The Mystique of Organ Transplantation

Thomas E Starzl, MD, PhD, FACS

The ability to replace faulty body parts with transplanted cells, tissues, and organs has forever altered the principles guiding the practice of medicine. I will first describe how basic science played an essential role in this revolution. But my second objective will be to show how surgeons successfully violated the preexisting rules of immunology and biology, thereby endowing transplantation with an enduring element of mystery. In the long run, surgeons who created the mystique also removed it, but not until a third of a century later.

THE DAWN OF MODERN TRANSPLANTATION: 1953 TO 1968

The basis for the scientific mystery can be understood only from a historic perspective. The chain of events began during the Battle of Britain when a 24-year-old Oxford zoologist named Peter Medawar was assigned to wartime duty with the Scottish plastic surgeon, Tom Gibson. The purpose of the alliance between the practicing surgeon and the basic scientist was to determine if skin from cadavers could be used to treat fire bomb victims of the Battle of Britain. The results of their studies, which were first done in humans and then confirmed in animals, showed that rejection of the skin is an immune reaction.1,2

The key observation came from experiments in which repeated skin grafts were transplanted from the same donor to a given recipient, placing each graft after the preceding one had been rejected. The transplants had a decremental survival. For example, the time to rejection might be 10 days for a first graft, 5 days for the next one from the same donor, and only a few hours after four or five preceding grafts (Fig. 1). Although immunology was still an infant science, this circumstantial evidence that rejection was an immune response prompted surgeons of the 1940s and early 1950s to envision mitigation of the response with total body irradiation. Experiments in animals included kidney and skin transplantation. Irradiation had no effect on graft survival but the modern era of transplantation was just around the corner.

The beginning of the modern era is usually dated to a brief report that appeared in the October 3, 1953 issue of the journal, Nature.3 The authors were Rupert Billingham, Leslie Brent, and the war-time investigator Peter Medawar, who by now was 34 years old. The men, who soon would be known as the "holy trinity" of transplantation immunology, described how they had isolated the leukocytes from the spleen or bone marrow of adult mice and injected these donor cells into the blood of newborn mouse recipients. Because the immune system of newborn mice was not yet developed enough to reject the infused cells, the donor leukocytes engrafted and were thought to have replaced the recipient immune cells (Fig. 2, outer cycle). This condition is known as complete donor leukocyte chimerism.

By 1955, similar leukocyte chimerism was produced in Bethesda at the National Institutes of Health cancer division, but this time, in adult mouse recipients whose otherwise normal immunity had been weakened by total body irradiation4 (Fig. 2, inner cycle). Once the donor leukocytes were engrafted, both the neonatal and irradiated adult mice were able to accept skin or other tissues from the original leukocyte donors but from no other donor. These were the first examples in the world of acquired donor-specific transplantation tolerance. The tolerance was clearly associated with the presence of donor leukocyte chimerism.

To surgeons, the logical next step appeared to be production of leukocyte chimerism by bone marrow transplantation before or at the same time as organ transplantation from the same donor. In 1958, John Mannick,5 then working in Cooperstown, NY, was able, with this strategy, to avoid canine kidney allograft rejection. But prolonged recipient survival could be accomplished only in a single beagle dog who lived for 70 days before dying of pneumonia. It also had become clear that avoidance of graft rejection would not be the only problem with co-
transplantation of bone marrow cells and an organ. In 1956, Medawar’s associates, Billingham and Brent,6 and the Danish scientist, Morten Simonsen,7 had shown that the successfully engrafted donor leukocytes could turn the tables and reject the recipients (graft-versus-host disease). This lethal complication could be avoided in the mouse tolerance models only if the donor and recipient had a good tissue match.

