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Thoracic Transplantation

SARA J. SHUMWAY MD
Associate Professor of Surgery
Surgical Director, Heart Transplantation
Cardiovascular and Thoracic Surgery
University of Minnesota Hospital and Clinic
Minneapolis, Minnesota

NORMAN E. SHUMWAY MD
Professor and Chairman Emeritus
Department of Cardiothoracic Surgery
Stanford University School of Medicine
Stanford, California

Chapter 39
The Enigma of Graft Acceptance

Thomas E. Starzl

Anthony J. Demetris

Noriko Murase

Camillo Ricordi

Massimo Trucco

The demonstration by Medawar (1,2) that rejection is an immunologic response is conceded by most early workers in the field to have been the seed of clinical transplantation (3). Medawar's conclusion about the nature of rejection was strengthened when it was shown that corticosteroids and total body irradiation (TBI), which already were known to weaken immunologic responses, modestly but significantly prolonged skin graft survival (4–6).

However, the relatively minor delay of rejection of rodent skin grafts with corticosteroids and TBI did not immediately excite dreams of clinical application. Nor did the 1953 article by Billingham, Brent, and Medawar that described permanent skin graft acceptance in a special circumstance not involving immunosuppression (7). The unique circumstance was the inoculation of fetal or perinatal mice with immunocompetent spleen cells. Instead of being rejected, these cells survived and endowed the recipient with the ability in later life to accept all allogeneic tissues (in this instance skin) from the original donor strain.

The impetus and rationale for these experiments came originally from the observation by Owen that the calf equivalents (called freemartin cattle) of human fraternal (dizygotic) twins were permanent hematopoietic chimeras if placental fusion and fetal cross circulation had existed in utero (Fig. 39.1) (8). Burnet and Fenner predicted that such chimerism and the ability to exchange other tissues could be induced by the kind of experiment eventually performed by Billingham, Brent, and Medawar (9).

At first, there was interest by clinicians in inocula-

tion of babies in utero or perinatally with a parental or other donor's immunocompetent cells on the off chance that tissues or organs from the donor might be needed at some time after birth (10). However, it was soon learned by Billingham and Brent with further experiments in mice that the penalty for the prophylactic infusion of such donor cells could be lethal graft versus host disease (GVHD) (11). Many of the inoculated mice became progressively emaciated (runt disease) and exhibited skin erosion, hair loss, diarrhea, diffuse pneumonitis, and characteristic changes in their lymphoid organs (11,12).

The GVHD lesions in the various host lymphoid and nonlymphoid organs were associated with the presence of donor immune cells. In essence, the transplanted immunologic apparatus proved capable of rejecting the host. In 1959, the three conditions for the occurrence of GVHD were summarized by Billingham: first, the presence of mature immunologically competent cells in the graft; second, sufficient time for these cells to react before they are rejected by the host; and, third, important histocompatibility antigens in the recipient, which are lacking in the transplant (12). Billingham and Brent both have described in their memoirs how the threat of GVHD posed by the tolerance-inducing immunocompetent donor cells was recognized late—undoubtedly dampening an initial rush of optimism about the feasibility of transplantation (13,14).

The definition of tolerance given by Billingham, Brent, and Medawar was that it "... represents the specific and systematic failure of the mechanism of
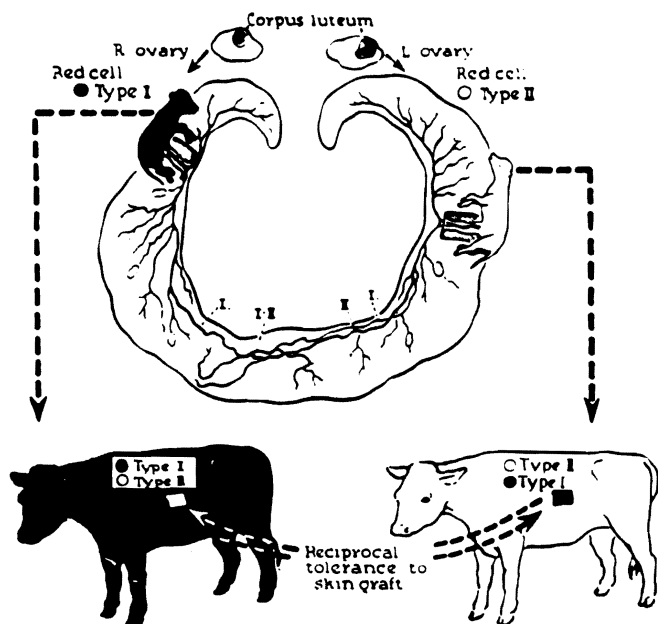
Fig. 39.1 Artist depiction (drawn in 1961) of Owens'
observation in freemartin cattle (8) and the subsequent
experiment described by Anderson D, Billingham RE,
Lamplin GH, and Medawar PB in the title of their article
"The use of skin grafting to distinguish between monozygotic
and dizygotic twins in cattle" (Heredity 1951;5:379–397).
(Reproduced by permission from Starzl TE, Butz GW Jr.
Surgical physiology of the transplantation of tissues and
organs. Surg Clin North Am 1962;42:55.)

