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Original Articles

Milestones in Transplantation: The Story so Far [06/12/2001]

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Introductory Note

The milestones in the following material were discussed at a historical consensus conference held at the University of California, Los Angeles (UCLA) to which 11 early workers in transplantation were invited: Leslie B. Brent (London), Roy Y. Calne (Cambridge, UK), Jean Dausset (Paris), Robert A. Good (St. Petersburg, USA), Joseph E. Murray (Boston), Norman E. Shumway (Palo Alto), Robert S. Schwartz (Boston), Thomas E. Starzl (Pittsburgh), Paul I. Terasaki (Los Angeles), E. Donnall Thomas (Seattle) and Jon J. van Rood (Leiden). Each person provided personal reflections which have been published in a special issue of the *World Journal o Surgery* (2000, Vol. 24: 755-843). However, the ultimate objective was to reach consensus on the key historical discoveries prior to 1975 that eventually allowed clinical transplantation to become a feasible and practical form of therapy. Carl Groth of Stockholm was invited to be the Chairman for these consensus deliberations, and to prepare the executive summary (1). Consensus landmarks in the summary were restricted to those made at least a quarter of a century ago. Although advances in the 1975-2000 period were not formally reviewed, the ones alluded to here appear destined for milestone status.

The concept of transplanting animal or human tissues and organs to patients is almost as old as recorded history (2). However, the first enduring contribution was the technology of blood vessel anastomosis developed by Carrel (3) (see Table I). Carrel recognized that transplanted organ allografts and xenografts were not permanently accepted, although he did not know why.

Table I: Nobel Prizes related to immunology/transplantation.

Year	Name	Accomplishment
1901	Emil Adolf Von Behring	Discovery of antibodies
1905	Heinrich Hermann Robert Koch	Cause and effect of microorganisms and infection
1908	Ilya Metchnikoff Paul Ehrlich	Champion of cellular immunity Side chain (receptor) concept; antimicrobial therapy Champion of humoral immunity
1912	Alexis Carrel	Vascular surgery and transplantation

1919	Jules Bordeau	Discovery of complement
1930	Karl Landsteiner	Discovered ABO blood group antigens
1960	Sir Frank MacFarlane Burnet Sir Peter Brian Medawar	Clonal selection hypothesis Acquired transplantation tolerance
1972	Gerald M. Edelman	Characterized immunoglobulins
	Rodney R. Porter	Clarified structure of antibody molecule
1980	Baruj Benacerrat Jean Dausset George Davis Snell	Discovered immune response genes Discovered first HLA antigen Discovery of major histocompatibility complex (MHC) in mice
1984	Niels Kaj Jerne Georges J.F. Kohler Cesar Milstein	Important immunologic hypotheses Hybridoma technology Hybridoma technology
1985	Michael Stuart Brown Joseph Leonard Goldstein	Hepatic control of cholesterol metabolism
1987	Susumu Tonegawa	Discovered somatic recombination of immunologic receptor genes
1988	Gertrude Belle Elion George Herbert Hitchings	Co-discovered 6-MP and azathioprine
1990	Joseph E. Murray E. Donnall Thomas	Kidney transplantation Bone marrow transplantation
1996	Rolf Zinkernagel Peter C. Doherty	Co-discovered the role of MHC restriction in adaptive immune response to pathogens

Source: Nobel Foundation, Stockholm.

The Technical Challenge

The kidney

Attempts at clinical renal xenotransplantation by vascular anastomoses were undertaken at the beginning of the 20th century in France and Germany using pig, sheep, goat and subhuman primate donors. None of the kidneys functioned for long, if at all, and the unmodified human recipients died a few hours to 9 days later. No further clinical xenotransplantations were tried again until chimpanzee (4) and baboon kidney xenografts were transplanted to human recipients under immunosuppression (5).

The first known attempt at transplantation of an organ allograft was reported from Kherson (the Ukraine) in 1936 by Yu Yu Voronoy (6); an English translation of the article was provided by Hamilton and Reid (7). The kidney, which was removed from a cadaver donor, never functioned. This was not surprising in view of multiple adverse factors: ABO incompatibility between the donor and recipient, a 6-hour delay at room temperature between donor death and kidney removal, and the recent suicide attempt of the recipient by mercury ingestion. In 1951, systematic clinical trials of kidney allotransplantation in unmodified human recipients were undertaken in France by Kuss, Teinturier and Milliez (8), Dubost *et al.* (9), and Servelle, Soulie, and Rougeulle (10). Most of the kidneys were obtained from criminals immediately after their execution by the guillotine, and some briefly excreted urine.

The first live donor kidney transplantation was performed in Paris by Michon *et al.* (11), using the extraperitoneal pelvic procedure developed by Kuss. This mother-to-son transplantation resulted in prompt kidney function that continued for 3 weeks before the allograft was rejected by the unmodified recipient. Kuss's procedure has been used worldwide ever since with an outstanding record of safety and reliability.

In the meantime, nine kidney allotransplantations were performed between March 30, 1951, and December 3, 1952, in patients whose pre- and post-transplantation dialysis was at the Peter Bent Brigham Hospital ("The Brigham") in Boston (12). In the first of these operations, performed by L.H. Doolittle in Springfield, Massachusetts, the

allograft was transplanted to the vacated renal fosa of the recipient after removal of the native organ. The next eight transplantations were performed at the Brigham. All eight of these allografts were placed by David Hume in the recipient anterior thigh. Some of the recipients received adrenal cortical steroids, and one of the transplanted kidneys produced urine for 5 months.

In December 1954, kidney transplantation from an identical twin donor recipient was carried out by Joseph E. Murray at the Brigham Hospital in Boston (13, 14). It was known from earlier research by plastic surgeons that skin grafts from identical twins were not rejected. To test genetic identity, reciprocal skin grafting was carried out prior to the kidney transplantation. Despite 82 minutes of warm ischemia, the isograft functioned immediately and for the next 25 years, until the death of the recipient from atherosclerotic coronary artery disease. Although the identical twin kidney transplantations did not provide fundamental new information about transplantation immunology, the cases exemplified the potential power of transplantation.

