviated chronologic summary of significant milestones in transplantation.

Viaspan, developed by Belzer and Southard[11] at the University of Wisconsin (see Chapter 178).

The next advancement in the field of transplantation came with the discovery of the immunosuppressive qualities of cyclosporine, described by Borel and colleagues.[12] Clinical trials of this agent were conducted in England by Calne and colleagues[13] and shortly thereafter in the United States by Starzl and colleagues.[14] The combination of cyclosporine and steroids was soon introduced into clinical transplantation, and the impact on liver, heart, and kidney transplantation was felt almost overnight. With the introduction of cyclosporine into clinical transplantation, survival rates for patients and grafts improved dramatically (see Chapter 178).

Nevertheless, allograft rejection and the consequences of the treatment of rejection continue to constitute one of the most common causes of retransplantation or death. Clinical rejection occurs in as many as 80% of recipients of solid-organ allografts, who are maintained on a cyclosporine and steroid regimen. In addition, a number of toxicities, including nephrotoxicity, may limit the optimal use of cyclosporine. Chronic renal damage and functional impairment have been shown to occur in transplant recipients, and hypertension requiring antihypertensive therapy occurs in the majority of these patients. Alterations in clinical immunosuppression to prevent or reverse these and other side effects have included (1) reduction of cyclosporine dose and (2) the addition of azathioprine, antilymphocyte antibodies, or other agents, with concomitant reductions in the cyclosporine dose. These methodols have their inherent dangers, increasing susceptibility to both rejection and infection.

Organ transplantation is now accepted as a therapeutic modality for treatment of various end-stage organ diseases. The cost of kidney transplantation has been paid by Medicare and the End-Stage Renal Disease Program for more than 15 years. The costs of liver, heart, and heart-lung transplantations have been paid by a majority of third-party insurances for more than 10 years; Medicare has also recognized the benefits of these procedures and has developed entitlement programs covering these procedures. Pancreas, lung, and intestinal transplantations have not yet been universally subscribed to by third-party payers, but as experience accumulates and the efficacy of these procedures is proved, it is likely that these procedures will also be covered by Medicare. As experience in organ transplantation grows and the experimental procedures also become accepted, the pressure exerted on a limited donor pool continues to increase. Donors who have not previously been used are being considered, and more attention is focused on artificial support systems and the area of xenotransplantation.

This chapter attempts to put into perspective some of the areas of research and development that may affect the future of transplantation. References to other chapters detail the developments in those areas that are worth mentioning in the context of future developments in the field of organ transplantation. It is not possible here to mention all of the various fields that may affect the future of transplantation, and omission of an area of interest does not in any way suggest that such an area is not important.

UPDATE ON SUCCESSFUL SOLID-ORGAN TRANSPLANTATION

In 1984, the National Organ Transplant Act (NOTA) was passed by the Congress of the United States. This act called for formation of an organ procurement network and a scientific registry (see Chapter 175). In 1987, the United Network of Organ Sharing (UNOS) was awarded a contract by the Health Resources and Services Administration to maintain a scientific registry for organ transplantation. One of the purposes of the registry is to collect and analyze data regarding the success of organ transplantation and the factors that are important in determining success. This registry represents one of the first attempts to examine the role of donor and recipient characteristics, as well as center-specific parameters, that

affect organ transplantation. The factors analyzed have included (1) age and race of the recipient, (2) risk factors in the recipient population, and (3) the medical urgency of the recipient population. These factors were then applied to national and center-specific outcomes. In 1992, the first Report of Center-Specific Graft and Patient Survival Rates was published[15]: it was followed by two additional analyses, in 1994[16] and 1997.[17] The data cited in the following pages have been abstracted from published UNOS statistics in the 1997 Annual Report for the United States experience.[18] In all organ transplant types, the registry has recorded an improvement in patient and graft survival during the period that has been analyzed. This improvement is in part related to better medical management, greater experience at the individual transplant centers, and changing recipient characteristics.

Kidney

The current 1-year patient survival rate for cadaveric kidney transplants is 94.7%, which is slightly lower, at 88%, by 3 years after transplantation; The 1- and 3-year graft survival rates are 86.6% and 72.1%, respectively. A patient whose kidney allograft fails can be put back on dialysis support, accounting for the significant differences between patient and graft survival rates.

The following factors appear to adversely affect the success of cadaveric kidney transplants:

1. Repeat transplants (~6% worse 3-year graft survival than first-time kidney transplant).
2. Blacks as recipients (~10% worse 3-year graft survival rates compared with white recipients; ~15% worse 3-year graft survival rates compared with Asian recipients).
3. Poor histocompatibility (HLA) matching (14% worse 3-year graft survival rates in the worst match compared with the best match).
4. Very young (<5 years of age) or very old (>65 years of age) recipients.

The corresponding biologic explanations for these risk factors are as follows:

1. Greater likelihood of sensitization of the recipient in repeated transplantation.
2. Worse matching characteristics in the black population than in white and Asian populations.
3. The role of histocompatibility, as determined by HLA matching, in the intensity of the rejection process.
4. Concurrent medical problems in the elderly and greater technical complications in the very young.

Living donors have been used for kidney transplantation since the earliest attempts at kidney transplantation. The overall graft and patient survival rates for recipients of living donor kidneys are better than for recipients of cadaveric kidney transplants. The 1- and 3-year patient survival rates for living donor recipients are 97.9% and 94.7%, respectively; the corresponding 1- and 3-year graft survival rates are 93.3% and 85.2%. Many of the risk factors that adversely influence graft survival in cadaveric kidney recipients also apply in living donor recipients. The biologic explanation for the better graft and patient survival rates in the living donor group compared with the cadaveric organ recipients is principally related to closer HLA matching and better-quality kidney allografts, with improved early graft function. The onset of early acute tubular necrosis after kidney transplantation, from either preservation or immunologic causes, has a deleterious effect on graft survival.

Liver

The current 1- and 3-year patient survival rates after liver transplantation are 87.0% and 77.4%, respectively; the corresponding graft survival rates are 79.1% and 66.4%. Unlike failure of kidney transplantation, failure of a liver graft results in a patient's death unless the patient undergoes retransplantation.

The risk factors associated with poorer outcomes in liver transplantation are as follows:

1. Older (>65 years of age) recipient age (3-year patient survival rate for this group is 8% less than the mean rate).
2. Repeat transplants (3-year survival rate for recipient of previous transplants was 23.5% less than for those receiving only one transplant).
3. Asian race (3-year survival rate for this group was 11.4% less than the mean rate).
4. Severity of medical illness at the time of liver transplantation (3-year survival rate of the most critically ill was 24.3% less than for those with little stigmata of chronic liver disease).
5. Primary diagnosis of malignant neoplasm (3-year survival rate for this group is 31.7% less than the mean rate).

For graft survival, the risk factors were similar, although the very young (<1 year of age) recipients had the lowest graft survival (3-year graft survival rate is approximately 9% less than the mean rate).

The corresponding biologic explanations for these risk factors are as follows:

1. Concurrent medical conditions in the elderly.
2. Greater severity of illness in those receiving more than one liver transplant.
3. Higher incidence of hepatitis B and C as well as the presence of primary liver tumors in the Asian recipients compared with other races.
4. Higher risk in sicker patients, related to other organ system involvement such as respiratory or renal failure.

In the pediatric population, a higher rate of technical complications in liver transplantation accounts for the higher graft loss in this group. For several risk factors, the effect on both patient and graft survival appears to occur in the immediate post-transplant period, without a disproportionate loss after the first 3 months.

Heart

The 1- and 3-year patient survival rates after heart transplantation are 85.1% and 76.2%, respectively; the corresponding graft survival rates are 84.5% and 74.2%. The similarity between patient and graft survival rates in heart transplantation is due to the limited retransplantations performed. For heart transplantation, the risk factors that adversely affect both patients and graft survival rates are as follows, listed in order of importance:

1. Very young age (<1 year); both patient and graft survival rates were 14% less in this age group compared with the mean.
2. Severity of medical illness at the time of heart transplantation; recipients with the most critical need for heart transplantation have a 9% worse 3-year outcome than those with minimum heart disease.
3. Women have approximately 3% to 4% worse patient and graft survival rates than men.

The corresponding biologic explanation for the first and third risk factors is the difficulty in obtaining heart grafts of appropriate size for children and for women. The second

factor can be explained by the presence of concurrent medical illnesses in those who are critically in need of heart transplantation, as well as the use of biomechanical devices to maintain such patients until transplantation.