immunological response which is brought about by
exposing embryos, or very young animals to anti-
genic stimuli, i.e. to stimuli which would have caused
older animals to have become sensitive or immune. It
is due to a primary central failure of the mechanism
of the immunological reaction, and not to some inter-
cession, at a peripheral level." (15) Twenty years later,
Medawar reflected that others had altered the mean-
ing of tolerance—on one hand by expanding beyond
what he and his colleagues had said, or on the other
by unnecessarily linking tolerance to a single mecha-
nism of clonal deletion (today called negative selec-
tion). These ambiguities of definition underscored the
poor understanding of what graft acceptance meant,
which by this time was being achieved with immuno-
suppression in patients and in various kinds of ani-
mal experiments. Medawar concluded in 1973: "The
balance of evidence upholds the belief that tolerance
is a state of essential nonreactivity—one in which ei-
ther a specific immune capability or the clone of cells
that exercises it is simply not present." (16)

The objective of producing specific and stable
allogeneic (medawarian) nonresponsiveness became

f transplantation when in 1955, Main
and Prehn simulated in adult mice an environment
which was likened to that in perinatal Billingham-
Brent-Medawar animals (17). The three steps were:
first, to cripple the immune system with supralethal
TBI, next to rescue it with allogeneic bone marrow
(creating a chimera), and finally to engraft skin from
the bone marrow donor. The experiments were
successful.

Main and Prehn believed (this was later verified)
that they had produced a bone marrow chimera:

> Should the injected homologous marrow cells and
> their descendants survive permanently, i.e.
> throughout the remaining life span of the host, the
> resultant animal would be a "pseudohybrid," ge-
> netically constituted as the recipient but with its
> bone marrow partially derived from [donor] cells.
> The transplanted marrow would not be capable of
> immunologic response to further grafts containing
> [donor] antigens. Since there is no reason to as-
> sume that the host would offer greater resistance
> to a bone-marrow graft than to a skin graft, the
> results of this experiment are consistent with the
> cellular repopulation theory of radiation protec-
> tion. For obvious reasons, much further work will
> be required before one could possibly expect a
> general solution to the homograft problem (17).

When the results of Main and Prehn were con-
firmed by Trentin, the prototype strategy for induc-
tion of tolerance in large animals and in humans
appeared at first to be obvious (18). Bad news was
close behind. Within the next few months it became
clear that GVHD similar to that described by Bill-
ingham and Brent in the perinatal mouse model
could be expected almost invariably after all bone
marrow engraftments that "took" except those from
donors with a perfect major histocompatibility com-
plex (MHC) match.

MEDAWARIAN VERSUS "NON-MEDAWARIAN" GRAFT ACCEPTANCE

Although the bubble had burst, Mannick and col-
leagues at Cooperstown, New York, produced bone
marrow chimerism in 1958 in an irradiated beagle
dog, followed by successful kidney allotransplanta-
tion from the original marrow donor (19). The animal
lived for 73 days before dying of pneumonitis and
was the first "successful" marrow-kidney chimera in
a large animal. However, efforts by Hume and associ-

ates and by others to extend the Main-Prehn irradiation plus bone marrow technology to mongrel dog donor–recipient combinations were totally unsuccessful (20). When dog lymphocyte antigen (DLA) typing was perfected in the late 1960s, Rapaport working with many of the original Cooperstown investigators explained why (21). Piece by piece, they compiled evidence that the irradiation strategy would work in dogs only when completely MHC-compatible marrow donors were used—usually litter mates. Apparently such matching had been achieved by accident in Mannick's dog. Under all other conditions, lethal GVHD, rejection, or both were to be expected.

Long before this, it had been demonstrated by Robert Good of Minneapolis and by E. Donnall Thomas (who moved in 1963 from Cooperstown to Seattle) that avoidance of lethal GVHD in human beings would require the same perfect tissue (HLA) matching as in the Cooperstown beagle dog colony if the knockout treatment approach were used of destroying and replacing the recipient immune system (22–24). This appreciation caused an early break in ranks between those interested in bone marrow transplantation for the treatment of hematologic disorders and those to whom the bone marrow was only the means to the transplantation of a needed solid organ of which the kidney was the sole candidate at the time.

From this point onward, the therapeutic philosophies of bone marrow and solid organ transplantation took separate pathways—one dependent and the other seemingly independent of classical tolerance induction. Much of the rest of this chapter will illustrate the folly of this dichotomy.

BONE MARROW TRANSPLANT AND "MEDAWARIAN TOLERANCE"

The requirement described earlier of a perfectly matched human donor meant that clinical bone marrow transplantation would be confined almost exclusively to siblings. Despite the consequent donor pool limitations, bone marrow transplantation for hematologic diseases and an assortment of other indications matured into accepted clinical therapy after the first clinical successes by Robert Good in 1968 (22–24). With the addition of continuous posttransplant maintenance immunosuppression with cyclosporine, FK-506, steroids, and antilymphoid antibodies that do not kill the bone marrow stem cells, the rigid lim-

its of donor acceptability set by the need for MHC compatibility have slowly expanded, but only slightly.