The extrarenal organs

Using vascular surgical techniques, animal research in transplantation was most highly focused on the kidney for most of the first half of the 20th century. The extrarenal vacuum rapidly was filled between 1958-1960 with the development in several laboratories of canine models with which to study all of the intra-abdominal and thoracic organs. Although each organ presented specific technical and physiologic issues, the core problems of immunosuppression, tissue matching and allograft preservation eventually were worked out mainly with the kidney and/or the liver and applied to other organs with minor modifications.

The Seminal Turning Points

The modern history of transplantation could be written from the vantage point of the first successful use in humans of allografts of the various organs and of bone marrow (Table II). However, a more accurate and complete picture can be obtained by reviewing how it was learned to harness destructive immunity enough to allow allograft survival. After Medawar's demonstration that rejection is an immune reaction (15), the feasibility of transplanting allografts hinged on two observations. The first was the discovery in 1953 by Billingham, Brent and Medawar (16, 17) that chimerism-associated neonatal tolerance could be induced in intrauterine and neonatal mice by the infusion of donor hematolymphopoietic cells (*i.e.,* splenocytes and bone marrow). The second seminal turning point was the recognition that human organ allografts were inherently tolerogenic when transplanted to immunosuppressed recipients (18).

Table II: First successful transplantation of human allografts (survival > 6 months).

Organ	City	Date	Physician/Surgeon
Kidney	Boston	January 24, 1959	Murray *et al.* (36)
Bone marrow	Paris	April 23, 1963	Mathe *et al.* (28)
Liver	Denver	July 23, 1967	Starzl *et al.* (71)
Heart	Cape Town	January 2, 1968	Barnard (131)
Pancreas*	Minneapolis	June 3, 1969	Lillehei *et al.* (132)
Lung**	Ghent	November 14, 1968	Derom *et al.* (133)
Abdominal multivisceral***	Pittsburgh	November 1, 1987	Starzl *et al.* (134)
Intestine alone	Paris	March 18, 1989	Goulet *et al.* (135)

*Kidney and pancreas allografts in uremic patient. **Patient died after 10 months. The first > 1-year survival of isolated lung recipient was not reported until 1987 (136). ***Small and large bowel plus liver, pancreas, stomach and duodenum. The patient died after 6 months.

Unfortunately, these two sets of observations led to a Y in the road, beyond which successful engraftment of bone marrow was explained by donor leukocyte chimerism-dependent mechanisms, as opposed to organ engraftment, which was attributed to chimerism-independent mechanisms. This egregious error precluded

genuine insight into the immunology of transplantation for nearly three decades and resulted in a systematic misinterpretation of research studies in transplantation (19). That a mistake of such magnitude could have been perpetuated for so long without a single challenge in the scientific literature is truly remarkable. This can be explained in part by the primitive state of immunology (see next section) at the time the false dogma became imbedded in the scientific literature and textbooks.

The Ascendancy of Immunology

The foundation of immunology had been laid at the turn of the century by the piecemeal discovery of the different components of the immune response and of the role of immunity in the defense against infectious disease. This wave of advances resulted in a large proportion of the first 30 Nobel prizes (Table I). The big leaps were succeeded by a period of "consolidation" that was reflected by a gap of 30 years between the 1930 Nobel prize for Landsteiner's discovery of ABO groups and the 1960 prize which was awarded to Burnet and Medawar (see Table I). Burnet with Fenner (20) had initially postulated that an individual produced antibodies only to those antigens to which (s)he had been exposed. Over the next decade, this clonal selection hypothesis was validated and extended by Burnet (21) to cellular immunity, thus providing a conceptual framework for the ontogeny of the immune system as well as for its function.

An important supporting strut in Burnet's hypotheses was the earlier study by Owen (22) of freemartin cattle, the bovine equivalents of human fraternal twins. Owen observed that permanent hematopoietic chimerism developed reciprocally in calves whose placentas had fused, thereby allowing intrauterine circulatory exchange. Working with Medawar, Anderson *et al.* (23) subsequently showed that these cattle also had reciprocal tolerance to skin grafts. The authors speculated that actively acquired tolerance could be induced deliberately by mimicking the conditions of cross circulation. When the neonatal tolerance experiments of Billingham, Brent, and Medawar (16,17) upheld the prediction, the observations were viewed as formal proof of the clonal selection theory of Burnet.

The Billingham-Brent-Medawar Experiments

In the original Billingham-Brent-Medawar experiments (16), acquired tolerance to skin allografts was induced in fetal and neonatal mouse recipients whose immunologically immature immune system was unable to reject the infused splenocytes from adult donors. In later experiments, bone marrow cells were used (17). The inoculated animals that survived to adult life had circulating donor leukocytes and developed specific nonresponsiveness to donor strain skin allografts, while evolving normal reactivity to third-party grafts. By 1957, however, it had been learned that the engraftment of immunocompetent donor leukocytes in immunologically defenseless recipients caused graft-*versus*-host disease (GVHD) that was avoidable or controllable only when there was a close genetic relationship between donor and recipient (*i.e.,* a good tissue match) (24-26).

In a logical extension of these experiments, adult mice were preconditioned for bone marrow transplantation with supralethal total body irradiation [TBI]). With engraftment of the donor bone marrow cells in these animals, the result was the same in principle as that achieved a decade later with human bone marrow transplantation to cytoablated recipient. However, the requirement for a good tissue match in mice (27) applied equally to humans (28-30). Mathe was the first to achieve prolonged survival after engraftment of allogeneic human bone marrow cells, but in spite of good histocompatibility between multiple familial donors and the cytoablated recipient, chronic graft-*versus*-host disease (GVHD) developed in this patient and caused his death after 2 years. Finally, in 1968, bone marrow cells were successfully transplanted from familial donors into two recipients whose immune deficiency diseases made cytoablation unnecessary (29, 30). Both patients are alive and well 32 years later.

