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TRANSPLANTATION

Abstract from 1970

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Preservation

Widespread and efficient use of organ homografts will not be possible without major developments in organ preservation that will allow banking for weeks or months. Ethacrynic acid and furosemide have a protective effect on rat kidneys if the drugs were administered at the beginning of the interval of vascular cross clamping. Canine kidney allografts were kept viable for 24 hours being perfused at low flow rates with a fluorocarbon in a cold salt solution emulsion. Surface cooling alone kept kidney autografts in good condition for 8 hours.

Excised canine hearts began to decay after 2 hours, whether left alone or perfused with oxygenated blood. In skin, there was no loss of antigenicity with freezing alone, but after freezing and lyophilization, histocompatability antigens could no longer be identified nor could second set reactions be induced. The clinical implication is that this kind of biologic dressing can be used without danger of recipient sensitization.

Diagnoses of Rejection

Urinary and serum concentrations of lactic dehydrogenase and alpha hydroxybutyric dehydrogenase were measured as indices of either physical or immunologic injury to renal homografts or autografts. There were elevations in both, but organ specificity was greater with alpha hydroxybutyric dehydrogenase. In cardiac transplantation, studies showed that the best diagnostic indicators of rejection are clinical observation and electrocardiography. Sepsis of the homograft in liver transplantation should not arbitrarily be ascribed to rejection, because hepatic abscesses and cholangitis were seen in experiments in the absence of an immunologic barrier. A decline in blood flow is a characteristic feature of all rejecting homografts.

Humoral Antibodies and Rejection

The classic view of rejection has been that destructive agents are mononuclear cells, but there is growing belief that circulating immunoglobulins play an important role in rejection. Sera from renal homograft recipients without overt rejection did not develop easily detectable antibodies, but in patients with clinically diagnosed rejection, cytotoxic antibodies were regularly found. When cytotoxic antibodies are present in a patient before transplantation, there is increased risk that the homograft may undergo immediate destruction.

Antilymphocyte Serum

Of all the immunosuppressive agents, heterologous antilymphocyte serum received the most attention. One study demonstrated that antisera could be raised against specific populations of lymphocytes. To date, there is no totally reliable in vitro test for assessing the immunosuppressive potency of antilymphocyte sera. The most specific risk of chronic therapy with heterologous serum or globulin is sensitization to the injected foreign protein. One study showed that tolerance to horse globulin could be produced with a course of intravenous antilymphocyte globulin, but other researchers warned that repeated infusion of high potency antilymphocyte serum causes local thrombosis and vasculitis.

Other Immunosuppressive Measures

In both humans and calves, mechanical lymphocyte depletion by thoracic duct drainage caused early rejection that was mild and late. Skin graft survival was prolonged using local homograft irradiation. Thiomycetin (thiamphenicol) significantly slowed rejection of canine renal homografts. Canine kidney recipients lived longer when treated with a protocol that substituted medroxyprogesterone for prednisone and also included azathioprine. Clinical trials with medroxyprogesterone substituting for prednisone are awaited with interest because of the excessively high morbidity rate after high dose prednisone therapy in kidney recipients. A study in rabbits showed that steroids caused increases in serum lipids,

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forming fat emboli that passed to terminal arterioles of bone and caused osteoporosis and osteonecrosis.

Graft Acceptance

One study showed a relation between sensitization and tolerance that depended on the dose of sensitizing antigen and the strength of the histocompatability barrier. Survival was prolonged in rats receiving kidney homografts when they were conditioned before the operation by intravenous administration of donor genotype spleen cells and injection of specific antidonor serum, suggesting that both tolerance and enhancement are factors in graft protection. It was reported that Kupffer cells of hepatic transplants became of host origin at all times after 3 months.

Graft Function

In dogs, reimplanted forelimbs almost never functioned normally, but gave auxiliary support. Perfused porcine spleens synthesized antihemophiliac globulin well enough to support the concept of splenic transplantation to treat hemophilia. Research reaffirmed the desirability of perfusing the portal system of liver homografts with venous blood from the splanchnic bed, but even when perfused, atrophy was usually observed if the hepatic transplants were used as auxiliary organs in dogs. It was reported that in liver transplantation, the homograft can synthesize protein of a new type, and that new gamma G globulin phenotypes were conferred on human recipients of orthotopic livers. In pulmonary transplantation, autografts had regeneration of efferent autonomic nerves by 2 to 6 months, but not of afferent autonomic nerves.