FIRST SUCCESSFUL CLINICAL BONE MARROW TRANSPLANTATION

Human tissue antigens (so-called HLA antigens) had not yet been discovered and would not be delineated well enough for another decade to permit the obligatory donor-to-recipient matching. After overcoming this obstacle, bone marrow transplantation was finally accomplished in 1968. The escalation from the original mouse tolerance model of 1953 to the first successful human bone marrow transplantations of 1968 was heralded as an ideal example of “translational” research. The drug-free HLA-matched humans, with their leukocyte chimerism and acquired tolerance were perfect analogues of the animals. But this would not be the means to the end of organ transplantation that surgeons had envisioned. Instead, bone marrow transplantation was destined to become a definitive treatment for immune deficiency diseases, blood disorders, and numerous other conditions.10

SUCCESSFUL TRANSPLANTATION OF ORGAN ALLOGRAFTS

In the meanwhile, renal transplantation in humans had become a widely used clinical service (albeit a flawed one) and by 1968 the first successful liver and heart transplantations also had been reported (Table 1). In contrast to the “bench to bedside” sequence of bone marrow transplantation, successful implantation of kidney allografts was accomplished initially in humans rather than in animals, first in Boston by Joseph E. Murray in January 1959 and then 5 months later by Jean Haber in Paris (Table 2). In these two patients, fraternal twin kidneys were transplanted into recipients who had been conditioned with sublethal total body irradiation without donor bone marrow infusion.11,14,15

Drug immunosuppression was not yet available. Nev-

<table>
<thead>
<tr>
<th>Organ</th>
<th>City (ref)</th>
<th>Date</th>
<th>Physician/surgeon</th>
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<tr>
<td>Kidney</td>
<td>Boston (11)</td>
<td>1/24/59</td>
<td>Murray</td>
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<tr>
<td>Liver</td>
<td>Denver (12)</td>
<td>7/23/67</td>
<td>Starzl</td>
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<td>Heart</td>
<td>Cape Town (13)</td>
<td>1/2/68</td>
<td>Barnard</td>
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<tr>
<td>Bone marrow</td>
<td>Minneapolis (8)</td>
<td>8/24/68</td>
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The first finding in kidney recipients treated in Denver in 1962 to 1963. 21 Highly appreciable, but enigmatic phenomena observed in survivors, a state of drug-free survival in which kidney rejection did not occur for long periods, if at all, when treatment from drug immunosuppression was suggested by two who were unrelated.16,17 (Table 2). The world’s first two long-surviving recipients of nonrelated kidneys were patients of Rene Kuss,17 a Paris urologist who in 1951 had described the renal transplant operation that has been used worldwide ever since.18

In the next major step, Murray19 achieved lifesupporting renal graft function for 17 months in a patient who was treated with azathioprine after receipt of a kidney from a genetically unrelated donor. The allograft was obtained from a patient on cardiopulmonary bypass whose heart beat could not be restored. This seventh patient (Table 2) provided the first example of prolonged survival under drug immunosuppression only. The stage for pharmacologic immunosuppression had been set by extensive preclinical studies in dogs of 6-mercaptopurine and azathioprine by Roy Calne (first in London and subsequently with Murray in Boston), and by Charles Zukoski (with David Hume in Richmond).

Other preclinical studies were done in surgical laboratories in Minneapolis, Denver, and elsewhere. Only about 5% of canine kidney recipients survived for as long as 100 days, but in a small subset of the long survivors, a state of drug-free allograft acceptance developed in which kidney rejection did not occur for long periods, if at all, when treatment was stopped. This was subsequently observed with far greater frequency after liver replacement.20

The tantalizing possibility of emancipating patients from drug immunosuppression was suggested by two highly appreciable, but enigmatic phenomena observed in kidney recipients treated in Denver in 1962 to 1963.21 The first finding was that kidney rejection was a regula-
Immunosuppression (1962-1963)  

<table>
<thead>
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<th>Prednisone</th>
<th>Azathioprine</th>
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Change in December 1963  

<table>
<thead>
<tr>
<th>Prednisone</th>
<th>Azathioprine</th>
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Figure 3. Left, empirically developed immunosuppression used for kidney transplant recipients at the University of Colorado in 1962 to 1963. Note the reversal of rejection with the addition of prednisone to azathioprine. More than a third of a century later, the crucial role of the timing of drug administration was clarified. Right, treatment revisions in immunosuppression made at the University of Colorado in December 1963 that unwittingly undermined basic mechanisms of tolerance induction. Pretreatment was deemphasized or eliminated, and high doses of prednisone were given prophylactically instead of as needed.

maintained on immunosuppression had been given a bone marrow infusion.