Immunosuppression for Organ Transplantation

Once rejection was identified by Medawar (15) as an immunological event, weakening the recipient response with TBI or with immunosuppressive drugs, became a logical strategy for mitigating or preventing the immune reaction. Cortisone, which did not depress bone marrow, was the first drug to prolong the survival of rabbit skin allografts (31), but the effect was modest. In contrast, observations in chickens reported by Cannon and Longmire (32) were of exceptional significance. However, the importance of this work was not recognized until many years later.

In control experiments, Cannon and Longmire showed that freshly hatched chicks permanently accepted skin allografts from different adult breeds in 6% of experiments. When a course of cortisone treatment was given, this incidence rose to over 20% without an increased mortality. The critical observation was that the skin allograft survival was of lifetime duration, *i.e.*, continued after discontinuance of the steroid course. This finding presaged the discovery in Denver a decade later that organs were inherently tolerogenic in patients treated with azathioprine and dose-maneuverable prednisone (18). Because the Cannon/Longmire studies had been long since passed over, however, they did not alter the pessimistic attitudes prevalent at the time about the feasibility of clinical organ transplantation.

Total body irradiation (TBI)

Instead, it was widely believed by the late 1950s that successful organ transplantation would not be possible without establishing donor leukocyte chimerism by the concomitant or preceding engraftment of donor bone marrow cells as had been shown to be feasible in cytoablated mice (27, 33). In practice, this approach proved to be impossible in large outbred animals (34). With the dog model, only a single irradiated beagle recipient survived for as long as 70 days following combined bone marrow and kidney transplantation (35).

Despite this discouraging record in animals, Murray *et al.* attempted the combined procedure at the Peter Bent Brigham Hospital (Boston) in two TBI-conditioned patients, both of whom died in less than a month. The next 10 human recipients in this trial were conditioned in 1958-60 with sublethal total body irradiation (TBI), followed by kidney transplantation alone (36, 37). All but one of the patients also died within 1 month. In the exceptional case, however, the irradiated recipient of a fraternal twin kidney survived for more than 20 years before dying of a malignant tumor. It was the first example in the world in any species including humans of successful organ transplantation from a genetically nonidentical donor.

It was suspected initially that placental fusion and cross circulation between the twins may have occurred *in utero* (as with Owen's freemartin cattle). The same reservation pertained to a second extended survival following fraternal twin kidney transplantation in Paris 5 months later (38). In the succeeding 3 years, however, the issue was settled by 4 more examples of survival ≥ 1 year in Paris. Two of these irradiated patients received kidneys from non-twin family members (39). The kidneys in the other 2 cases were from non-related donors (40)(Table III). Because none of the 6 kidney allograft recipients who had ≥ 1 year survival had been given adjunct donor bone marrow cells, it was concluded that donor leukocyte chimerism was not a necessary condition for successful kidney transplantation.

Table III: Kidney transplantation ≥6 months' survival as of March 1963.

Case (reference)	City	Date	Donor	Survival (months)+
1 (36, 137)	Boston	January 24, 1959	Fraternal twin	>50
2 (38, 39)	Paris	June 29, 1959	Fraternal twin	>45
3 (40)	Paris	June 22, 1960	Unrelated*	18 (died)

3 (40)	Paris	June 22, 1960	Unrelated	15 (died)
4 (38, 39)	Paris	December 19, 1960	Mother*	12 (died)
5 (40)	Paris	March 12, 1961	Unrelated*	18 (died)
6 (38, 39)	Paris	February 12, 1962	Cousin*	>13
7 (36, 137)	Boston	April 5, 1962	Unrelated	10

*Adjunct steroid therapy. +The kidneys in patients 1, 2 and 6 functioned for 20.5, 25 and 15 years, respectively. Patient 7 rejected his graft after 17 months and died 7 months after return to dialysis.

Pharmacologic immunosuppression

Because the failure rate using TBI was overwhelming, the prospects for developing kidney transplantation as a clinical service remained grim until the end of 1962. A sea change began with the testing of drugs whose myelotoxicity initially prompted their use as a substitute for TBI. In 1963, Willard Goodwin of Los Angeles belatedly reported a case of a mother-to-daughter kidney transplantation that had been carried out in September 1960, after first producing severe bone marrow depression with large doses of methotrexate and cyclophosphamide. During the 143 days of survival, the recipient developed several rejections that were reversed with prednisone (41).

Although this was the first example of extended survival of a human kidney recipient without the use of TBI, the case did not significantly impact the field because its existence was not generally known. In the meanwhile, it was learned in studies of rabbit skin transplantation (42, 43) and of kidney transplantation in dogs (44-46) that the drug 6-mercaptopurine (6-MP) and its imidazole derivative azathioprine, were immunosuppressive at submyelotoxic doses. Both agents permitted only about 5% long-term survival of canine kidney allografts (37), but the transplanted kidneys in some of the long-surviving animals continued to function long after discontinuance of immunosuppression (47-50). The observation was reminiscent of the finding in newly hatched chicks treated with a short limited course of cortisone that had been described a decade earlier (32).

Realizing that neither 6-MP nor azathioprine alone would permit more than an occasional clinical success, Calne and Murray (51) tested azathioprine in combination with other myelotoxic drugs at the Brigham canine laboratory. Prompted by the personal communication from Goodwin about the effect of steroid therapy (52), prednisone was also added to azathioprine. When the azathioprine-prednisone combination appeared to be no more effective than azathioprine alone (45, 53), the decision was made to use azathioprine with the myelotoxic agents azaserine and actinomycin for the Boston clinical trials. Only one of the first 10 drug-treated kidney recipients survived. The exceptional patient received a non-related kidney on April 5, 1962, and remained dialysis-free for 17 months until the allograft was lost to chronic rejection. Thus, this patient became the seventh 1-year kidney allotransplant survivor in the world. More importantly, he was the first to achieve this milestone without TBI (Table III).