Pancreas

Pancreas transplantation has been used in the following three scenarios:

- Pancreas alone, as treatment of type I juvenile-onset diabetes mellitus, without overt renal failure
- Pancreas combined with simultaneous kidney transplantation (SPK), for diabetic patients with renal failure
- Pancreas after successful kidney transplantation (PAK)

Overall, the current 1- and 3-year graft function rates for all pancreas transplants are 79.6% and 67.4%, respectively. In the national experience, patients undergoing SPK have fared the best, with 1- and 3-year graft survival rates of 80.8% and 70.5%, compared with 63.5% and 40.2%, respectively, for pancreas alone; and 72.8% and 41.6%, respectively, for PAK. The biologic explanation for these differences lies in the difficulties in assessing pancreas rejection. In pancreas transplantation, elevation of the serum glucose is often a late sign of rejection, because only 10% of the islet mass may be present before overt diabetes mellitus reappears. Thus, reversal of rejection may not recover sufficient islet function to ensure long-term graft function. In SPK, the kidney has been used as a "window" to assess pancreas rejection. It has been assumed that treatment of kidney rejection will also treat rejection of the pancreas, which is occurring at the same time. In PAK, monitoring of pancreas rejection has been less successful; usually, the HLA types of the original kidney donor and of the pancreas donor are significantly different.

The 1- and 3-year patient survival rates for pancreas transplantation are 93.2% and 86.4%, respectively. For SPK, the corresponding survival rates are 93.2% and 86.4%, which are approximately 1.5% to 11.7% worse than for patients who receive kidney grafts alone. The higher morbidity and mortality rates associated with adding a pancreas transplant at the time of kidney transplantation are related to technical factors in pancreas grafting, such as a higher rate of infections after pancreas transplantation.

Heart-Lung

The number of heart-lung transplants has actually fallen, in part because of a shift of some candidates to just lung transplantation. The patient survival rate is almost identical to the graft survival rate, because retransplantation is rare. The 1- and 3-year survival rates are 74% and 51%, respectively. Because experience with this procedure is limited, detailed analysis of the risk factors is meaningless.

Lung

The patient survival rate after lung transplantation is also similar to the graft survival, owing to the limited experience with retransplantation. The 1- and 3-year patient survival rates are 77% and 58%, respectively; the corresponding graft survival rates are 76% and 55%, respectively. Males tend to have poorer patient and graft survival rates (approximately 4% to 5% lower 1- and 3-year survival rates than for women). The biologic explanation for this difference is not clear but may be related to differences in the indications for lung transplantation between men and women.

Controversies

One of the principal controversies about the meaning of the data cited in this discussion is the effect on national policies regarding organ allocation and possible restriction of transplant services to selected groups of recipients.[19-21] Some researchers have argued that the transplant community should restrict transplants to the patients who have the greatest likelihood of long-term survival, whereas others have viewed transplantation as a means to provide life-saving therapy to patients who have the most to gain (i.e., the most critically ill). For example, the greatest net benefit of liver transplantation is for those patients whose outcomes without transplantation are poor. The net benefit is the difference in survival between those who receive transplants and those who do not. Data derived from UNOS have already shown that this difference (i.e., net benefit) is greater for the sicker patients (on the order of 50% at 1 year) compared with the patients in whom transplantation was performed on the most elective basis (i.e., no difference in survival between those who received transplants and those who remained on the waiting list for more than 2 years).[22]

On April 2, 1998, the U.S. Department of Health and Human Services (DHHS) issued final regulations regarding organ allocation.[23] These regulations require that policies that govern organ allocation must embody the following principles:

1. Equity for patients awaiting organ transplantation as measured by waiting time.
2. Access to transplant center data for patients.
3. A "level playing field" through definition of standard listing and status criteria.
4. Reaffirmation of the role of government oversight as public advocacy.
5. Encouragement of patient participation in transplant issues.

These principles have been advanced by impartial panels. For example, in 1977, the American Medical Association Council on Ethical and Judicial Affairs[24] affirmed that, "Organs should be considered a national, rather than a local or regional, resource. Geographical priorities in the allocation of organs should be prohibited."[24] In 1984, NOTA called for the fair and equitable national allocation of organs. The 1986 recommendations by the U.S. Task Force on Organ Transplantation clearly stated the need to avoid using geography as the basis for organ distribution. Currently, these issues are being debated within the transplant community, and UNOS is leading the opposition to adoption of these regulations; the likelihood that these regulations will be implemented is still unknown. However, in a 1998 editorial published in *The Lancet*, Horton[25] wrote, "UNOS would better serve the transplant community if it abandoned its stance and began working with DHHS to draw up allocation policies that are practical and fair."

IMMUNOLOGIC ADVANCES

Several new developments in the area of immunology and immunosuppression promise to affect organ transplantation in the near future. Chapter 178 has been devoted to the discussion of immunosuppressive agents; attention is given here to two areas that will influence our immunosuppressive management of recipients of solid-organ transplants.

Chimerism

Billingham and colleagues[26, 27] first associated tolerance to skin grafting with hematopoietic mixing or chimerism in freemartin cattle and subsequently verified this finding by injecting

viable allogeneic spleen cells into fetuses of the recipient strain. Ildstad and Sachs[28] demonstrated the ability to duplicate mixed allogeneic lymphodendritic chimerism and subsequent tolerance by allogeneic bone marrow transplantation. The concept of natural microchimerism, which develops after solid-organ transplantation, was first suggested by clinical observations of acquisition of delayed-type hypersensitivity in recipients after successful kidney transplantation.[29] This hypothesis was documented years later, after technologic advances allowed for detection of small numbers of donor cells (outside the grafted organ) through the use of immunostaining or polymerase chain reactions (PCRs), in which donor deoxyribonucleic acid is amplified.[30] This pattern of migration of donor-derived cells after transplantation was subsequently found in experimental animal models[31] and in other human organ transplant models, such as liver and small-bowel transplantation.

The functionality of these cells was suggested in a study by Starzl and coworkers,[30] in which an unexpected benefit of transplantation of the liver for type IV glycogen storage disease resulted in reversal of the deposition of the insoluble defective polysaccharide. In this study, donor-derived cells were detected in the heart and other tissues in two patients receiving liver transplants for type IV glycogen storage disease. Similar findings were noted in a transplant recipient with a deficiency of the lysosomal enzyme B glucocerebrosidase, which causes type 1 Gaucher's disease.

A more systematic survey of long-term survivors after liver transplantation was performed with the use of immunostaining and polymerase chain reaction.[32] Of a group of 22 surviving liver transplant recipients who had received their transplants more than 10 years before being studied, all demonstrated systemic tissue microchimerism. The immunologic privilege of the liver, its ability to induce systemic hyporesponsiveness and to protect other organs from rejection, may lie in the relative abundance of migratory cells in the liver compared with other organs, such as the kidney and heart. If this hypothesis is correct, strategies can be developed to identify the cell type and the optimal source of these cells and then to enhance their migration, in an attempt to accentuate the immunomodulating effect of the cells on the recipient immune response.

The effect of the migratory donor cells on the recipient immune response is not clear. It is likely that a number of factors determine the ultimate effect of these cells on allograft survival. First, if insufficient immunosuppression is given in the early phases after transplantation, these cells may be immunogenic and may accentuate the rejection process. Second, if the recipient is made immunoincompetent shortly before transplantation, either by cytoablation or by an imbalance in the number of immunocompetent donor cells given, a graft-versus-host disease (GVHD) process may occur, in turn further suppressing the immunocompetence of the recipient.[33] Third, if an appropriate balance of immunosuppression is given, along with a sufficient number of migratory cells or their precursor stem cells, a phase in which the donor cells can migrate and take residence in the recipient follows a phase in which peripheral anergy or coexistence may occur.[34] Put into an immunologic perspective on tolerance (ranging from chronic infection to autoimmune diseases to transplantation), the outcome of immune effector functions after antigen exposure depends on the dose, timing, route, and localization of the antigen.[35]

A number of observations have suggested these cells are of bone marrow origin. Donor-specific blood transfusions (DSTs) have been shown to enhance long-term graft survival in living donor kidney transplants since the 1970s. Cochrum and associates[36] reported that DSTs improved 1-year graft survival by 57% to 95%. Even in the cyclosporin era, Salvatierra and coworkers[37] reported a 1-year graft survival of 93% in DST-treated patients, compared with 82% in non-DST-treated patients. In addition, Reed and colleagues[38] reported a benefit of DST in reducing both rejection and the need for steroids.[38] Protocols have been developed to infuse donor bone marrow at the time of solid-organ transplantation. Several groups, including ours at the University of Pittsburgh, are currently using cadaveric bone marrow infusion, along with solid-organ transplantation from the same donor, in attempts to enhance the chimerism observed after solid-organ transplantation alone.[39-41]

University of Pittsburgh Experience

At the University of Pittsburgh, since 1992, 226 primary allograft recipients have received perioperative infusion of a single dose of 300 to 500 million unmodified donor bone marrow cells per kg of body weight. The mean recipient and donor ages are 40 years and 29 years, respectively, and follow-up periods have ranged from 3 to 2023 days.