Graft-versus-host disease had never been seen despite the systematic use of organs from HLA-mismatched donors. Taken together, these observations ostensibly ruled out leukocyte chimerism as a factor in organ engraftment. In a nutshell, the seed planted by Medawar1,2 in 1943 and nurtured a decade later by Billingham and colleagues3 apparently had differentiated along two completely unrelated pathways: one of organ and the other of bone marrow transplantation (Fig. 5). An additional conclusion now was reached by group consensus that fundamentally different mechanisms must be involved in the two kinds of engraftment.

In effect, this conclusion detached organ transplantation from the scientific anchor of leukocyte chimerism that directly linked the mouse tolerance models and human bone marrow transplantation. With acceptance of
the chimerism exclusionary premise, there was an epistemologic collapse, a "failure to understand."27 The enigma of organ engraftment was remanded to the basic research laboratories for resolution. Medawar28 himself was puzzled. In commenting on the frustrating search for the increasingly mystical mechanisms of organ alloengraftment, he concluded that, "... the spectacle of a scientist locked in combat with the forces of ignorance is not an inspiring one if, in the outcomes, the scientist is routed."

One of the scientists who tackled the problem was a young Philadelphia surgeon named Clyde Barker who joined forces with a member of the English holy trinity (Rupert Billingham) after Billingham migrated from England to the University of Pennsylvania in 1967. In 1968, Barker and Billingham29 published a key contribution to basic transplant immunology. This was the demonstration that skin grafts were not rejected if they were placed on an island of recipient skin that had been separated from lymphatic drainage (Fig. 6). With their elegant but simple experiment, they had elucidated one of the two mechanisms of allograft acceptance, namely the failure of the immune system to recognize the presence of antigen that does not reach host lymphoid organs, ie, "immune ignorance."

More than a quarter of a century passed before the validity and importance of this mechanism were fully established. Clonal exhaustion-deletion, the other seminal mechanism of alloengraftment, also was a casualty of group think. In 1969, the concept of exhaustion and deletion of the clonal antidonor response (also called "clone stripping"), was depicted schematically to explain liver and other kinds of organ engraftment (Fig. 7).30 With dismissal of the two simple and easily understood mechanisms of exhaustion-deletion and immune ignorance, alternative mechanisms of alloengraftment abounded, too numerous to do more than list (Table 3).

The "proof of principle" period, during which the feasibility of clinical organ transplantation was established, ended in 1968 with a downside. The bright note, of course, was the birth of a new surgical specialty (organ transplantation) and of a new medical discipline (bone marrow transplantation). The dark legacy was the intellectual divorce of bone marrow and organ transplantation. There also was a therapeutic legacy: a modified version of the treatment strategy that had been developed originally with azathioprine and prednisone (Fig. 3, left). The principal changes were in drug timing and quantity (Fig. 3, right).

Beginning in 1964, large prophylactic doses of prednisone were given from the time of operation instead of administering steroids only when needed. This strategy of heavy prophylactic immunosuppression with azathioprine and prednisone was used at most centers until well into the 1980s, without or with the antilymphocyte globulin, which was introduced clinically in 1966.31 In addition, pretreatment with azathioprine, which had been used in the 1962 to 1963 patients, was deemphasized or abandoned (Fig. 3, right). Implications of these modifications were not recognized until the end of the 20th century.

**MATURATION OF TRANSPLANTATION**

The years between 1969 and 1980 bracketed a bleak period. In the view of critics, the heavy mortality and, particularly, the devastating morbidity caused by steroid dependence, made organ transplantation (even of kidneys) as much a disease as a treatment. Prospects improved dramatically with the clinical introduction of cyclosporine in 1980,32,33 followed a decade later by tacrolimus.34,35 The new drugs were associated with step-
wise improvements with all organs, but their impact was most conclusively demonstrated with liver and heart transplantation.