"NON-MEDAWARIAN SOLID ORGAN ACCEPTANCE"

With Total Body Irradiation

Solid organ transplant surgeons were quick to abandon efforts to produce specific allogeneic unresponsiveness with bone marrow. In Boston, Murray and colleagues used the Main-Prehn principle of recipient preparation in their first two attempts at human kidney allotransplantation in 1958, but eliminated the bone marrow component for the next 10 recipients, using sublethal TBI alone (25,26). Eleven of the 12 irradiated recipients, including the 2 given supralethal irradiation and bone marrow, died after 0 to 28 days. The sole survivor, the recipient of a fraternal twin kidney in January 1959 after being treated only with preoperative sublethal irradiation, lived for more than 20 years and was the first example of a successful transplantation beyond the identical twin (25–27).

Five months later in Paris, Hamburger and associates added a second successful fraternal twin case (28). Although neither the American nor French fraternal twin recipient was exposed iatrogenically to donor bone marrow, the possibility remained that their individual placentas had cross-circulated with those of their kidney donors, partially or even completely mimicking the conditions of in utero tolerance induction described in freemartin cattle by Owen (8). This clearly could not be the explanation for success in an extraordinary further kidney transplant experience in France during 1960 and 1961 using TBI without bone marrow reconstitution. Hamburger and coworkers succeeded with kidney transplantation from a sibling and a cousin; after undergoing retransplantation 18 years later the latter patient now is a member of the French parliament and the longest surviving kidney allograft recipient (32 years) from that heroic and primitive era (28–30).

Also in Paris, Rene Kuss had long-term survival of three of six irradiated patients treated with kidney transplantation from June 1960 through 1961 (31,32). This was an extraordinary achievement because two of Kuss's long-surviving patients were given nonrelated kidneys (the first in June 1960) that functioned for 17 and 18 months. During the critical period of

1959 through most of 1962, the cumulative French experience was the principal justification to continue clinical kidney transplantation trials (30,33). By showing that bone marrow chimerism was *not* a necessary condition for prolongation of kidney grafts, the stage was set for the transition to drug therapy.

Those examining this period historically have been inclined to consider irradiation-induced and drug-induced graft acceptance as different phenomena (26,27,34). However, it seems certain that the Boston and Paris fraternal twin kidney recipients, as well as the five long-surviving nontwin French recipients, had achieved to variable degrees the same kind of graft acceptance that later was seen in tens of thousands of drug-treated patients after all kinds of whole organ transplantation.

That this could happen with irradiation (but not why) was shown years later in beagle dog experiments reported to the American Surgical Association by Felix Rapaport with the title "Induction of unresponsiveness to major transplantable antigens in adult mammals." The descriptive subtitle was a capsule summary of the original Main-Prehn therapeutic concept: "A Recapitulation of Ontogeny by Irradiation and Bone Marrow." In these experiments, the recipient's own (*not allogeneic*) bone marrow was reinfused for immunologic reconstitution after supralethal irradiation (21). If a kidney or any of the other solid organs from a well-matched allogeneic donor were transplanted at the correct time after the autologous bone marrow infusion, the allogeneic organ was accepted by the reinfused and repopulating recipient marrow, which in its original native state would have rejected it.

Curiously little attention was paid at the time or subsequently to Rapaport's important discovery. The conditions in Rapaport's autologous bone marrow experiments were analogous to those in Murray's fraternal twin and the historic French recipients whose own in situ sublethally irradiated and recovering bone marrows had not been normally reactive to allogeneic grafts (26,29,31). The conditions also resembled those that recently have allowed the production of mixed allogeneic chimerism (see later).

With Drug Therapy

In view of the historic developments through 1960, it was not surprising that the search for immunosuppressive drugs was focused on myelotoxic agents that were viewed at first as "space makers" for the new marrow, and thus the pharmacologic equivalent of

TBI. In fact, cyclophosphamide and busulphan still are used in this context to prepare patients for bone marrow transplantation. Goodwin and colleagues of Los Angeles achieved sublethal bone marrow "burn out" with methotrexate and cyclosphamide in a living-related kidney recipient in September 1960, who subsequently developed rejection that was treated with prednisone. This was the first example of protracted human kidney graft survival with drug treatment alone (35).

Kidney transplant surgeons were quick to learn that myelotoxicity should be avoided, not deliberately imposed. The most important step in this evolution was the discovery by Schwartz and Dameschek that 6-mercaptopurine was immunosuppressive without bone marrow depression in nontransplant models (36) and the demonstration by them and Meeker and colleagues that this drug could mitigate skin graft rejection in rats (37,38). Subsequently, Calne and Zukoski and coworkers independently showed that 6-mercaptopurine could delay the rejection of canine kidneys (39,40). However, all that had been achieved so far was delay of the inevitable rejection. This soon would change.