Since April 1996, 39 organ recipients have been included in a protocol involving three daily sequential perioperative infusions of unmodified BM cells (200 million cells/kg/day) from day 0 to 2 post-transplantation. The mean recipient age in this group is 45 years, and the follow-up period has ranged from 4 to 790 days. Control subjects were those organ transplant recipients for whom bone marrow was not available (n = 131). Standard immunosuppression in this study consisted of tacrolimus and steroids. Mycophenolate mofetil (MMF) was used in 53 study patients and 17 control patients. In addition to serial monitoring of clinical parameters, peripheral blood from the recipients was screened for the presence of donor cell chimerism (by flow cytometry and PCR) and cellular immune responses (by mixed leukocyte reaction [MLR]). Infusion of bone marrow cells was safe in all cases, and of the 55 grafts (21%) lost in study patients, none could be attributed exclusively to bone marrow infusion. Thirty-one (24%) of the control patients experienced loss of the allograft. A slightly higher incidence (77% versus 63%) of mild to moderate acute cellular rejection (ACR) was observed in the control group.

Heart recipients demonstrated a statistically significant (P = .006 by Fisher's exact test) decrease in rejection episodes after BM infusion. Sixty-two per cent of study patients (as opposed to 18% of control patients) were free of rejection (grade 3A or higher) in the first 6 months after transplantation. Lung transplant recipients in the study group also showed a statistically lower incidence of obliterative bronchiolitis (3.8%) than the control group (31%).

Mild, easily reversible GVHD was observed in 1% (n = 2) of patients receiving single bone marrow infusions. Contrarily, fulminant GVHD was encountered in 1 of 39 recipients of multiple bone marrow infusions. This individual was a liver recipient, and in patients undergoing liver transplantation, we have reinstituted the single infusion strategy. In those patients evaluated at least 1 year after transplantation, a slightly higher incidence of steroid-free existence was noted in the bone marrow study group (53% versus 40%). This finding was associated with a higher incidence of multilineage donor cell chimerism in study patients (95% of total study group) compared with the controls (53% of total control group). In an evaluation using one-way MLR assays, donor-specific hyporeactivity was witnessed in 57% of bone marrow–augmented liver, lung, and kidney recipients compared with 44% of controls.

Ricordi and colleagues[42] are conducting a similar study at the University of Miami. They have modified the infusion protocol to include the use of one or more donor bone marrow infusions during the early post-transplantation period. This group has suggested that multiple bone infusions are

associated with a lower incidence of acute rejection and higher levels of chimerism.[42] In both of these trials, the conclusive clinical endpoint will be to determine the ability to wean bone marrow-infused and control transplant recipients from immunosuppression completely and to determine the incidence of the subsequent development of chronic rejection.

Sirolimus (Rapamycin)

Sirolimus (rapamycin) is a natural fermentation product (macrolide antibiotic) with immunosuppressant properties that acts by inhibiting growth factor signal transduction.[43] Its specific mechanism of action is the blockade of T and B cell responses to stimulating cytokines, thus preventing cell cycle progression in the phase of the cell cycle G_1 and subsequent cellular proliferation.[44] Like cyclosporine, sirolimus is metabolized in the cytochrome P_{4503A} pathway and is a substrate for p-glycoprotein countertransport.[45] Animal studies have shown that when combined with standard immunosuppressive therapy, sirolimus allows for the reduction of individual drug dosages and leads to a remarkable decrease in the incidence of acute rejection.[46] Although some studies found sirolimus levels to be consistently higher when the drug was administered concomitantly with cyclosporine,[45] others found no pharmacokinetic interaction between the two agents.[47] Sirolimus prevented accelerated atherosclerosis in synergism with mycophenolic acid and reduced transplant vasculopathy.[48, 49] This drug has also been said to have a putative beneficial role in the treatment and prevention of obliterative bronchiolitis when used in combination with other immunosuppressive agents in heart-lung transplantation.[50]

Toxicities associated with sirolimus include thrombocytopenia, leukopenia, increased cholesterol levels, elevated triglyceride levels, anorexia, vomiting, diarrhea, diabetes mellitus, myocardial necrosis, and testicular atrophy.[44, 51-54] Hypertension, nephrotoxicity, and hepatotoxicity have not been reported,[52, 55] although potentiation of cyclosporine nephrotoxicity by sirolimus has been reported.[56] In vitro studies suggest that it may also have neurotoxic potentials.[57] Sirolimus is now in phase III trials for renal transplant recipients; preliminary results indicate a decrease in acute rejection when sirolimus is used in combination with cyclosporine.[58]

SDZ RAD (40-O-(2-hydroxyethyl)-rapamycin), a rapamycin analog, also acts by inhibiting growth factor-driven cell proliferation. Although it has less in vitro activity compared with sirolimus, SDZ RAD has similar immunosuppressive properties when given orally.[59, 60] Clinical trials of this agent are currently under way.

APPLICATIONS TO NEW ORGAN TRANSPLANTATION

One way to assess the impact of a new immunosuppressive agent in transplantation is the ability to successfully transplant organs that were not considered feasible for transplantation with standard immunosuppression. This was certainly the situation when cyclosporine was introduced to liver transplantation. Chapters 182 to 184 deal with the topics of lung, pancreas, and intestinal transplantation, respectively; a brief reference to the impact of tacrolimus in these organ transplants is provided here.

Intestinal Transplantation

Success with intestinal transplantation using cyclosporine immunosuppression has been sporadic.[61-63] A growing experience of small-bowel transplantation, either alone or combined with other abdominal organs, with tacrolimus immunosuppression has been accumulated at the University of Pittsburgh,[64-66] the University of Miami,[67] the University of Nebraska,[68] and the University Hospital in London, Ontario.[69] At the University of Pittsburgh, small-bowel allografts have been transplanted alone (n = 37), along with livers (n = 50), or as part of multivisceral clusters (n = 17) using tacrolimus immunosuppression. Of the 98 patients who received these 104 allografts, 48% were alive at a mean follow-up of 32 months. The actuarial 1- and 5-year patient survival rates are 72% and 48%, respectively. Graft function was satisfactory, with 91% of survivors being enterally sustained and the other 9% relying on supplemental parenteral nutrition. Rejection was common; 90% of the patients had at least one episode of rejection of the intestinal allograft. The high incidence of rejection may be altered by the addition of some of the newer immunosuppressive agents discussed in Chapter 178.

Lung Transplantation

Lung transplantation is a rapidly developing procedure that has been limited for technical, preservation, and immunologic reasons. A prospective, randomized trial of primary adult pulmonary transplantation using tacrolimus immunosuppression was conducted at the University of Pittsburgh.[70] Sixty-six patients were randomly assigned to receive tacrolimus immunosuppression, and 66 patients to receive cyclosporine immunosuppression. Although 1- and 2-year patient survival rates were not statistically significantly different for the two regimens, there was a trend toward increased survival in the tacrolimus group. In addition, obliterative bronchiolitis developed in significantly fewer patients in the tacrolimus group (22%) than in the cyclosporine group (38%).