With the low rate of success, however, kidney transplantation was still viewed with pessimism. This was relieved by two observations at the University of Colorado, first in canine kidney recipients and then in a series of 10 consecutive human cases compiled in 1962-63. The addition of high doses of prednisone to baseline therapy with azathioprine resulted not only in reversal of established rejection, but also in the variable induction of donor specific tolerance (18). Nine of the 10 human recipients had prolonged survival and 2 remain alive today with excellent function of their original grafts in their 38th post-transplant year. These 2 patients (cases 2 and 3 in the original series), who bear the longest continuously functioning allografts in the world, have been free of all immunosuppression for 5 and 36 years respectively.

These results, and especially the easily taught treatment principles with which they were accomplished, fostered a whirlwind of activity in the United States and Europe. While dozens of new kidney transplant centers were established, the mechanisms by

which the allografts had been "accepted" remained unknown. However, the conclusions that the kidney allografts had induced variable donor-specific tolerance, and that engraftment depended on alterations in the transplanted organ plus a loss of specific responsiveness by the recipient (18, 49) has stood the tests of time and of experimental verification.

These conclusions continued to generate controversy for three decades. Commenting on mechanisms, Medawar (54) suggested that a progressive replacement of the vascular endothelium of the graft vessels by endothelium of host origin may have been made possible by weakening the allograft reaction with immunosuppression. Neither this hypothesis nor the suggestion that "a protective" host antibody had come to cover the endothelial layer (attributed to Calne by Medawar [54]) has proved to be correct.

The enigmatic phenomena of the reversal of rejection and the development of variable donor-specific tolerance observed after kidney transplantation were soon demonstrated with other organs. Furthermore, canine recipients of orthotopic liver allografts self-induced tolerance under short-term azathioprine therapy much more frequently than renal allografts (55). Soon thereafter, examples of spontaneous engraftment and self-resolving rejection crises in the absence of treatment were reported following liver transplantation in untreated outbred pigs (56-58). Spontaneous tolerance has since been demonstrated in rats (59, 60) and mice (61).

Eventually, it was shown that heart (61, 62) and kidney allografts (63) could also self-induce donor-specific tolerance in selected mouse strain combinations. Exhaustion and deletion of an antigen-specific clone was one of the mechanisms proposed for allograft acceptance as early as 1964 and again in 1969 (Fig. 1), but this was difficult to defend in the absence of chimerism. The failure to evolve an intellectual framework with which to explain organ engraftment contrasted with bone marrow transplantation in which the association of tolerance with donor leukocyte chimerism was readily apparent.

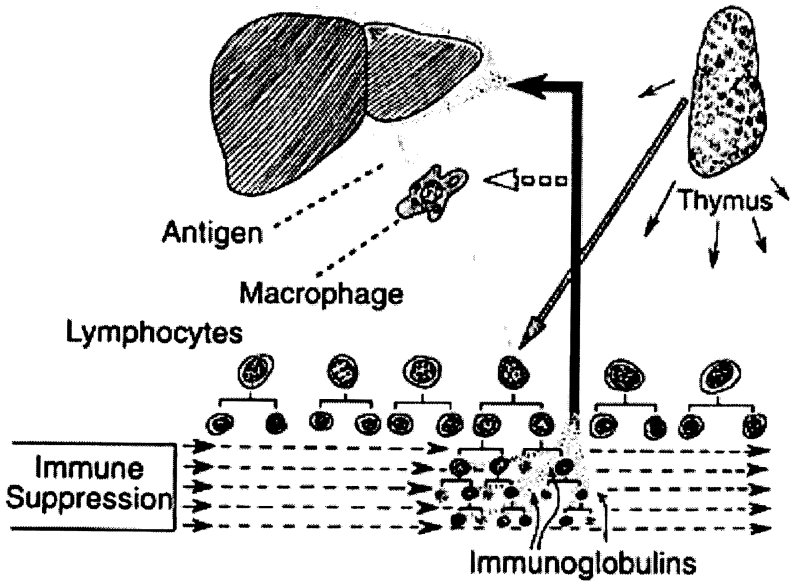

Fig. 1

Thus, organ transplantation became disconnected from a scientific base, creating an image that was judged at times to be dubious--scientifically, ethically and practically. A widely-expressed opinion was that chronic immunosuppression would lead inevitably to lethal infectious complications and/or the development of malignant tumors (Fig. 2). These complications did, in fact, prove to be common. Infection was

exemplified by the cytomegalovirus (CMV), which normally has low pathogenicity, but which has been responsible for many post-transplantation deaths as a coinfection with *Pneumocystis carinii. De novo* neoplasms, and particularly the Epstein Barr virus-associated B-cell malignancies, were prototype examples of the loss of tumor surveillance (64). As it turned out, these problems were manageable.

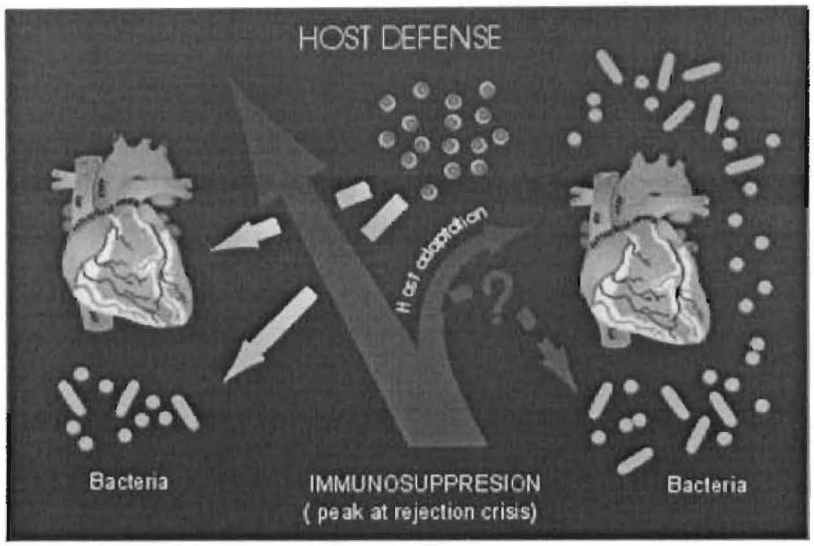

Fig. 2

Antilymphoid strategies

Successful kidney transplantation was first accomplished several years before the lymphocyte had any known function, and almost a decade before the distinction between T- and B-lymphocytes was made. After Gowan¨s studies in rats demonstrated the defects in the immune response caused by lymphoid depletion with thoracic duct drainage (TDD) (65, 66), TDD was used in Stockholm in 1963 and subsequently to precondition human kidney recipients (67). Although moderately effective, TDD was inconvenient and expensive. When Woodruff and Anderson (68) added antilymphocyte serum (ALS) to TDD, the effects were additive, but this combination was never used clinically.