Occasional examples of long-term or seemingly permanent allograft acceptance were observed throughout 1962 and 1963—defined as long survival of transplanted mongrel dog kidneys after a 4- to 12-month course of 6-mercaptopurine (or its imidazole derivative, azathioprine) was stopped (Fig. 39.2) (41–44). Since then, each new major immunosuppressive agent (or drug cocktail regimen) including cyclosporine and FK-506 has generated excited claims of the same phenomenon. However, the most potent agents for induction of this state have been the polyclonal antilymphoid sera (ALS) and globulin (ALG) purified from them (45,46). Although variable in its incidence, the graft acceptance seen with all these modalities was indistinguishable and thus was not a treatment-specific phenomenon.

This new kind of ultimately drug-free graft acceptance in dogs was easier to produce than with TBI (which had been impossible in outbred animals), but the number of absolute examples was (and is) extremely small in contrast to what can be achieved today in small rodents. In summarizing his research with Calne, Alexandre, Sheil, and others using 6-mercaptopurine and azathioprine, Murray described a 20-day mortality of approximately 50% and a 3-month mortality of 90% in their best series of 120 mongrel dogs given daily treatment; eventually a
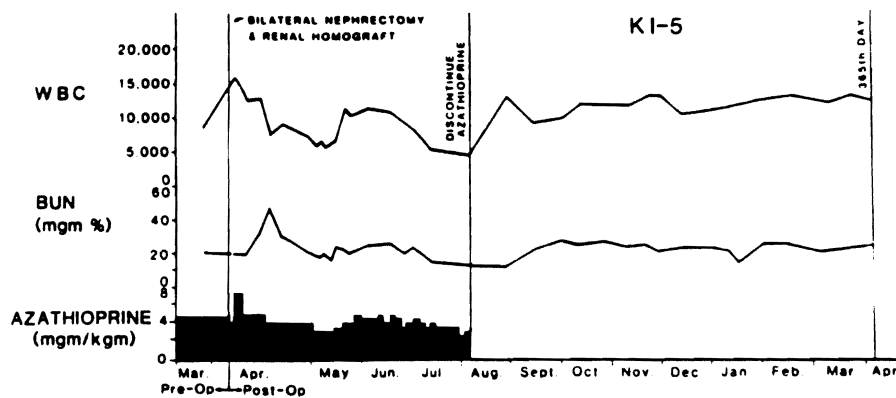
Fig. 39.2 The original caption for this figure was "Demonstration of a high degree of tolerance in a dog treated with azathioprine four months after homotransplantation and bilateral nephrectomy. Good renal function has continued for more than a year after stopping immunosuppressive therapy." This was a mongrel recipient in 1962 of an unrelated kidney whose eventual survival was almost a decade. (Reproduced by permission from Starzl TE, Experience in renal transplantation. Philadelphia: WB Saunders, 1964:164–170.)

handful of surviving animals was the distillation from 1000 experiments (26,43). The results in our Colorado research laboratory were similar but with one striking difference (33,47). Adrenocortical steroids were shown to reverse rejection in 88% of our dogs, sometimes in spectacular fashion, before the steroids usually caused fatal peptic erosions of the gastrointestinal tract (47).

The animals proudly displayed as chronic survivors were those precious few who had run the gauntlet of continuous therapy to the point where the drugs were discontinued. After withdrawal of treatment, more than two thirds of the small residual group of canine survivors rejected their kidneys. The survivors that emerged drug free from this ruthless biologic filter did not begin to approach 5% of the starting animals in any of the three laboratories (Boston, Richmond, and Denver) in which canine experiments with 6-mercaptopurine or azathioprine were actually done. It was on this dismal record that the clinical kidney transplant trials of the early 1960s were based. In a display of optimism that would not be tolerated in today's clinical research climate, the rare exception was given more weight than the customary failure. Thus, the poor results came as no surprise when the drugs were first used for patients in the same way as had been tried in the dogs (26,48).

REJECTION REVERSAL AND "TOLERANCE"

Because of the pessimism that resulted from these initial trials, the outcome when azathioprine and prednisone were combined at the University of Colorado exceeded everyone's expectations (49,50) and precipitated a revolution in transplantation. Success

hinged on two crucial observations. First, it was shown in human beings that acute rejection was not, as had been commonly assumed, one of nature's most powerful and persevering processes. It usually could be reversed with prednisone—confirming what had been seen and belatedly reported in dogs (47). Although other components of "cocktail" immunosuppression have changed, the value of steroids for management of acute rejection has not diminished through the years.

The second and more fundamental observation was that something changed during the first weeks and months after successful kidney transplantation in the relation of the recipient to the graft. The pattern of recovery, in which the amount of drug treatment often became progressively less was the strongest testimony that such a host–graft change had occurred at an early time, allowing the lifetime rehabilitation of some of the patients. Of the first 64 patients in the Colorado series compiled between 1962 and March 1964, 16 survived for the next 25 years; 2 eventually stopped all immunosuppression without rejection for 25 and 27 years, thus mimicking completely the phenomenon occasionally seen in dogs (30,50).