In light of the lung allograft shortage, the utility of single lung transplantation rather than double lung transplantation for pulmonary hypertension has been established,[71] although other indications, such as cystic fibrosis, preferentially require double lung transplantation.[72] The organ shortage is reflected by a high incidence of deaths of patients on the waiting list.[73] Other approaches to increasing lung allograft availability are the development of living related donor bilateral lobar lung transplantation.[74, 75] The results reported from the University of Southern California revealed survival outcomes similar to those for transplantation from cadaveric donors, with no donor mortality and a complication rate of 10%.[74]

Islet Cell Transplantation

For treatment of type I diabetes mellitus, islet cell transplantation would be preferable to whole pancreas transplantation. The morbidity and mortality associated with transplantation of the exocrine component of whole pancreas are well known. A number of investigators have developed automated systems of enzymatic and mechanical separation that have improved previous methods of islet cell isolation.[76, 77] Application of this technique has proved to be applicable to clinical situations through infusion of purified islets into liver allografts, during combined liver-islet transplantation, and has resulted in long-term islet function in selected cases of surgically induced diabetes mellitus.[78] The early experience with islet transplantation for treatment of juvenile-onset type I diabetes mellitus was less successful, although C-peptide secretion was almost uniformly observed.[79] With improved isolation and immunosuppression, several cases of exogenous insulin freedom have been noted in patients undergoing combined kidney-islet cell transplantation.[80] The technical and immunologic factors are still being investigated.[81, 82] However, its general clinical appli-

cation awaits demonstration of improvements in long-term insulin freedom.[83]

ADVANCES AND INNOVATIONS IN SUPPORT SYSTEMS

Extracorporeal Liver Assist Device

Acute liver insufficiency can present as either fulminant hepatic failure (FHF) or primary nonfunction (PNF) of liver allografts after liver transplantation. The clinical presentation is acute liver failure complicated by hepatic encephalopathy either in a previously healthy person (FHF) or after liver transplantation (PNF). Survival with either FHF or PNF is poor, particularly for patients suffering advanced encephalopathy with development of the hepatorenal syndrome, systemic lactic acidosis, and severe coagulopathy. The morbidity and mortality of FHF and PNF cannot be underestimated. The mortality rate of FHF is 60% to 95%; it is higher for FHF owing to virus or toxic exposure, and lower in patients with FHF owing to acetaminophen overdose. PNF occurs in up to 10% of patients after liver transplantation; contributing factors include donor instability and length of preservation times, although many cases of PNF have no predisposing factors. Mortality associated with PNF is approximately 40% to 50%, even after retransplantation.

Management of both PNF and FHF is challenging and is aimed at prevention and treatment of complications, including infections, brain edema, hemodynamic instability, pulmonary and renal failure, acid-base disturbances, and coagulopathy. Orthotopic liver transplantation has increasingly been used for selected patients with FHF, whereas orthotopic retransplantation is the only procedure of choice in patients with PNF.

The concept of using mechanical devices to maintain patients with PNF or FHF until transplantation is an attractive one. Nevertheless, dialysis and charcoal hemoperfusion are not of proven benefit because (1) the multiple biochemical functions are not being replaced and (2) their use has not decreased the mortality in patients with FHF.[84, 85] Three proposed systems have used a hybrid device containing metabolically active liver cells.

Laboratory models have attempted to use primary hepatocyte cultures isolated from either animal or human livers; however, the inability to grow such cells in vitro has limited the practicality of this source of cells. Rozga and coworkers[86] have used a liver support system consisting of plasma separation and perfusion through a charcoal filter and hollow-fiber cartridge with porcine hepatocytes attached to collagen-coated dextran microcarrier beads, which are then placed into the extracapillary space of a hollow-fiber cartridge. Plasma is passed through the intracapillary space, and the porcine hepatocytes are separated from the plasma by the cartridge membrane. In a case report of a patient with FHF, total hepatectomy with extracorporeal liver support was able to support the patient until orthotopic liver transplantation could successfully be completed. In this case, intracranial pressures and serum ammonia were thought to be controlled with the use of the porcine hepatocyte-extracorporeal liver assist device (ELAD) system.

Watanabe and colleagues,[87] in a phase I clinical trial, reported encouraging results with the extracorporeal bioartificial liver. Their experience involved a total of 31 patients. Of 18 patients with a diagnosis of FHF, 16 were maintained successfully until transplantation and one until recovery of native liver function. Of three patients with PNF, all were successfully maintained by the device until retransplantation. The remaining 10 patients had a diagnosis of acute exacerbation of chronic liver disease. Two of these were successfully maintained until recovery and transplantation. The other eight did not qualify for transplantation but were successfully treated with the bioartificial liver prior to their deaths. There were decreases in ammonia, bilirubin, and transaminase levels after treatment with the liver assist device. Coagulation parameters, including prothrombin time, did not improve. There was an improvement (increase) in the ratio of branched chain amino acids to aromatic amino acids.

The system described by Sielaff and colleagues[88] at the University of Minnesota is similar in concept to the porcine hepatocyte-ELAD described above but uses a three-compartment collagen gel entrapment of porcine hepatocytes in the lumens of hollow-fiber cartridges. Blood is passed through the extracapillary space, but the hepatocytes are protected from immune damage by the cartridge membrane.[89] This system is being launched for phase I human trials.

The third ELAD system is based on the use of a subclone of HepG2 (HepG2/C3), a human hepatoblastoma cell line that expresses nearly normal levels of several central metabolic pathways and has the morphology and polarity characteristic of human hepatocytes.[90] HepG2/C3 can be grown in hollow-fiber cartridges, with the intention of developing an extracorporeal liver assist device (C3-ELAD). Six human patients have been treated with C3-ELAD.[91] All of the patients had advanced encephalopathy at the start of therapy, and all were in an intensive care unit (ICU). The devices were used for between 24 hours and 6 days. Improved clinical status, such as mental status, was noted in three of the six patients. One patient recovered completely from FHF, apparently as a result of C3-ELAD. Two other patients first improved but died of non-liver-related causes, one from sepsis 3 days after discontinuation of C3-ELAD therapy, and the other of brain death following a period of hypotension not thought to be related to C3-ELAD therapy. The remaining three patients died, one during an unrelated diagnostic procedure, one owing to technical inability to continue C3-ELAD therapy, and one because of advanced metabolic derangements related to liver failure.

A larger trial of this device was performed at the King's College Hospital, where a randomized trial of C3-ELAD was compared with a control regimen. Although the patients receiving C3-ELAD therapy were noted to have a higher level of improvement in encephalopathy, survival was not different.[92] Unfortunately, the unknown risk that immunosuppressed patients may be inoculated with tumor cells in the advent of a break in the hollow fibers, along with logistic difficulties, ended this trial.

Hepatocytes obtained from livers considered unsuitable for transplantation have been isolated and determined to be viable.[93] All organs had been excluded primarily because of steatosis, although advanced donor age was a significant secondary consideration. These cells exhibited decreased length of viability in cell culture compared with hepatocytes obtained from fresh surgical specimens. However, evidence of hepatocyte-specific function was shown by their ability to metabolize diazepam and lidocaine. Intrasplenic hepatocyte transplantation has been shown to improve the survival of laboratory animals with liver failure and to lead to an improvement in associated physiologic liver-based abnormalities.[94] The exact use of isolated hepatocytes and their human application is still to be determined.

Strom and coworkers[95] have used human hepatocytes to maintain patients with FHF until liver transplantation. Five hepatocyte-treated patients were successfully maintained until liver transplantation, with improvement in cerebral perfusion and cardiac stability. In one published report, the use of allogeneic hepatocytes partially reversed a liver disease due to an inborn error of metabolism, Crigler-Najjar syndrome.[96]

Some investigators have expressed concern for risk of disease transmission from the use of animal hepatocytes, although human blood does not directly contact the animal hepatocytes. Swine herds can be maintained in specific pathogen-free facilities, and known pathogens, such as *Brucella*, can be screened out. The discovery of a pig endogenous retrovirus (PERV)[97] that can be transmitted to human cells in culture, however, has raised safety concerns.[98] Given the issues related to organ shortage and the need for support of patients with acutely failing livers, cautious exploration of bioartificial liver support devices appears warranted, in order to identify those areas that will require further study. Technologic advances and better screening tools are likely to identify donor livers that may harbor latent infections.

Bioartificial Pancreas

Unlike totally artificial pancreas devices (in which exogenous insulin is placed into a pump and delivered via a glucose sensor),[99] bioartificial pancreas devices rely on the presence of the natural glucose homeostasis mechanisms of the beta cell. Biohybrid artificial pancreatic devices have been made in which cultured cell lines derived from pancreatic islet cells were placed within three-layered encapsulating microbeads, were inserted subcutaneously, and functioned in a rat model.[100] Bioartificial constructs with transformed cells have also been suggested as an alternative, given the scarcity of islets cells.[101] Encapsulated xenografted islets were found to control carbohydrate metabolism in pregnant diabetic animals and may eliminate the higher incidence of fetal malformations observed in diabetic pregnancies.[102]

Artificial Heart Assist Devices

Owing to the success of heart transplantation, artificial heart assist devices have been used primarily to maintain patient survival until the time of transplantation. Power supplies and the complications arising from the interaction of a foreign surface with the blood remain major issues still to be resolved. Animal studies have employed electromechanical artificial hearts of reduced dimensions driven by transcutaneous energy systems that may evolve into devices of permanent implantation for humans. Several systems (Novacor, Thoratec, HeartMate) have been tested in humans, primarily as bridges to heart transplantation.[103-105] Although these devices are effective in restoring cardiac output and reversing the sequelae of cardiogenic shock, major complications have included driveline infection and thromboembolic strokes.