In 1966, heterologous antilymphocyte globulin (ALG) was introduced clinically for lymphoid depletion (69). In preclinical studies, horse anti-dog antilymphocyte serum (ALS) was raised and the active component was shown to be a gamma globulin moiety (70). After demonstrating that the refined horse anti-dog ALG inhibited or reversed kidney and liver rejection in dogs, horse anti-human ALG was raised and given to human kidney recipients as a short-term adjunct to azathioprine and prednisone. After the "triple drug" therapy was shown to be effective in the kidney trial, the same treatment was used in 1967 for the first successful liver transplantations (71).

Within 24 months after the first successful liver replacement, many extrarenal transplant programs (*i.e.,* heart, lung and pancreas) were begun, using triple drug therapy. Although isolated successes were recorded (Table II), most of these new programs closed because of the high mortality. Nevertheless, ALG played an important role in the first successful extrarenal organ transplant procedures and served as the therapeutic model for strategies using the more standardized antibody preparations made possibly by the hybridoma technology of Kohler and Milstein (72).

OKT3, a monoclonal antibody directed against all T-lymphocytes was introduced clinically in the early 1980s (73) and is still part of the immunosuppressive armamentarium. Other monoclonal antibody preparations have been developed more

recently, some of which are humanized hybrids and directed at such diverse targets as T-cell subsets, adhesion molecules and receptors for T-cells or interleukin-2 (IL-2). Their diversity notwithstanding, the use of all of the monoclonal antibodies is guided by the same treatment principles that were developed with the crude ALG.

T-Cell Directed Drugs

Borel *et al.* (74) showed that cyclosporine depressed cellular immunity by acting with relative specificity on T-lymphocytes without depressing the bone marrow and without obvious toxicity to other organs. Borel *et al.* also reported that the new drug prolonged skin allograft survival in mice, rats and guinea pigs. Kostakis *et al.* (75), Calne and White (76) and Green and Allison (77) then demonstrated that cyclosporine could prevent or delay heart, kidney, liver or pancreas rejection in rats, rabbits, dogs and pigs. After cyclosporine was introduced clinically, the dose-limiting nephrotoxicity of the drug became apparent as well as its neurotoxicity, diabetogenicity, cosmetic side effects and propensity to induce B-cell lymphomas (78, 79). At lower doses, and in combination with prednisone (80), the prognosis with transplantation with all kinds of organs, and especially the liver (81), was dramatically improved (Fig. 3).

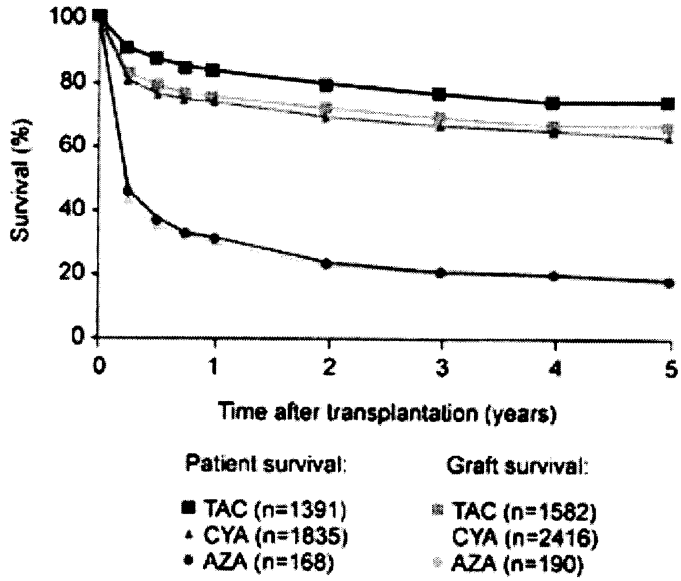

Fig. 3

Tacrolimus was first used clinically as a replacement for cyclosporine in patients who were intractably rejecting liver allografts (82, 83). When the drug was found to rescue >75% of rejecting hepatic allografts and other kinds of rejecting organs, trials began using tacrolimus as the baseline agent from the outset (84). Tacrolimus did not have cyclosporine's cosmetic side effects, but the nephrotoxicity, neurotoxicity and diabetogenicity profiles were similar. As with cyclosporine, these problems were dose-related and manageable by using tacrolimus in combination with prednisone or in more complex drug cocktails. Survival of various kinds of grafts and their recipients was improved (Fig. 3) and it became possible for the first time to offer intestinal transplantation as a clinical service (85). Most recently, tacrolimus has been combined with rapamycin, with unprecedented control and prevention of rejection.

Organ Preservation

Very little research had been done on preservation of organs at the time clinical kidney transplantation suddenly and unexpectedly became a widely used form of treatment in the early 1960s. Total body hypothermia of live donors was used initially to minimize ischemic injury to the excised kidney (86), but this potentially dangerous

to minimize ischemic injury to the excised kidney (86), but this potentially dangerous practice was promptly supplanted by infusion of chilled fluids into the allograft renal artery immediately after donor nephrectomy (87). Intravascular cooling of liver allografts with chilled lactated Ringer's solution had been developed much earlier in canine liver transplant experiments and had dramatically increased the chance of survival (88). Today, intravascular cooling derived from the *in situ* techniques of Marchioro *et al.* (89) remains the first step in the preservation of all cadaveric organs.