The reversibility of rejection and change in host–graft relationship eventually were verified with all other transplanted organs, beginning with the liver (51–53). Although its transplantation is technically difficult, the liver has appeared to enjoy an immunologic privilege in that it is more resistant than any other organ to antibody-mediated rejection, more capable in some species of prolonged survival without treatment (first noted in the pig and later rat and mouse), more apt to remain rejection free when treatment is stopped, and seemingly more capable of

shielding contemporaneously transplanted organs of the same donor from rejection (52–58). This last quality has been called tolerogenicity. In fact, each of the solid organs including the heart has similar qualities but its own peculiarities of timing, vigor, and reversibility of rejection although these characteristics may be difficult to quantitate except where they can be studied simultaneously in the same recipient as with heart–lung or multivisceral abdominal transplantation (59–64).

Despite these differences between organs, the central therapeutic dogma governing their transplantation has changed very little in the 28 years since it was delineated for the kidney—except that the available drugs or biologic adjuvant procedures have improved (Table 39.1) (26,45,49–51,65–76). The dogma calls for daily treatment with one or two baseline drugs with further immune modulation by the highly dose-maneuverable adrenocorticosteroids to whatever level is required to maintain stable graft function. This means that every patient goes through a trial and potential error experience as drugs are weaned to maintenance levels. However, as early as 1964, it already was realized that with different tissues and organs the laws governing the onset, treatability, and reversal of rejection would apply generically (51).

CELL MIGRATION AND CHIMERISM

The "graft acceptance" defined above has been an immunologic enigma (43,77,78). An ancient clue that cell migration is central to the process was the reduced allogenicity noted by Woodruff and Woodruff in thyroid tissue during a period of privileged sanctuary in the anterior chamber of the guinea pig eye before subcutaneous transplantation (79,80). Such

Table 39.1

Central Therapeutic Dogma	Baseline Agents
1. Baseline therapy with one or two drugs	1. Azathioprine
	2. Cyclophosphamide
2. Secondary adjustments with steroids with or without antilymphoid agents	3. Cyclosporine
	4. FK506
3. Case-to-case trial (and potential error) of weaning	

"adaptation" in combination with clonal attrition was used in 1963 to explain two crucial observations of the reversibility of rejection, and the later change in the host–graft relationship that often made it possible to lighten maintenance immunosuppression (see previous section). After transplantation, previously negative tuberculin, histoplasmin, and other skin tests in these bellwether patients always became positive to antigens that had provoked positive reactions in their donors. The results were correctly interpreted as adoptive transfer of donor cellular immunity "by leukocytes in the renal vasculature and hilar lymphoid tissue." (81) This clear statement of cell migration into recipient tissues was not easy to defend nearly 30 years ago because the kidney was thought to be "lymphoid cell-poor." The flash of insight faded away until it was rediscovered three decades later.

In the meanwhile, the reversal of rejection and an altered host–graft relationship were soon documented after transplantation of all of the other commonly engrafted organs—using a variety of drug treatment regimens. In 1969, karyotyping studies of long-surviving human liver allografts obtained from cadaveric donors of the opposite sex showed that while the hepatocytes and endothelium of major blood vessels retained their donor specificity, the entire macrophage system including the Kupffer cells was replaced with recipient cells (82,83). It was not known where the departed donor cells had gone, but their continued presence somewhere in the body was evidenced by the acquisition and maintenance in the recipient blood of new donor-specific immunoglobulin (Gm) types (83,84), anti-red blood cell alloantibodies when donors with ABO nonidentity were used (85), and new soluble class I HLA antigens (86). Although secretion of the new HLA types was attributed by Davies and colleagues (86) to the transplanted hepatocytes, these molecules also are known to come from bone marrow-derived macrophages or dendritic cells (87–89) and thus undoubtedly had, in part, the same extrahepatic sources after completion of cell migration as the additional Gm types and anti-red cell antibodies.

The chimeric structure of the transplanted liver was considered to be a unique feature of this organ until the demonstration of lymphoid and dendritic cell replacement (with recipient cells) under FK-506 immunosuppression in transplanted rat and human intestine (64,90,91). The two-way traffic was the same whether the bowel was engrafted alone or as a part of a multivisceral allograft that also contained the liver,

stomach, and pancreas. In the rats, the replaced donor lymphoid and dendritic cells homed through vascular routes to widely distributed host lymphoid tissues, creating a state of mixed allogeneic chimerism—free of lethal or even detectable GVHD except in special strain combinations in which there is a poorly understood imbalance between the graft and recipient immune systems (92,93).

Similarly, GVHD has been a relatively minor problem in human beings after cadaveric small bowel or multivisceral allotransplantation (94). This was not surprising because *mixed* allogeneic or xenogeneic chimerism already had been shown to be GVHD resistant after bone marrow transplantation (95,96). A possible explanation for the GVHD resistance is the "exhaustive differentiation" described by Webb, Morris, and Sprent (97). Thus powerful immune responses of coexisting donor and recipient immune cells, each to the other, could first cause reciprocal clonal expansion followed by peripheral clonal deletion (97). If this is true, the current clinical dogma for bone marrow transplantation of deliberately "unbalancing" the donor–recipient equation by host cytoreductive procedures bears reassessment. It seems clear that to the extent this is done, the marrow donor must have a perfect HLA match. To the extent that it is not done, the need for HLA matching is diminished.