The U.S. Food and Drug Administration (FDA) granted approval for the use of a Ventricular Assist Device (VAD) System in patients recovering from open-heart surgery. The device, manufactured by Thoratec Laboratories of Pleasanton, Calif., has already been approved for use as a bridge prior to cardiac transplantation. This makes the VAD System the only device clinically available in the United States for the treatment of patients on a short-term or long-term basis. It remains to be determined whether these devices will be cost effective compared with heart transplantation and prolonged medical therapy.[106]

EXPANSION OF THE DONOR POOL

Non-Heart-Beating Donors

Before the acceptance of brain death, the heart function of any organ donor was required to have ceased before the organs could be procured for transplantation. In some countries where brain death legislation has not been passed, non-heart-beating donors (NHBDs) represent the principal source of organs for transplantation. Four distinct populations of NHBDs have been identified and are classified as follows by Kootstra[107]:

1. Uncontrolled cardiopulmonary arrest—dead on arrival.
2. Uncontrolled cardiopulmonary arrest—declared dead following unsuccessful cardiac resuscitation in hospital.
3. Cardiac arrest under conditions of removal from life support, without fulfillment of criteria for brain death.
4. Cardiac arrest in a brain-dead patient.

The first situation is unlikely to generate usable organs, because the warm ischemic time would be unknown. In the second scenario, reasonable expectation of organ survival would require either immediate procurement of organs or infusion of preservation solution into the individual pronounced dead in a setting where family consent may not be available. A pilot study of such a procedure has been initiated by the Regional Organ Bank of Illinois; it is based on in situ perfusion of the abdominal aorta with a double-balloon catheter.[108] The fourth situation does not require any consideration except expedient procurement of organs, because consent for organ donation has generally been obtained.

The third situation is that of using organs from a patient who does not yet fulfill brain death criteria but for whom a desire has been expressed, by either the patient or the family, for the removal of life support so that the patient may become an organ donor after the declaration of death. Such a protocol has been developed by the University of Pittsburgh Medical Center and the Center for Organ Recovery and Education, which is the organ procurement organization associated with the geographic area of Pittsburgh. This protocol was developed in response to a perception that some families wished to have the right to terminate life support and to donate organs. After lengthy review, a protocol was implemented in 1992.

The details of this protocol are worth discussing, because they may form a foundation for further developments in this field. The interest in using NHBDs is indicated by the development of protocols among organ procurement organizations (OPOs) in the United States and the potential controversies they generate.[109, 110] In 1997, in Cleveland, Ohio, the lack of clarity in one set of protocols for organ procurement generated controversy that resulted in their withdrawal by the OPO concerned.[111] In addition, the lack of uniform criteria and policies for NHBDs was the topic of the 1997 hearings conducted by the Institute of Medicine (IOM) and of its subsequent report.[112] This issue is particularly important given the growing utilization of NHBDs in the United States. According to the 1997 Annual UNOS Report, NHBDs accounted for 1% of all cadaveric donors from 1994 to 1996 (162 of 15,874).[18]

A number of principles have been suggested for a NHBD protocol to ensure that patients, health care providers, and potential recipients are safeguarded.[112] The most important recommendations are summarized here:

1. Written, locally approved NHBD protocols.
2. Public openness of NHBD protocols.
3. Case-by-case decisions on anticoagulants and vasodilators.
4. Family consent for premortem cannulation.
5. Safeguards against conflict of interest—separate times and personnel for important decisions.
6. Determination of death in controlled NHBDs as cessation of cardiopulmonary function for at least 5 minutes as indicated by electrocardiographic and arterial pressure monitoring.
7. Family options (e.g., attendance at life support withdrawal) and financial protection.

A report by Yokoyama and coworkers[113] from Japan revealed that the 1-year graft survival rates of 110 kidney allografts taken from NHBDs were similar to those reported for organs from brain-dead NHBDs. At the University of Pittsburgh, similar results have been obtained for both kidney and liver allografts taken from NHBDs.[114] Cho and associates[115] compared the early function and survival of more than 200 kidneys from NHBDs with those of more than 8700 control grafts from donors with heart beats. Graft survival rate at 1 year was 83% for the former and 86% for the latter; 48% of recipients of NHBD kidneys required dialysis within the first week after transplant, compared with 22% of the control group. Despite the delayed function, however, survival rates were high for both groups. Primary failure rate was 4% for NHBD kidneys and 1% for kidneys from donors with heart beats. Among NHBD kidneys, grafts from donors who died as a result of trauma had a statistically significant better 1-year survival than grafts from donors who had died of other causes.[115]

Xenotransplantation

To understand the trends in the development of xenotransplantation, we must realize that potential donors for cross-species transplantation into humans can be separated into two groups. Donors can be considered either discordant or concordant. A *discordant* combination is characterized by the presence of a preformed antibody in the recipient, usually at high titers, that reacts and causes hyperacute rejection of the donor organ. *Concordant* combinations are generally characterized by low or nonexistent antibodies, so that the resultant rejection process resembles that of an allograft.[116] For example, transplants from primates into humans are usually concordant, whereas cross-species transplant from a pig into a human would be discordant.

In animal studies, the organ rejection in concordant combinations usually occurs in a different time frame from that in discordant combinations. Livers and hearts from an untreated discordant combination are rejected by antibodies within minutes to hours after revascularization. These organs would be rejected days to weeks after transplantation in an untreated concordant combination. We also realize that liver grafts are less susceptible to antibody-mediated injury than kidney or heart grafts. This difference has been used in some attempts at clinical xenotransplantation, with the expectation that liver xenografts may be more likely to succeed than other types of xenografts.

If one follows this train of thought, liver xenografts may be envisioned in three different clinical settings. The first would be to use liver xenografts as a temporary support, either until the liver recovers from injury or as a "bridge" to transplantation. These organs could be perfused outside the recipient, in an ex vivo manner. Such an approach was reported in a number of early experiences in the 1960s and again by the Johns Hopkins University and Duke University groups.[117] In the second situation, heterotopic liver xenotransplantation might be envisioned as a bridging method until an appropriate human liver is found. Finally, permanent orthotopic replacement of a diseased liver with a liver xenograft can be considered as a definitive procedure that may potentially expand the donor pool.

Several general therapeutic considerations may be taken into account in xenotransplantation. The first deals directly with the selection of an appropriate donor species. If an initial high titer of cytotoxic antibodies is noted in the recipient, these antibodies must be depleted. The depletion can be achieved either specifically, by immunoabsorption, or nonspecifically, by removal of plasma immunoglobulins. Once a suitable environment is created, in which the likelihood of hyperacute rejection is reduced, one must sustain a low titer of cytotoxic xenoantibody levels in the early post-transplant period, usually by pharmacologic methods. The second consideration is to minimize the inflammatory cascades that amplify the immune system. Specifically, complement activation leads to a number of inflammatory mediators that are difficult to control. Agents that can interfere with this cascade and the subsequent inflammatory mediators must be developed. Finally, sustained suppression of cell-mediated rejection is important to minimize the long-term damage to the xenograft that may occur via lymphocyte-derived cytokines.

Other considerations regarding the success of xenotransplants depend on the compatibility of proteins between the donor and recipient species[118] as well as potential for infectious diseases.[119] These are areas of immense interest to physicians studying xenotransplantation. Although three liver xenotransplantations have failed, the longest remaining viable for 72 days, important facts about the pathology, immunology, compatibility, and physiology were obtained and will aid in future attempts.[120-122] Although the baboon-to-human xenotransplants were encouraging, the limiting factors in the pursuit of concordant xenotransplantation are "humanization of primates," limited availability, donor size incongruity, and the theoretical risk of transmitting infectious agents. These concerns have prompted a quest to seek alternative sources of animals for clinical xenotransplantation.