Because more potent baseline or adjunct agents became available, they were simply folded into the modified formula of heavy prophylactic immunosuppression that had been inherited from the previous generations. Used in various combinations, the better drugs fueled the golden age of transplantation of the 1980s and 1990s. Acute rejection became almost a non-problem. But the unresolved issues now were chronic rejection, risks of immunosuppression, and drug-specific toxicity.

**RELATION OF ALLOENGRAFTMENT TO TOLERANCE**

I have emphasized that organ engraftment was attributed until recently to mechanisms that did not involve either the presence or role of leukocyte chimerism. It was known that organs contain large numbers of passenger leukocytes and that these “nonparenchymal cells” were largely replaced in the successfully transplanted organ allograft by recipient leukocytes of the same lineages (Fig. 8A). The missing donor cells were thought to have undergone immune destruction with selective sparing of the specialized parenchymal cells. In mirror image, the ideal result in the bone marrow transplant recipient had

**Table 3. Mechanisms of Acquired Tolerance and Alloengraftment**

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<thead>
<tr>
<th>Seminal mechanisms</th>
<th>Alternative mechanisms</th>
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<tr>
<td>Clonal exhaustion-deletion</td>
<td>Special cells → T-regulatory, suppressor, veto</td>
</tr>
<tr>
<td>Immune ignorance</td>
<td>Antibodies → Idiotypic, enhancing</td>
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<tr>
<td></td>
<td>Cytokine → Self-perpetuating profiles</td>
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<tr>
<td></td>
<td>Graft secretions → Soluble HLA antigens</td>
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<tr>
<td></td>
<td>Antigen presentation → Defective or deviant</td>
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<tr>
<td></td>
<td>Anergy → Absence of second signal</td>
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been perceived to be complete replacement of recipient immune cells (i.e., total hematolymphopoietic chim­er­ism) (Fig. 8B).

A flaw in the overarching dogma shown in Figures 8A and 8B was revealed in the early 1990s with our discov­ery of small numbers of donor cells (microchimerism) in the tissues or blood of long-surviving organ recipi­ents36,37 (Fig. 8C). At about the same time, it was inde­pendently demonstrated in Seattle that there was essen­tially always a small residual population of recipient hematolymphopoietic cells in bone marrow recipients, who previously had been considered to have complete leukocyte chimerism38 (Fig. 8D). Now it was evident that organ engraftment and bone marrow cell engraft­ment were mirror image versions of leukocyte chimer­ism, differing primarily in the reversed proportion of donor and recipient cells (compare Figs. 8C and 8D).39

The microchimeric cells in organ recipients clearly were progeny of donor precursor or pluripotent stem cells that had survived a double immune reaction years or decades earlier, just after transplantation. Both organ and bone marrow cell engraftment now could be ex­plained by “... responses of coexisting donor and recipi­ent cells, each to the other, causing reciprocal clonal exhaustion, followed by peripheral clonal deletion.”36,37

Exhaustion-deletion of the host-versus-graft response was the reason for reversal of rejection and development of variable tolerance that had been first observed in kid­ney recipients 30 years earlier, and eventually in all other kinds of organ recipients.
The host-versus-graft response (upright curve in the middle panel of Fig. 9) was the dominant one in most cases of organ transplantation, but with the occasional exception of graft-versus-host disease (the inverted curve). Host irradiation or other methods of cytoablation for conventional bone marrow transplantation simply transferred immune dominance from the host to the graft, explaining the high risk of graft-versus-host disease in bone marrow recipients. All of the major differences between the two kinds of transplantation were caused by recipient cytoablation.

In their classic studies of 1968, Barker and Billingham had demonstrated that transplant antigen that did not reach host lymphoid organs was not recognized to be present (immune ignorance). The only mobile antigen in organs consisted of passenger leukocytes. In the current paradigm, selective migration of these cells to host lymphoid organs is an absolute requirement for the seminal tolerance (and engraftment) mechanism of clonal exhaustion-deletion of the antigraft response (Fig. 10).