Recent reports have suggested that some variant of mixed chimerism is an essential feature of all successful transplantations, no matter what the organ (98–100). In experimental studies, heart and liver allografts and xenografts in FK506-treated rat recipients were repopulated by recipient lymphoid and dendritic cells. The donor cell traffic leaving the allografts in the other direction homed to the host lymphoid organs including the medulla of the thymus, with distribution of dendritic cells to nonlymphoid tissues as well. The ubiquitous peripheralization of donor dendritic and other cells after heart and liver transplantation was qualitatively similar (but far less extensive) to that described earlier after transplantation of the small bowel and was similar in principle to the donor cell distribution in irradiation-induced fully xenogeneic (rat to mouse) chimeras (92,93,98).

Thus, cell migration is a striking generic phenomenon with all kinds of transplants. The donor cells departing the solid organ grafts and the recipient cells entering them include the passenger leukocytes that were suggested by Snell and proved by Steinmuller

to be the principal cause of allograft immunogenicity (101–103). Steinman and Cohn (104–107) delineated the most important of these cells as a distinct family of bone marrow-derived antigen presenting dendritic leukocytes that are distributed throughout the body including the interstitium of the kidney, heart, and other organs once thought to be nearly devoid of immunologically active cells (108,109).

With the expectation of ameliorating rejection, numerous techniques to deplete the intensely immunogenic dendritic cells have been described, particularly after Hart, Winearls, and Fabre and Batchelor and colleagues showed how their presence in rat renal allografts elicited strong primary T-cell-dependent alloimmunity (110–117). Observations by Larsen, Austyn, and Morris and by others showing rapid bloodstream movement of allograft dendritic cells to the spleen have emphasized the role of central as opposed to intragraft mechanisms of sensitization (118–121). Single-donor dendritic cells at the center of large clusters of recipient T cells in the spleen as well as in rat liver allografts graphically denoted an efficient amplification system of T-cell activation in both locations (121).

The primary or derivative objective in much of the foregoing research was reduction of graft antigenicity, a potentially self-defeating strategy and a violation of a fundamental tenant of Billingham, Brent, and Medawar that ". . . the stimulus that confers tolerance must be fully antigenic." (15) In untreated rats, Prop and coworkers of Holland have shown that the lymphoid-poor heart is less vigorously rejected than the lung that contains rich bronchus-associated lymphoid tissue (BALT) (61,62). However, this order of susceptibility to rejection was reversed with one or two postoperative doses of cyclosporine that commonly induced permanent acceptance of the lung but never of the heart. The authors explained the paradox by the greater ease and volume of the lung's lymphoid and dendritic cell migration in much the same context as in the intestinal repopulation discoveries (64,90). The finding by Fung and associates of mixed chimerism in human heart–lung allografts also was consistent with the Dutch observations (122).

The fine margin between immunization and tolerance was illustrated by Armstrong and colleagues who found an association between the rate of dendritic cell replacement and the survival of renal allografts transplanted to rats after they had been lightly immunized by blood transfusions from the donor strain (123). Such observations and those cited

throughout this chapter have suggested that tolerance induction can be efficiently and safely accomplished across formidable histocompatibility barriers without excessive alteration of the natural immunologic substrate of either host or graft, explaining our omission of recipient pretreatment and a "nonintervention" policy in the preparation of human intestinal and multivisceral allografts (124).

With clinical solid organ transplantation, and we believe with bone marrow transplantation as well, the treatment strategy can be redefined in terms of achievement of two-way cell migration while using powerful medications or other means to avoid the graft destruction or GVHD that is normal and inevitable without such therapeutic intervention. We believe that each new immunosuppressive regimen of the last 30 years has allowed this phenomenon to be accomplished with greater consistency, but rarely to the extent that treatment can be stopped altogether. However, failure to be drug free does not mean that the graft acceptance is by a different process than originally described by Billingham, Brent, and Medawar who noted that "Every degree of tolerance is possible, from that which allows a homograft to live only a few days beyond its normal expectation . . . to that in which it is permanently accepted and incorporated into its host." (15)

The framework of understanding that these earlier investigators constructed (7,15) was amazingly modern and lacked only the knowledge of immunologic cell migration and relocation that artfully concealed itself from inquiring eyes for more than a third of a century. With appreciation of the two-way cell migration, virtually every detail of graft acceptance as it is induced by drugs or other methods can be reconciled with the original Billingham-Brent-Medawar model of actively acquired tolerance, accommodating in addition Woodruff's explanation of adaptation that included as one possibility replacement of certain elements of graft, for example connective tissue stroma and vascular endothelium (7,80).

It is fascinating to realize how close Medawar himself came to this truth when he wrote in 1965 that ". . . foreign kidneys do sometimes become acceptable to their hosts for a reason other than acquired tolerance in a technical sense . . . One possible explantation is the progressive and perhaps very extensive replacement of the vascular endothelium of the graft by endothelium of host origin, a process that might occur insidiously and imperceptibly during a homograft

reaction weakened by immunosuppressive drugs" (125). Woodruff's and Medawar's ideas stand today, modified by a fresh understanding of the nature of the exchanged cells, insight about where they travel, and the need to associated the cell migration with (not dissociate it from) the fundamental definition of tolerance.