Pigs are available in sufficient quantities, are similar in anatomy and physiology to humans, and can be bred under conditions where they can be genetically modified. These factors have prompted the consideration of this species as a source for clinical xenotransplantation, but hyperacute rejection mediated by naturally occurring antibodies (also called "preformed xenoantibodies") presents a formidable challenge. Because of the difficulty in controlling hyperacute rejection, novel approaches are required to overcome this barrier to successful discordant xenotransplantation.

One strategy that has been utilized is the removal of preformed antibodies from the recipient's blood prior to transplantation (a process known as *plasmapheresis*). Although this approach has been utilized in ABO blood type-incompatible human-to-human transplants with some success, its application in xenotransplantation has been limited, owing to the rapidity with which the preformed xenoantibodies are produced, resulting in rapid restoration of xenoantibody levels and leading to hyperacute rejection.[122] Strategies to eliminate xenoantibody production have not been successful, and alternative approaches must therefore be taken. Preformed xenoantibodies play a vital role in mediating hyperacute rejection, but they are not the effector molecules responsible for the observed damage in discordant xenografts. Antibody binding to the xenograft results in activation of another family of proteins, complement, which is normally present in circulating blood. These proteins exist in an inactive form but are activated when antibody binds to the target cells, resulting in damage to the cell. Normally, this process is self-limited by a process of inactivation by a group of cell surface-associated proteins called *complement inhibitory proteins*. Why, then, is activated complement in the discordant xenograft not rendered inert by these complement inhibitory proteins?

Complement inhibitory proteins can interact only with complement of the same species and not with that of different species *(homologous species restriction)*; this limitation may play an important role in liver xenotransplantation, because the liver is the primary source of complement synthesis.[123] Thus, following pig-to-human xenotransplantation, activated human complement will not be inactivated by the complement inhibitory proteins found on pig cells. One unique approach to this problem entails expressing human complement

inhibitory proteins on pig cells; this has been achieved by generating genetically modified pigs that carry the genes for human complement inhibitory proteins. Organs obtained from these transgenic pigs enjoy prolonged survival when transplanted across discordant barriers, suggesting that the human complement inhibitory proteins inserted genetically into the pig organ can overcome hyperacute rejection.[124-126] In these recipients, however, xenograft rejection still occurs by less understood mechanisms, including antibody-directed cell-mediated cytotoxicity. This finding suggests that additional approaches must be employed to overcome the immunologic barrier of xenotransplantation.[127]

SUMMARY

The field of transplantation has grown tremendously in the 45 years since the first successful human organ transplant. A better understanding of the immune mechanisms that cause graft damage, as well as new immunosuppressive agents, has helped put transplantation in a therapeutic realm. Unfortunately, with the success of transplantation, the scarcity of donor organs remains one of the principal limitations for broader applications. More than 7000 patients die every year while waiting for an organ; in the United States, for every individual who receives a transplant, three others are added to the waiting list. Efforts are constantly made to expand the donor pool, either by the use of donors who do not fulfill the criteria once applied to living donors or by xenotransplantation. Each of the next advances in the expansion of the donor pool is likely to generate controversy and will require careful scientific approaches to ensure the safety of the recipients. Other developments in the areas of bioartificial—totally artificial support devices and xenotransplantation—are of significant interest, because their successful development will address the organ shortage problem.

References

1. Carrel A: La technique opératoire des anastomose vasculares et la transplantation des viscères. Lyon Médicine 1902; 98:859.
2. Voronoy VV: Blocking the reticuloendothelial system in man in some forms of mercuric chloride intoxication and transplantation of the cadaver kidney as a method of treatment for the anuria resulting from the intoxication. Transplant Sci 1991; 1:71.
3. Merrill JP, Murray JE, Harrison JH, et al: Successful homotransplantation of the human kidney between identical twins. JAMA 1956; 160:277.
4. Gibson T, Medawar PB: Fate of skin homografts in man. J Anat 1942; 77:299.
5. Calne RY, Murray JE: Inhibition of the rejection of renal homografts in dogs by Burroughs Wellcome 57-222. Surg Forum 1961; 12:118.
6. Starzl TE, Marchioro TL, Waddell WR: The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet 1963; 117:385.
7. Reemtsma K, McCracken BH, Schlegel JU, et al: Renal heterotransplantation in man. Ann Surg 1964; 160:384.
8. Report of the Ad Hoc Committee of the Harvard Medical School: A definition of irreversible coma. JAMA 1968; 205:537.
9. Starzl TE, Miller C, Broznick B, et al: An improved technique for multiple organ harvesting. Surg Gynecol Obstet 1987; 165:343.
10. Collins GM, Bravo-Shugarman M, Terasaki PI: Kidney preservation for transportation. Lancet 1969; 2:1219.
11. Belzer FO, Southard JH: Principles of solid organ preservation by cold storage. Transplantation 1988; 45:673.
12. Borel JF, Feurer C, Gubler HU, et al: Biological effects of cyclosporin A: A new antilymphocytic agent. Agents Actions 1976; 6:468.
13. Calne RY, Rolles K, White DJG, et al: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreata, and 2 livers. Lancet 1979; 2:1022.
14. Starzl TE, Weil R, Iwatsuki S, et al: The use of cyclosporin A and prednisone in cadaveric kidney transplantation. Surg Gynecol Obstet 1980; 151:17.
15. U.S. Department of Health and Human Services (DHHS): 1991 Report of Center-Specific Graft and Patient Survival Rates. Washington, DC, DHHS, 1991.
16. U.S. Department of Health and Human Services (DHHS): 1994 Report of Center-Specific Graft and Patient Survival Rates. Washington, DC, DHHS, 1994.
17. U.S. Department of Health and Human Services (DHHS): 1997 Report of Center-Specific Graft and Patient Survival Rates. Washington, DC, DHHS, 1997.
18. United Network for Organ Sharing (UNOS) and the U.S. Department of Health and Human Services (DHHS): 1997 Annual Report: The U.S. Scientific Registry of Transplant Recipients and the Organ Procurement and Transplantation Network. Washington, DC, DHHS, 1997.
19. Gaston RS, Ayres I, Dooley LG, et al: Racial equity in renal transplantation: The disparate impact of HLA-based allocation. JAMA 1993; 270:1352.
20. Bronsther O, Fung JJ, Tzakis A, et al: Prioritization and organ distribution for liver transplantation. JAMA 1994; 271:140.
21. Burdick JF, Klein AS, Harper AM, et al: How should livers be allocated in the United States? *In:* Clinical Transplants 1996, Cecka JM, Terasaki PI (Eds). Los Angeles, UCLA Tissue Typing Laboratory, 1997, p 321.
22. Edwards EB, Bennett LE, Daily OP, et al: The relative risk of mortality for UNOS Status 3 liver recipients: A comparison of the risk post-transplant to the risk on the waiting list (Abstract). Presented at the 16th International Congress of the Transplantation Society, Barcelona, August 25-30, 1996.
23. U.S. Department of Health and Human Services: Organ Procurement and Transplantation Network (42 CFR, § 121)—Final Rule. RIN: 0906-AA 32, Docket No: 98-HRSA-01. 63 Federal Register 16295-16338 (1998).
24. American Medical Association (AMA) Council on Ethical and Judicial Affairs: Code of Medical Ethics—Current Opinions with Annotations. Chicago, AMA, 1997, pp 31-32.
25. Horton R: Changing the U.S. transplant system (Editorial). Lancet 1998; 352:79.
26. Billingham R, Lampkin G, Medawar P, et al: Tolerance of homografts, twin diagnosis, and the freemartin condition in cattle. Heredity 1956; 6:201.
27. Billingham RE, Brent L, Medawar PB: "Actively acquired tolerance" of foreign cells. Nature 1953; 172:603.
28. Ildstad ST, Sachs DH: Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. Nature 1984; 307:168.
29. Wilson WEC, Kirkpatrick CH: Immunologic aspects of renal homotransplantation. *In:* Experience in Renal Transplantation. Starzl TE (Ed). Philadelphia, WB Saunders, 1964, p 239.
30. Starzl TE, Demetris AJ, Trucco M, et al: Chimerism after liver transplantation for type IV glycogen storage disease and type I Gaucher's disease. N Engl J Med 1992; 328:745.
31. Murase N, Demetris AJ, Woo J, et al: Lymphocyte traffic and graft-versus-host disease after fully allogeneic small bowel transplantation. Transplant Proc 1991; 23:3246.
32. Starzl TE, Demetris AJ, Trucco M, et al: Systemic chimerism in human female recipients of male livers. Lancet 1992; 340:876.
33. Starzl TE, Demetris AJ, Trucco M, et al: Cell migration and chimerism after whole organ transplantation: The basis of graft acceptance. Hepatology 1993; 17:1127.
34. Nagler A, Ilan Y, Amiel A, et al: Systemic chimerism in sex-mismatched liver transplant recipients detected by fluorescence in situ hybridization. Transplantation 1994; 57:1458.
35. Starzl TE, Zinkernagel RM: The regulation of immune function by antigen migration and localization: With particular reference to infectious and transplantation tolerance. N Engl J Med, in press.
36. Cochrum KC, Salvatierra O, Belzer FO: Correlations between MLC stimulation and graft survival in living related and cadaver transplants. Ann Surg 1974; 180:617.
37. Salvatierra O, Metzer J, Vincenti F: Donor-specific blood transfu-