Heterotransplantation

Treatment with antilymphocyte serum significantly prolonged graft survival in skin transplantation from rats or hamsters to mice. When porcine kidneys were transplanted to goats, organ survival was one day in untreated recipients, but it increased to 14 days when antilymphocyte globulin and cytosine arabinoside were given. Pretreating rabbit kidneys with a collagen emulsion prepared from calf skin slowed the rejection process by 10 times in transplantation to dogs.

Surgical Technique

In the Shumway-Lower technique for orthotopic cardiac transplantation in dogs, using a Teflon coupler to reconnect the aorta and pulmonary artery lowered ischemia time and eliminated hemorrhage encountered in suturing the canine aorta.

Clinical Notes

The longest followup period is now 5 months for patients still living after combined pancreatic and renal transplant. Six patients have survived a year or more after liver transplant, and it appears the prime indication for liver transplantation in the future will be for benign diseases, such as biliary atresia and cirrhosis. Between six and ten patients have lived for a year or more after cardiac transplantation, the longest survival period being 17 months. ***What's New in Surgery, "Transplantation," Surg Gynecol Obstet, 1970, 130:316–321.***

Author's Retrospective

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My "What's New" comments in the 1970 issue of *Surgery, Gynecology, and Obstetrics* (now *Journal of the American College of Surgeons*) reflected the recent birth of this new specialty. Essentially all of the citations were from papers presented at the 1969 Surgical Forum, where fresh ideas are presented by young investigators.

The center pole of transplantation, then as now, is immunosuppression, with supporting struts of rejection, mechanisms of graft acceptance (and tolerance), preservation, tissue matching, and physiologic implications of allogeneic organ function. Major advances in each of these areas were made between 1959 and 1968, allowing the movement of organ transplantation to the clinics.

These areas all were subjects of reports at the Surgical Forum in the consolidation year of 1969. Organ preservation, the "orphan" of transplantation, was given special attention in my report, which bemoaned the fact that the only substantive progress had been with short-term "static" conservation based on hypothermia rather than technologies that would permit organ banking for weeks or months. Although banking at a practical level is no closer today

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than it was 28 years ago, the sophisticated technology is there and may be picked up again. In the meanwhile, "short term" became "medium term" static preservation using the Collins/Terasaki (1969) and University of Wisconsin solutions (1987). Ironically, the latter accomplishment by the late Folkert Belzer rendered obsolete the same Belzer's asanguineous perfusion technique (1968). Compared with other progress in transplantation, preservation remains an orphan.

By 1969, the concern of the 1950s and early 1960s about the adverse effects of organ denervation had been allayed. It also was generally conceded that normal physiologic processes were so closely shared by members of the same species that serious metabolic handicaps would not be imposed on recipients unless the donor (particularly of a liver) had undetected metabolic abnormalities. An important ripple effect of hepatic transplantation was the discovery (1960–1967) that splanchnic venous blood contained specific liver-supporting ingredients that prevented liver atrophy and were required for optimal hepatic regeneration. Although this was still controversial in 1969, the demonstration in transplant-derived models that insulin is a major liver growth factor (1973–1976) was the beginning of the special discipline of hepatotrophic (growth factor) physiology. This discipline continues to burgeon today and influences decisions in both transplantation and nontransplant surgery.

Although it is worth reflecting on what has happened in these broad areas in the nearly 3 decades since the 1969 Surgical Forum, the greatest changes have been in applied immunology. Without these, clinical transplantation would have atrophied from the state to which it had dramatically risen from zero, only to become mired at an unsatisfactory plateau by the end of the 1960s. All of the major organs had been successfully transplanted, but none reliably enough to be a legitimate clinical service. The impending ordeal of brownian movement would last for a decade.

IMMUNE SUPPRESSION

Less than 6 years after the systematic use of azathioprine and prednisone was first reported, the search was already on in 1969 for a replacement for the relatively weak and myelotoxic azathioprine (eg, thiampherical, an analogue of chloromycetin). It would be 10 years before the arrival of cyclosporine (Sir Roy Calne, 1978), followed by tacrolimus (1989), which also required use with dose-maneuverable adrenal cortical steroids. Studies of a third (adjunct) agent, polyclonal antilymphocyte globulin, dominated sessions at the 1969 Surgical Forum.