Although many details remain to be clarified, it may be said now that no matter how disparate the MHC compatibility or what the solid organ, the consequence with effective immunosuppression is the rapid formation of a composite graft (chimera) within a short time after transplantation. The product of nature's workmanship is similar in the various organ allografts and except for greater difficulty of attainment, it appears much the same in xenografts. The accompanying recipient changes are profound and contain the elusive key to understanding what tolerance really means at a cellular and molecular level.

The similar nature of the donor cell migration pattern after successful solid organ transplantation under drug induction versus the migration pattern in radiation-induced xenogeneic bone marrow chimeras means that the "classical tolerance" defined so precisely by Billingham, Brent, and Medawar (and bone marrow transplanters) versus the ambiguous "graft acceptance" familiar to transplant surgeons are merely variants and stages of the same thing. In either case, clinical success means that a characteristic lymphoid and dendritic cell chimerism exists that may be stable without further treatment, stable only when continued immunosuppression is provided, or unstable in the direction either of rejection or GVHD.

THE CONCATENATION HYPOTHESIS

Although cell migration is an invariable initiating event, it is clear that drug-free graft acceptance is not synonymous with permanent chimerism. However, it is evident that early chimerism can lead to self-perpetuating changes in the host immune response. The word concatenation suggests that multiple linked immunologic pathways are involved in solid organ graft acceptance. The list has grown from an original suggestion in 1964 that there was a combined effect of clonal deletion and Woodruff's adaptation (51). Five years later, the list of possible mechanisms had burgeoned (126). "Enhancement" was added by borrowing a concept that originally came from studies of tumor immunology by Kaliss (127). The hy-

pothesis was that antigraft antibodies protected the transplanted organ either by shielding it (peripheral enhancement) or by feedback inhibition of the lymphoid organs that secreted these antibodies (central enhancement).

Another potential factor discussed in 1969 (126) about which little was known at the time was a defect in antigen processing by the macrophage system, caused by the circumstances of transplantation with immunosuppression; this concept was developed by Nossal (128). Apparently unaware of the concatenation hypothesis, Levey subsequently presented an almost identical multifactorial theory stressing "the delicate balance between tolerance and immunity in terms of antigen, antibody, and cells" (129). Since then, the fifth possibility of a role for special (suppressor) cells that block immune reactions has been added (130,131).

The concatenation hypothesis has defied both verification and repudiation, as exemplified in experiments by Murase and colleagues in which ACI rat hearts or livers were transplanted to LEW recipients under short-course treatment with FK-506 (60). Reflecting the immunologic advantage enjoyed by the liver under many experimental circumstances (see earlier) essentially all of the hepatic grafts were accepted permanently, whereas the hearts typically were rejected 40 to 80 days after a 2-week course of FK-506 was completed. Neither kind of transplantation was associated with suppressor cell dominance or any other decisive single change sufficiently striking to explain why transplanted organs appeared to have been forgotten by the body.

REUNIFICATION THROUGH MIXED CHIMERISM

The realization that mixed chimerism is a natural consequence of cell migration and repopulation has reunited bone marrow and solid organ technology after an estrangement of nearly 30 years. Observations following transplantation of the intestine were particularly illuminating because they provided unmistakable microscopic evidence of chimerism with an organ heavily endowed with immunocompetent cells. Despite this, GVHD did not occur. Two analogous circumstances with GVHD resistance could be found in seemingly unrelated research begun in the 1970s by Slavin and Strober and their associates at

Stanford and in the 1980s by Ildstad and Sachs at the National Cancer Institute (Bethesda).

Via Total Lymphoid Irradiation

In Slavin and Strober's original experiments at Stanford, specific transplantation tolerance to skin and hearts was induced in adult mice and rats using a combination of fractionated total lymphoid irradiation (TLI) and donor-specific bone marrow (132,133). The radiotherapy regimen called TLI that had been developed by Kaplan for the treatment of human malignant lymphomas was known to be immunosuppressive but because it spared part of the central lymphoid organ system, it did not cause leukopenia or other overt myelotoxicity (134,135). Unlike the outcome in the classic experiments that destroyed the host immune system with TBI, allogeneic bone marrow engraftment after TLI did not cause GVHD (17,18). Myburgh and coworkers confirmed these findings in baboons after kidney and liver allotransplantation (136,137).

Eventually, five clinical trials with TLI were conducted—at Stanford, the University of Minnesota, Louvain, Belgium, Rome, and at Witwatersrand University in Johannesburg (138–142). All except the South African patients (some of whom were liver recipients) underwent renal transplantation exclusively. The results were summarized by Myburgh at the international congress of the Transplantation Society in Sydney, Australia in August 1988 (143). His report revealed a striking change in therapeutic intention in the course of the trials. These were foreshadowed by new laboratory experiments showing that, on the average, TLI *without bone marrow* was as effective as the two modalities together (142,144,145). As a consequence, bone marrow was omitted altogether in the clinical cases from Stanford (n=25), Belgium (n=20), and Rome (n=30) or used only occasionally in Minneapolis (5 of 22) and with decreasing frequency in Johannesburg (number unstipulated but known to include liver recipients).