sions versus cyclosporine—the DST story. Transplant Proc 1987; 19:160.
38. Reed A, Pirsch JD, Armbrust MJ, et al: A comparison of donor-specific and random transfusions in living-related renal transplantation and their effect on steroid withdrawal. Transplant Proc 1991; 23:1321.
39. Rao AS, Fontes P, Zeevi A, et al: Enhancement of donor cell chimerism in whole organ allograft recipient by adjuvant bone marrow transplantation. Transplant Proc 1995; 27:3387.
40. Ricordi C, Karatzas T, Selvaggi G, et al: Multiple bone marrow infusions to enhance acceptance of allografts from the same donor. Ann N Y Acad Sci, 1995; 770:345.
41. Shapiro R, Rao AS, Fontes P, et al: Combined kidney/bone marrow transplantation—evidence of augmentation of chimerism. Transplantation 1995; 59:306.
42. Ricordi C, Karatzas T, Nery J, et al: High-dose donor bone marrow infusions to enhance allograft survival: The effect of timing. Transplantation 1997; 63:7.
43. Suthanthiran M, Strom TB: Mechanisms and management of acute renal allograft rejection. Surg Clin North Am 1998; 78:77.
44. Kelly PA, Gruber SA, Behbod F, et al: Sirolimus, a new, potent immunosuppressive agent. Pharmacotherapy 1997; 17:1148.
45. Kaplan B, Meier-Kriesche HU, Napoli KL, et al: The effects of relative timing of sirolimus and cyclosporine microemulsion formulation coadministration on the pharmacokinetics of each agent. Clin Pharmacol Ther 1998; 63:48.
46. Stepkowski SM, Tian L, Wang ME, et al: Sirolimus in transplantation. Archiv Immunol Ther Exp 1997; 45:383.
47. Ferron GM, Mishina EV, Zimmerman JJ, et al: Population pharmacokinetics of sirolimus in kidney transplant patients. Clin Pharmacol Ther 1997; 61:416.
48. Goggins WC, Risher RA, Cohen DS, et al: Effect of single-dose rapamycin-based immunosuppression on the development of cardiac allograft vasculopathy. J Heart Lung Transplant 1996; 15:790.
49. Schmid C, Heemann U, Azuma H, et al: Rapamycin inhibits transplant vasculopathy in long-surviving rat heart allografts. Transplantation 1995; 60:729.
50. Fahrni JA, Berry GJ, Morris RE, et al: Rapamycin inhibits development of obliterative airway disease in a murine heterotopic airway transplant model. Transplantation 1997; 63:533.
51. Goodyear N, Napoli KL, Murthy JN, et al: Radioreceptor assay for sirolimus in patients with decreased platelet counts. Clin Biochem 1997; 30:539.
52. Murgia MG, Jordan S, Kahan BD: The side effect profile of sirolimus: A phase I study in quiescent cyclosporine-prednisone-treated renal transplant patients. Kidney Int 1996; 49:209.
53. Brattstrom C, Sawe J, Tyden G, et al: Kinetics and dynamics of single dose of sirolimus in sixteen renal transplant recipients. Ther Drug Monit 1997; 19:397.
54. Brattstrom C, Wilczek H, Tyden G, et al: Hyperlipidemia in renal transplant recipients treated with sirolimus (rapamycin). Transplantation 1998; 65:1272.
55. Andoh TR, Burdmann EA, Fransechini N, et al: Comparison of acute rapamycin nephrotoxicity with cyclosporine and FK506. Kidney Int 1996; 50:1110.
56. Andoh TF, Lindsley J, Franceschini N, et al: Synergistic effects of cyclosporine and rapamycin in a chronic nephrotoxicity model. Transplantation 1996; 62:311.
57. Serkova N, Christians U, Flogel U, et al: Assessment of the mechanism of astrocyte swelling induced by the macrolide immunosuppressant sirolimus using multinuclear nuclear magnetic resonance spectroscopy. Chem Res Toxicol 1997; 10:1359.
58. Kahan BD for the Rapamune U.S. Study Group: A phase III comparative efficacy trial of Rapamune in renal allograft recipients (Abstract). Presented at the 17th World Congress of The Transplantation Society, Montreal, July 12-17, 1998.
59. Schuler W, Sedrani R, Cottens S, et al: SDZ RAD, a new rapamycin derivative: Pharmacological properties in vitro and in vivo. Transplantation 1997; 64:36.
60. Schuurman HJ, Cottens S, Fuchs S, et al: SDZ RAD, a new rapamycin derivative: Synergism with cyclosporine. Transplantation 1997; 64:32.
61. Deltz E, Schroeder P, Gebhardt H, et al: Successful clinical small bowel transplantation: Report of a case. Clin Transplant 1989; 3:89.
62. Grant D, Wall W, Mimeault R, et al: Successful small bowel/liver transplantation. Lancet 1990; 335:181.
63. Schroeder P, Goulet O, Lear PA: Small bowel transplantation: European experience (Letter). Lancet 1990; 336:110.
64. Todo S, Tzakis AG, Abu-Elmagd K, et al: Cadaveric small bowel and small bowel-liver transplantation in humans. Transplantation 1992; 53:369.
65. Todo S, Tzakis AG, Abu-Elmagd K, et al: Intestinal transplantation in composite visceral grafts or alone. Ann Surg 1992; 216:223.
66. Abu-Elmagd K, Reyes J, Todo S, et al: Clinical intestinal transplantation: New perspectives and immunologic considerations. J Am Coll Surg 1998; 186:512.
67. Weppler D, Khan R, Fragulidis GP, et al: Status of liver and gastrointestinal transplantation at the University of Miami. *In:* Clinical Transplants 1996. Cecka JM, Terasaki PI (Eds). Los Angeles, UCLA Tissue Typing Laboratory, 1997, pp 187-202.
68. Vanderhoof JA, Langnas AN: Short-bowel syndrome in children and adults. Gastroenterology 1997; 113:1767.
69. Asfar S, Atkison P, Ghent C, et al: Small bowel transplantation: A life-saving option for selected patients with intestinal failure. Dig Dis Sci 1996; 41:875.
70. Keenan RJ, Konishi H, Kawai A, et al: Clinical trial of tacrolimus versus cyclosporine in lung transplantation. Ann Thorac Surg 1995; 60:580.
71. Gammie JS, Keenan RJ, Pham SM, et al: Single- versus double-lung transplantation for pulmonary hypertension. J Thorac Cardiovasc Surg 1998; 115:397.
72. Mendeloff EN, Huddleston CB, Mallory GB, et al: Pediatric and adult lung transplantation for cystic fibrosis. J Thorac Cardiovasc Surg 1998; 115:404.
73. Grover FL, Fullerton DA, Zamora MR, et al: The past, present, and future of lung transplantation. Am J Surg 1997; 173:523.
74. Barr ML, Schenkel FA, Cohen RG, et al: Bilateral lobar transplantation utilizing living related donors. Artif Organs 1996; 20:1110.
75. Starnes VA, Barr ML, Cohen RG, et al: Living-donor lobar lung transplantation experience: Intermediate results. J Thorac Cardiovasc Surg 1996; 112:1284.
76. Ricordi C, Lacy PE, Finke EH, et al: An automated method for the isolation of human pancreatic islets. Diabetes 1988; 37:413.
77. Lakey JR, Warnock GL, Brierton M, et al: Development of an automated computer-controlled islet isolation system. Cell Transplant 1997; 6:47.
78. Carroll PB, Rilo HL, Alejandro R, et al: Long-term (>3-year) insulin independence in a patient with pancreatic islet cell transplantation following upper abdominal exenteration and liver replacement for fibrolamellar hepatocellular carcinoma. Transplantation 1995; 59:875.
79. Ricordi C, Tzakis AG, Carroll PB, et al: Human islet isolation and allotransplantation in 22 consecutive cases. Transplantation 1992; 53:407.
80. Secchi A, Socci C, Maffi P, et al: Islet transplantation in IDDM patients. Diabetologia 1997; 40:225.
81. Jaeger C, Brendel MD, Hering BJ, et al: Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. Diabetes 1997; 46:1907.
82. Rastellini C, Shapiro R, Corry R, et al: An attempt to reverse diabetes by delayed islet cell transplantation in humans. Transplant Proc 1997; 29:2238.
83. Sutherland DE: Pancreas and islet cell transplantation: Now and then. Transplant Proc 1996; 28:2131.
84. Hughes RH, Williams R: Clinical experience with charcoal hemoperfusion and resin hemoperfusion. Semin Liver Dis 1986; 6:164.
85. O'Grady JG, Gimson AES, O'Brien CJ, et al: Controlled trials of charcoal hemoperfusion and prognostic factors in fulminant hepatic failure. Gastroenterology 1989; 94:1186.
86. Rozga J, Podesta L, LePage E, et al: Control of cerebral edema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. Lancet 1993; 342:898.
87. Watanabe FD, Mullon CJP, Hewitt WR, et al: Clinical experience with a bioartificial liver in the treatment of severe liver failure: A phase I clinical trial. Ann Surg 1997; 225:484.
88. Sielaff TD, Nyberg SL, Rollines MD, et al: Characterization of the three-compartment gel-entrapment porcine hepatocyte bioartificial liver. Cell Biol Toxicol 1997; 13:357.