Two basic strategies for extending organ graft survival after initial cooling were also developed with kidneys and livers, and applied to other organs. In one, *ex vivo* perfusion techniques were used to simulate normal physiologic conditions as pioneered by Carrel and Lindberg (90). Using blood for priming, the technology was modified by Ackerman and Barnard (91). Because these perfusion methods were too complex for general use, Belzer, Ashby and Dunphy (92) developed a simplified asanguinous perfusion technique, which eventually was abandoned in favor of the second option of "slush" preservation.

Slush preservation consists of intravascular infusion with chilled fluids, followed by immersion of the organ in the fluid and simple refrigeration. Collins, Bravo-Shugarman and Terasaki (93) replaced the original lactated Ringer's solution with a perfusate that resembled the electrolyte composition of intracellular fluid. Renal allograft preservation with the "Collins solution" was reliable for at least a day, and preservation of the liver was adequate for approximately 6 hours. Nearly 20 years passed before the advent of the University of Wisconsin (UW) solution allowed the safe preservation of livers for 24 hours (94, 95) with a doubling of the safe time for kidney preservation. The UW solution made the exchange of organs between different cities or countries a reality.

Before 1980, cadaveric organ procurement and kidney procurement were essentially synonymous. With the emergence of extrarenal organ transplantation, flexible techniques were developed with which the kidney, liver, heart, lung, pancreas and even intestine could be removed separately or in combinations (96, 97). These flexible techniques involve cooling of all organs *in situ* and removal in a bloodless field, followed by *ex vivo* dissection. Taken together, the improvements in organ procurement and preservation have allowed the efficient use of donor organs, an especially important advance in view of the world-wide shortage of essentially all kinds of allografts.

Immunologic Screening

Matching cadaveric donors against a list of prospective recipients was not possible until effective methods of organ preservation became available. Consequently, it had been predicted in the early 1960s that tissue matching and organ preservation would have to develop in parallel if long-term engraftment of tissues and organs was to succeed with any degree of reliability and predictability. Instead, immunologic screening of donors and recipients played a very little role in the developmental period of organ transplantation during the volatile period of 1959-1968.

The crossmatch principle

As it turned out, the greatest impact of pretransplant immunologic screening has been the prevention of hyperacute rejection by observation of ABO compatibility guidelines and the routine use of the cytotoxicity crossmatch.

ABO incompatibility. Hyperacute rejection was first observed more than 30 years ago when ABO-mismatched renal allografts were transplanted into patients who had preformed antigraft ABO isoagglutinins (98). After such kidneys were lost on the operating table, arteriograms of the infarcted organs showed nonfilling of the small vessels. The gross findings correlated histopathologically with widespread thrombotic occlusion of the microvasculature.

It was concluded that high-affinity ABO isoagglutinins in the recipient sera had bound to A or B antigens in the graft vessels and parenchymal cells. The guidelines formulated from this experience (Table IV) were designed to avoid such antibody confrontations (98). The ABO rules also apply to heart, liver, and other kinds of organ transplantation. However, not all organs placed in an environment made hostile by antigraft isoagglutinins meet the same fate. In addition, it was learned at an early time that the liver is more resistant to antibody attack than other organs (99).

*Table IV: Direction of acceptable organ transfer when the donor and recipient have different ABO red cell types (98).**

O to non-O	Safe
Rh- to Rh+	Safe
Rh+ to Rh-	Relatively safe
A to non-A	Dangerous
B to non-B	Dangerous
AB to non-AB	Dangerous

*For organ transplantation, O is universal donor and AB is universal recipient. With the transplantation of bone marrow allografts, or of lymphoid-rich organ allografts (*e.g.*, intestine or liver), enough antihost isoagglutinins may be produced by the allograft to cause serious or lethal hemolysis in a significant number of cases (humoral GVHD). Consequently, the rules summarized in this table are fully applicable only with leukocyte-poor organs like the kidney and heart.

Preformed antidonor cytotoxins. Hyperacute rejection of a kidney by an ABO compatible recipient was reported for the first time by Terasaki, Marchioro and Starzl (100). Terasaki's observation that the serum of the recipient of a live donor kidney contained preformed antigraft lymphocytotoxic antibodies was promptly confirmed in similar cases by Kissmeyer-Nielsen *et al.* (101) and by others (102, 103). The evidence of a cause and effect relation in Terasaki's single first case was so clear that he recommended and immediately introduced his now universally applied lymphocytotoxic crossmatch test (100, 104).

Tissue matching

The importance of the genetically determined major histocompatibility complex (MHC) in determining the immune response to allografts was established at a very early time by investigations in inbred mice (105, 106). The possibility of clinical tissue matching did not begin to emerge, however, until the discovery of the first human leukocyte antigen (HLA) (107), and the discovery in the same year of antileukocyte antibodies (soon shown to be HLA directed) in the sera of pregnant women (108).

The report in 1964 of the microcytotoxicity test, with which HLA antigens could be detected serologically with minute quantities of sera (109) was a further critical development in moving forward with the detection and classification of the antigens. It was anticipated that long-term organ engraftment would be achievable only with a high degree of donor/recipient HLA match, and that there would be a stepwise deterioration in outcome with every level of HLA mismatch. The importance of HLA matching was immediately fulfilled with bone marrow transplantation, in which anything less than a perfect or near perfect match between the donor and recipient resulted in GVHD or else rejection of the graft (28-30,110).

Inexplicably at the time, Terasaki promptly recognized that kidney transplantation was not dependent on tissue matching. This was evident in a retrospective study of long-surviving kidney recipients and their volunteer live donors (111). This was followed by a prospective trial in which kidney donors were selected on the basis of the best available HLA match for recipients who were treated with azathioprine and prednisone, with or without adjunct ALG (112). Although HLA-matched (zero mismatched) allografts had the best survival and function, no cumulative adverse

effect of mismatching in the kidney recipients could be identified (113). This imprecise prognostic discrimination also pertained to cadaver kidney transplantation (114) and has been evident in analyses up to the present time. The absence of a large and consistent matching effect unless there is a perfect or near perfect match has always been the same (115). Furthermore, the difference in clinical outcome with completely matched *versus* variably HLA-mismatched allografts has been surprisingly small.