Although antilymphocyte globulin had been used clinically since 1966 and was credited with extension of transplantation from the kidney to extrarenal organs (eg, liver, heart), numerous controversial questions remained: its refinement from animal serum, the appropriate potency assay, toxicity (including oncogenicity), and optimal treatment scheduling. Evidence was presented at the 1969 meeting that lymphocyte subpopulations could be deleted or "blindfolded" according to the choice of lymphoid cells used to immunize the animal serum donor. This possibility was fully realized in the 1980s and 1990s with the engineering with hybridomas of monoclonal antilymphoid antibodies and, more recently, antibodies directed with laser precision to cell receptors and other increasingly discrete targets.

GRAFT ACCEPTANCE VERSUS REJECTION

Until 1969, the collective immunologist's view of rejection, not shared by many experienced transplant clinicians, was that rejection was a pure expression of cellular immunity. Yet, between 1965 and 1969, antibody-associated hyperacute rejection had been identified (Paul Terasaki), and Yoji Iwasaki and David Talmage had demonstrated (with an antiglobulin consumption test) seemingly harmless antibodies after virtually every successful kidney transplantation. Hypotheses were being formulated that included a role for "enhancing antibodies" to explain organ allograft acceptance by mechanisms other than the donor leukocyte chimerism that Billingham, Brent, and Medawar had found to be a necessary condition for acquired neonatal immunologic tolerance.

The chimerism-exclusionary theories were unchallenged for 30 years, beginning in 1962. This effectively disconnected the development of organ transplant surgery from the scientific base enjoyed by bone marrow transplantation, which ironically was not done successfully until 1968, 9 years after Joseph Murray's first successful kidney allotransplantation. A golden opportunity to avert the epistemologic col-

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lapse was missed at the 1969 Surgical Forum, when the full significance was not appreciated of the most important paper of the Congress, given by Kashiwagi. In the first longterm surviving female recipients of male livers, Kashiwagi reported the observation by his collaborator, K. A. Porter (London), that the nonparenchymal leukocyte population of human hepatic allografts had invariably been replaced by female recipient cells of the same lineages, converting the liver to a male/female composite. The products of hepatocyte synthesis changed permanently to donor phenotypes, allowing assurance that hepatic-based inborn errors of metabolism would be cured by liver replacement. Paradoxically, new gamma-globulin phenotypes of donor origin also were conferred upon these recipients. Although this meant that the replaced donor leukocytes had migrated and engrafted peripherally, the concept was so alien in 1969 and for years afterward that no one thought to look peripherally for the donor cells. When they were found in 1992 in kidney and liver recipients 10–30 years after transplantation, it was realized that donor leukocyte migration and chimerism were fundamental to the acceptance of all varieties of organ allografts.

The story was not completed until 1997, when transplantation immunity and infectious immunity were reconciled by Starzl and Zinkernagel.[1] It can now be stated with conviction that acquired tolerance to noncytopathic microorganisms and acceptance of allografts are variations on the same theme and are dependent on the same mechanisms. The donor-specific nonreactivity is explained primarily by donor leukocyte-driven clonal exhaustion/deletion (activation-associated tolerance). This process and its subsidiary mechanism of "immune indifference" are governed by antigen migration and localization, as opposed to the antigen per se.

In addition, most of the enigmas that made organ transplantation appear to follow different rules than bone marrow transplantation were resolved. With the hematolymphopoietic cytoablation (with irradiation or cytotoxic drugs) used to precondition bone marrow transplant patients, the recipients were made vulnerable to a one-way graft-versus-host reaction unless the donor was HLA matched. In contrast, the "nullification" occurring after organ transplantation with a double immune reaction (graft-versus-host and host-versus-graft [rejection]) allowed hepatic, heart-lung, intestinal, and abdominal multivisceral grafts to be transplanted to nonconditioned recipients with impurity, and with "blindfolding" of the HLA-matching effect. The surprisingly poor prognostic discrimination of HLA matching in kidney transplantation could be discussed without rhetoric for the first time, allowing a consensus development to begin for resolving the 30-year controversy about the value of HLA matching, which was just surfacing in 1969.

Without the insights into mechanisms of graft acceptance that have evolved recently, it is very difficult to envision moving on to the successful use of xenografts from phylogenetically close, much less more distant (discordant), species. Having moved inside a seamless conceptual world, it has been easier to look out than it was trying to see in from the murky outside.[2]

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