89. Nyberg SL, Platt JL, Shirabe K, et al: Immunoprotection of xenocytes in a hollow fiber bioartificial liver. ASAIO J 1992; 38:M463.
90. Thrift RN, Forte TM, Cahoon BE, et al: Characterization of lipoproteins produced by the human liver cell line, HepG2, under defined conditions. J Lipid Res 1986; 27:236.
91. Sussman NL, Chong MG, Koussayer T, et al: Reversal of fulminant hepatic failure using an extracorporeal liver assist device. Hepatology 1992; 16:60.
92. Ellis AJ, Hughes RD, Wendon JA, et al: Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. Hepatology 1996; 24:1446.
93. Hewitt WR, Corno V, Eguchi S, et al: Isolation of human hepatocytes from livers rejected for whole organ transplantation. Transplant Proc 1997; 29:1945.
94. Kobayashi N, Ito M, Nakamura J, et al: Hepatocyte transplantation improves liver function and prolongs survival in rats with decompensated liver cirrhosis (Abstract). Presented at the American Society of Transplant Physicians, Chicago, May 13–15, 1998.
95. Strom SC, Fisher RA, Thompson MR, et al: Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 1997; 63:559.
96. Fox IJ, Chowdhury JR, Kaufman SS, et al: Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med 1998; 338:1422.
97. Akiyoshi DE, Denaro M, Zhu H, et al: Identification of a full-length cDNA for an endogenous retrovirus of miniature swine. J Virol 1998; 72:4503.
98. Patience C, Takeuchi Y, Weiss RA: Infection of human cells by an endogenous retrovirus of pigs. Nature Medicine 1997; 3:282.
99. Jaremko J, Rorstad O: Advances toward the implantable artificial pancreas for treatment of diabetes. Diabetes Care 1998; 21:444.
100. Kawakami Y, Inoue K, Hayashi H, et al: Subcutaneous xenotransplantation of hybrid artificial pancreas encapsulating pancreatic B cell line (MIN6): Functional and histological study. Cell Transplant 1997; 6:541.
101. Benson JP, Papas KK, Constantinidis I, Sambanis A: Towards the development of a bioartificial pancreas: Effects of poly-L-lysine on alginate beads with BTC3 cells. Cell Transplant 1997; 6:395.
102. Hunter SK, Wang Y, Weiner CP, et al: Encapsulated beta-islet cells as a bioartificial pancreas to treat insulin-dependent diabetes during pregnancy. Am J Obstet Gynecol 1997; 177:746.
103. Koul B, Solem JO, Steen S, et al: HeartMate left ventricular assist device as bridge to heart transplantation. Ann Thorac Surg 1998; 65:1625.
104. Griffith BP, Kormos RL, Nastala CJ, et al: Results of extended bridge to transplantation: Window into the future of permanent ventricular assist devices. Ann Thorac Surg 1996; 61:396.
105. Holman WL, Bourge RC, Spruell RD, et al: Ventricular assist devices as a bridge to cardiac transplantation. A prelude to destination therapy. Ann Surg 1997; 225:695.
106. Cloy MJ, Myers TJ, Stutts LA, et al: Hospital charges for conventional therapy versus left ventricular assist system therapy in heart transplant patients. ASAIO J 1995; 41:M535.
107. Kootstra G: The asystolic, or non-heartbeating, donor. Transplantation 1997; 63:917.
108. Anaise D, Smith R, Ishimaru M, et al: An approach to organ salvage from non-heartbeating donors under existing legal and ethical requirements for transplantation. Transplant Proc 1990; 22:290.
109. Youngner SJ, Arnold RM: Ethical, psychosocial, and public policy implications of procuring organs from non-heart-beating cadaver donors. JAMA 1993; 269:2769.
110. Arnold RM, Youngner SJ (Eds): Ethical, psychosocial, and public policy implications of procuring organs from non-heart-beating cadavers. Kennedy Institute of Ethics Journal 1993; 3:103.
111. Funk J, Mazzolini J: Clinic puts controversial transplant plan on hold. *Cleveland Plain Dealer*, April 4, 1997, p 1.
112. Non-Heart-Beating Organ Transplantation: Medical and Ethical Issues in Procurement. Washington, DC, Division of Health Care Services, Institute of Medicine, National Academy Press, 1997.
113. Yokoyama I, Uchida K, Tominaga Y, et al: Ten-year experience in the use of double balloon catheter for kidney procurement from non-heart beating donors in cadaveric kidney transplant. Clin Transplant 1993; 7:258.
114. Casavilla A, Ramirez C, Shapiro R, et al: Experience with liver and kidney allografts from non-heart beating donors. Transplantation 1995; 59:197.
115. Cho YW, Terasaki PI, Cecka JM, et al: Transplantation of kidneys from donors whose hearts have stopped beating. N Engl J Med 1998; 338:221.
116. Calne RY: Organ transplantation between widely disparate species. Transplant Proc 1970; 2:550.
117. Chari RS, Collins BH, Magee JC, et al: Brief report: Treatment of hepatic failure with ex vivo pig-liver perfusion followed by liver transplantation. N Engl J Med 1994; 331:234.
118. Celli S, Valdivia LA, Fung JJ, et al: Early recipient-donor switch of the complement type after liver xenotransplantation. Immunol Invest 1997; 26:589.
119. Michaels M, Simmons R: Xenotransplant-associated zoonoses. Transplantation 1994; 57:1.
120. Starzl TE, Fung J, Tzakis A, et al: Baboon-to-human liver transplantation. Lancet 1993; 341:65.
121. Manez R, Kelly RH, Marino IR, et al: Complement activation correlates with graft damage in baboon-to-human liver xenotransplantation. Transplant Proc 1994; 26:1249.
122. Makowaka L, Cramer DV, Hoffman A, et al: The use of a pig liver xenograft for temporary support of a patient with fulminant hepatic failure. Transplantation 1995; 59:1654.
123. Valdivia LA, Fung JJ, Demetris AJ, et al: Donor species complement after liver xenotransplantation: The mechanism of protection from hyperacute rejection. Transplantation 1994; 57:918.
124. White DJ, Yannoutsos N: Production of pigs transgenic for human DAF to overcome complement-mediated hyperacute xenograft rejection in man. Res Immunol 1996; 147:88.
125. Heckl-Ostreicher B, Binder R, Kirschfink M: Functional activity of the membrane-associated complement inhibitor CD59 in a pig-to-human in vitro model for hyperacute xenograft rejection. Clin Exp Immunol 1995; 102:589.
126. Fodor W, Williams BL, Matis LA, et al: Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection. Proc Natl Acad Sci U S A 1994; 91:11153.
127. Starzl TE, Rao AS, Murase N, et al: Will xenotransplantation ever be feasible? J Am Coll Surg 1998; 186:383.