Terasaki's observations of the University of Colorado kidney cases nearly three decades ago breathed life into the still struggling fields of liver, heart and lung transplantation. It was a relief to know that the selection of donors with random tissue matching would not result in an intolerable penalty. A quarter of a century passed before it could be explained why HLA matching was critical for bone marrow, but not organ, transplantation (see next section).

Allograft Acceptance and Acquired Tolerance Involve the Same Mechanisms

Until the last decade, a transplanted organ was viewed as a defenseless object of immune attack by the host, subject to rejection of varying severity depending on the degree of histoincompatibility (Fig. 4, upper left). In contrast, the cytoablated host after bone marrow engraftment was viewed as the defenseless object of a unidirectional graft-*vs.*-host (GVH) immune reaction (Fig. 4, upper right). In the early 1990s, we discovered evidence suggesting that both of these perceptions were incorrect in that a double immune reaction occurred with both kinds of transplantation. This insight began with a study of long-term human survivors of liver, kidney and other organ transplantations.

It was discovered that all of these organ recipients had small numbers of donor leukocytes in their blood or tissue (microchimerism) (116-118). At about the same time, it was shown that cytoablation of bone marrow recipients does not completely destroy host leukocytes (119, 120) as had been previously assumed. From these findings, we concluded that a bidirectional immune reaction had taken place in both bone marrow and solid organ recipients, and that this occurred with maximum intensity in the first few post-transplant days or weeks (Fig. 5). The differences between organ transplantation and bone marrow transplantation (Table V), which had been assumed for three decades to be caused by separate mechanisms, lay instead in the relative strength of the opposing immune reactions. Furthermore, reciprocal interactions of coexisting donor and recipient leukocyte populations were necessary for alloengraftment with both kinds of transplantation (Fig. 4, lower).

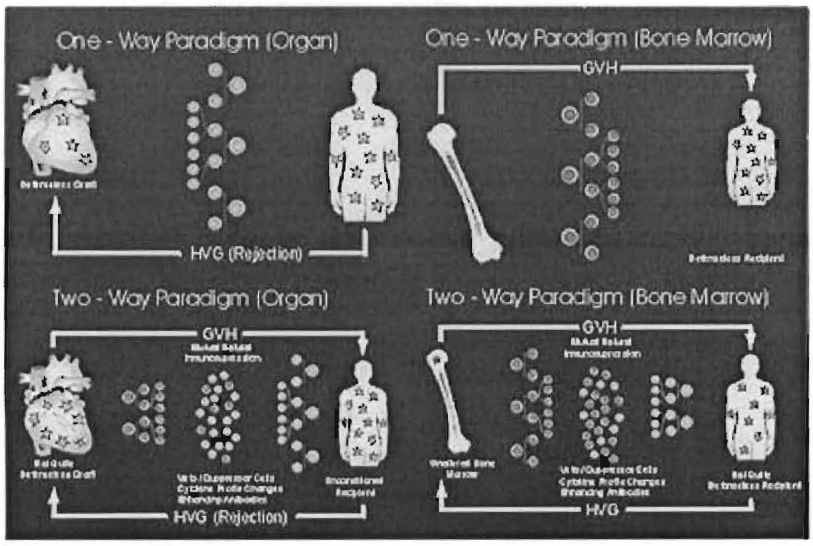

Fig. 4

Table V: Differences between conventional bone marrow and organ transplantation (121).

Bone marrow		Organ
Yes	← Recipient cytoablation*→	No
Critical	← MHC compatibility→	Not Critical
GVHD	← Principal complication→	Rejection
Common	← Drug-free state→	Rare
Tolerance	← Term for success→	"Acceptance"**

*Note: All differences derive from this therapeutic step which in effect establishes an unopposed GVH reaction in the bone marrow recipient whose countervailing immune reaction is eliminated. **Or "operational tolerance".

After organ transplantation, there is an acute migration of immunogenic multilineage "passenger" leukocytes from the graft, selectively at first to host lymphoid organs. In the meanwhile, host cells replaced most, but not all, of the passenger leukocytes in the graft (Fig. 6). Initially, the coexisting donor and recipient cells in widespread organized lymphoid collections generate widespread host-*versus*-graft (HVG) and graft-*versus*-host (GVH) immune activation that may proceed to reciprocal clonal exhaustion-deletion and variable degrees of donor and recipient specific nonreactivity (*i.e.,* tolerance) (Fig. 5). Maintenance of the acutely induced state require persistence of the microchimerism (121-123). The same events in mirror image occur with bone marrow engraftment.