The early Barker-Billingham observations, as it turned out, had an extra dimension. After 2 or 3 weeks, cells that have escaped destruction in the host lymphoid organs or have bypassed them move to protected nonlymphoid sites where they can be forgotten by the immune system. This more subtle version of immune ignorance is thought to be essential for perpetuation of the low-level microchimerism of organ recipients and maintenance of the exhaustion-deletion induced at the outset in complex ways that are discussed elsewhere. Suffice it to say here, the spectrum of clinical outcomes after both kinds of transplantation depended on variable combinations of clonal exhaustion-deletion and immune ignorance.

After an estrangement of more than a third of a century, the conceptual separation of bone marrow and organ transplantation was ended. How this unified view of transplantation fit into the larger framework of general immunology was considered subsequently in a review written in collaboration with Rolf Zinkernagel, whose 1996 Nobel Prize (with Peter Doherty) was awarded for basic studies of the adaptive immune response to noncytopathic pathogens. The review clarified the analogies between the immunologic mechanisms of transplantation vis-à-vis those of infections, with particular emphasis on the regulation of these mechanisms by the migration and localization of the respective antigens.

CLINICAL IMPLICATIONS
Although the mystery of alloengraftment was solved, it was not clear how the insight into immunologic mechanisms could be used to improve the treatment of transplant recipients? This question was addressed in a second review in 2001. By now, it required little imagination to envision how the heavy multidrug immunosuppression given in most centers from the time of transplantation could systematically inhibit the seminal tolerance mechanism of clonal exhaustion-deletion and thereby commit the recipient to unnecessarily high longterm immunosuppression to prevent emergence of the suboptimally deleted antidonor clone (Fig. 11, top).

We suggested that this undesirable consequence could
Figure 11. Top: If clonal activation is unduly inhibited by excessive posttransplant immunosuppression, exhaustion-deletion is variably precluded, and longterm graft survival is unnecessarily high immunosuppression. Bottom: Conversion of rejection (thick dashed arrow) to an immune response that can be exhausted and deleted by combination of pretreatment and minimalistic posttransplant immunosuppression. GVH, graft-versus-host; HVG, host-versus-graft; Tx, transplantation. (Reprinted from: Starzl TE. Tolerogenic immunosuppression for organ transplantation. Lancet 2003;361:1502-1510, with permission.)

Figure 12. Course of a cadaver kidney recipient in July 2001, after pretreatment with 5 mg/kg antilymphocyte globulin. Biopsy-proved rejection in the third week was treated with infusions of 1.0 and 0.5 prednisone. Daily tacrolimus (Tac.) (fully shaded area) was begun on the day after operation and spaced to every other day or longer intervals after 6.5 months. Open balls in the upper panel indicate trough levels of tacrolimus. Tacrolimus doses have been given once per week for almost 3 years. (Reprinted from: Starzl TE. Chimerism and tolerance in transplantation. Proc Natl Acad Sci 2004; 101[Suppl 2]:14607-14614, with permission.)

by minimum posttransplant immunosuppression with tacrolimus monotherapy (see Fig. 12 caption). With this strategy, the complications of longerterm immunosuppression have been considerably reduced with survival results equivalent to those under conventional heavy immunosuppression.45-48

VIEW OF THE FUTURE

Although completely drug-free tolerance undoubtedly will be most easily achieved in recipients of HLA-matched organs, our already extensive experience suggests that the burden of chronic immunosuppression can be systematically reduced in the vast majority of patients with the foregoing mechanism-driven approach to management. Xenotransplantation will have to be developed within the same immunologic framework, but this will require the creation with transgenic tech-
nology of a better interspecies tissue match. Although the all-important step of knocking out the α-Gal gene in pigs has been accomplished, it is not yet known what additional changes must be made before porcine organs can be reliably transplanted to humans. Where stem cell biology will fit into future transplantation plans remains unknown, but it clearly will have to conform to the same immunologic rules.

Whatever the future holds, progress will require the continued collaboration of basic and clinical scientists. What does this really mean? I will conclude by expressing my belief that the distinction of basic science from the science of clinical medicine is artificial. No advance in surgery ever illustrated this more clearly than the phoenix-like rise of clinical organ transplantation during which truly dominant discoveries came from the laboratory (bench to bedside) and equally important breakthroughs drove the equation in the opposite direction (bedside to bench). The magic was in the mix.

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REFERENCES


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