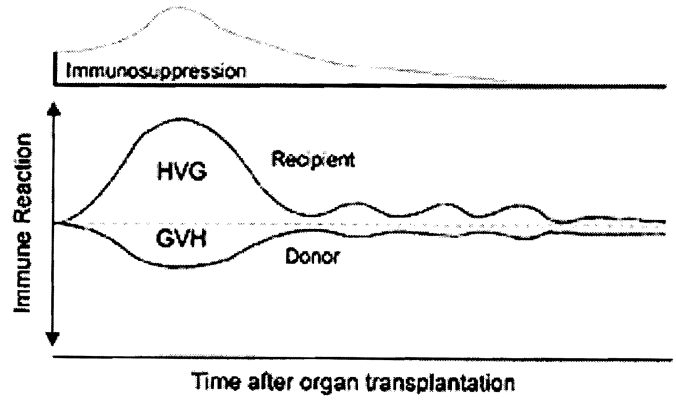

Fig. 5

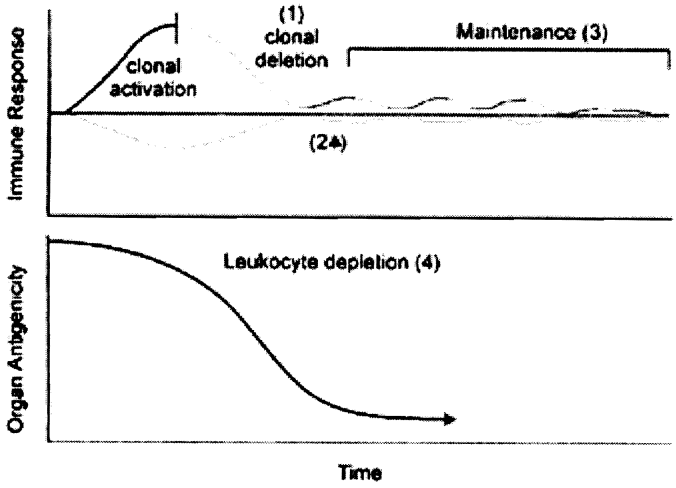

Fig. 6

With either kind of transplantation, immunosuppression allowed the acute induction to proceed by preventing either the donor or recipient cell population from destroying the other before the clonal deletion could occur. The organs' passenger leukocytes that survive the initial immune reaction migrate secondarily to areas other than the lymphoid organs, thereby escaping attack by the host immune system (immune indifference) (124). These "sheltered" leukocytes may then "leak" periodically to the host lymphoid organs, thus maintaining clonal exhaustion-deletion at a level compatible with allograft survival (121)(Fig. 6). The greatest cell migration occurs from the leukocyte-rich liver, accounting for its unusual tolerogenicity; but the same events occur after transplantation of all organ grafts. The modulation of the host immune response by these donor cells explained the poor discrimination of HLA matching for organ transplantation.

The Immune Reaction to Infectious Microorganisms is the Same as That Against Allografts and Xenografts

In 1974-75, Zinkernagel and Doherty discovered that one of the biologic roles of the major histocompatibility complex (MHC) is the adaptive immune response directed against intracellular noncytopathic or weakly cytopathic microorganisms (Fig. 7). Because the cost of total elimination of all cells infected with this kind of pathogen could be the death or invalidism of the host, a means has evolved by which the immune response can be terminated by antigen-specific clonal exhaustion-deletion, thereby allowing survival of both the microorganism and the host (125-127). Clonal deletion is governed by migration and localization of the microorganisms. In the same traffic pattern as that of migratory leukocytes, the pathogens move preferentially at first to host-organized lymphoid tissues (*e.g.,* lymph nodes, spleen) (128). After either an infection or after transplantation, host cytolytic T-lymphocytes recognize the mobile antigen in a MHC-restricted context (121).

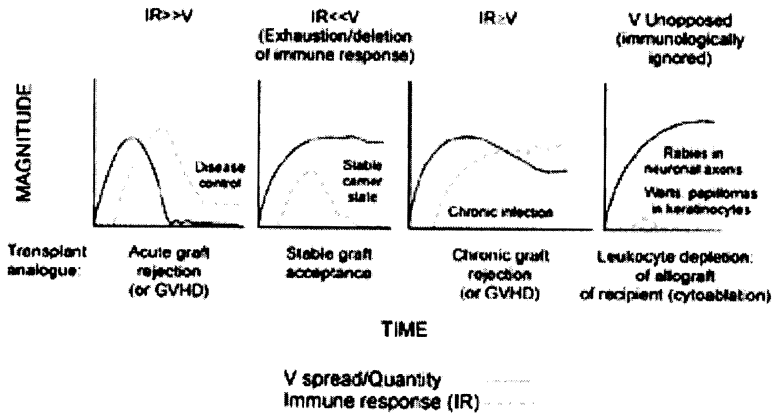

Fig. 7

Thus, a perfect state of allograft acceptance can be compared to a continuously high load of non-cytopathic microorganisms that may lead to a pathogen-specific immunologic collapse (*i.e.,* an asymptomatic carrier state) (Fig. 7, second panel). At the other extreme, acute irreversible rejection may be compared to dramatic, efficient control of the pathogen by antigen-specific effector T-cells (Fig. 7, first panel). Degrees of chronic rejection of the allograft correspond to variable incomplete clonal exhaustion-deletion (Fig. 7, third panel). In mirror image, the infectious disease analogy with bone marrow transplantation after pretreatment with cytoablation is infection by microorganisms that avoid migration to host lymphoid organs (*e.g.,* rabies and wart viruses) and therefore do not induce an efficient immune response (immune indifference) (Fig. 7, right).

In contrast to the MHC-restricted response to noncytopathic organisms, the response to cytopathic microorganisms includes activation of the innate response effectors such as interferons, macrophages, gamma/delta T-cells, natural killer (NK) cells, B-cells that may continue to secrete antibodies without T-cell help, early interleukins and phagocytes (127). The principal but probably not the only target of this uncontrollable reaction when the organs of lower mammals (*e.g.,* of pigs) are transplanted to humans is the galactose α 1-3 galactose epitope (α Gal) found in the Golgi apparatus of the cells (129,130). If such discordant animal organs, or even those from more closely related species such as baboons are to be transplanted clinically, it will be necessary to change the antigenic profile of the xenograft to one that is recognized by the human immune system as a noncytopathic microorganism (*i.e.,* comparable to an allograft). Until this is accomplished by the creation of transgenic animals, xenotransplantation will not be a viable option.

Conclusions

Over the last 40 years, progress in manipulating the mechanisms involved in the immune response has steadily increased the probability of allograft acceptance and acquired tolerance. However, the penalty has been a variable weakening of the host's ability to mount an immunologic response to pathogens, to maintain tumor surveillance, or (probably) to carry out other subtle homeostatic functions. Herein lies one of the primary challenges to the future of transplantation. Hopefully, from continued study of transplantation *per se*, as well as advances in related fields, more effective solutions will be found to overcome the current barriers to allografts or even xenografts with minimum perturbation to all other facets of immune function.

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