By the end of the multiyear clinical trial period, TLI had become a competitor with cyclosporine as a front-line immunosuppressant rather than a means to the end of deliberate bone marrow chimerism. Because of its inconvenience, expense, and morbidity, TLI lost the race. What was left when the smoke cleared was a group of surviving patients in each clinical series with thoroughly documented donor-specific allogeneic tolerance that was explained with

different versions of the concatenation hypothesis. Only now is it clear how chimerism (and genuine tolerance) can occur as the result of cell migration and repopulation, with or without bone marrow.

Via Total Body Irradiation

In investigations with TBI beginning in 1984, mixed syngeneic-allogeneic and syngeneic-xenogeneic (mouse plus rat) bone marrow chimerism was achieved using ex vivo syngeneic marrow instead of keeping intact a protected portion of the autologous marrow in situ as had been done in the Slavin-Strober models. After preparing mouse recipients with lethal TBI and reconstituting them with the mixed allogeneic (or xenogeneic) plus syngeneic marrow, stable multilineage mixed chimerism was present with the lifetime production and coexistence from both host and donor origin of platelets, red blood cells, T cells, B cells, natural killer (NK) cells, and antigen-presenting cells (95,146–149). In individual mixed chimeras, the level of allogeneic chimerism varied widely, from 1% to 98%. However, any level of donor lymphoid chimerism was associated with complete systemic medawarian donor-specific transplantation tolerance.

Just as with successful intestinal transplantation or in the Slavin-Strober experiments, the mixed chimeric state was remarkably free of the GVHD classically occurring when the stem cells of the host are completely replaced with those of the allogeneic donor (fully allogeneic chimerism) (Fig. 39.3) (150–155). The doubly reconstituted chimeras were tolerant to skin and heart grafts from either contributor to the hematopoietic chimerism (cotolerance) (95,146–149), while maintaining better overall immunocompetence than that in the fully allogeneic chimeric state (146,151). It was shown recently that these mixed chimeras had ubiquitous distribution of donor immunologic cells (especially dendritic cells) through the lymphoid and all other tissues of the recipient—much the same as after successful liver or intestinal transplantation (92,93,98,156).

The recent information about mixed allogeneic and xenogeneic chimerism undoubtedly will stimulate clinical trials in which donor immunologic cells will be infused perioperatively at the time of solid organ transplantation—an iatrogenic simulation of the events that occur naturally during the cell migratory phase following any kind of transplantation. The benefit (and risks) will depend on factors such as donor cell load and timing, the quality of immunosuppression, and the degree of genetic disparity between donor and recipient. The infused allogeneic (or xenogeneic) marrow cells will have the same destination as those that leave the graft.

The clinical exploitation of this approach should have potential value and minimum hazard if the conventional continuous immunosuppression that has been developed empirically, as opposed to knockout therapy of the recipient immune system, provides an acceptable environment for the transplanted immunocompetent cells without preliminary destruction of the recipient immunologic apparatus with supralethal irradiation, drugs, and probably without the need for TLI. As with intestinal transplantation, aggressive conditioning of the recipient to "make space" for the arriving donor cells may not be necessary and may even be harmful.

The use of immunocompetent donor cells to facilitate engraftment of solid organs will complete the cycle that began with the original Billingham-Brent-Medawar spleen cell inoculation technique, which has taken many forms since it was first modified by Main and Prehn (17). The simplest version was infusion of the donor white blood cell buffy coat perioperatively as an adjunct to kidney transplantation under azathioprine and prednisone. For nearly 25 years, Monaco has advocated that bone marrow is superior to all other sources of "tolerogenic" cells (157,158). In
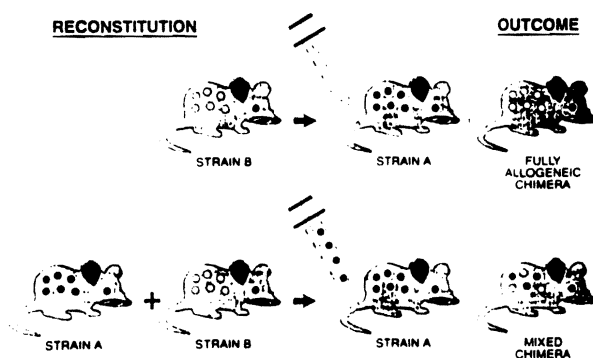

Fig. 39.3 Transplantation of bone marrow from one strain (B) into a genetically different recipient (A) results in fully allogeneic chimerism (B to A). The immune system of the recipient is totally replaced by that of the donor. The recipient chimeras are tolerant but not fully immunocompetent and exhibit susceptibility to GVHD. When syngeneic (host-type) marrow is coadministered with the allogeneic donor bone marrow, stem cells from both the donor and host coengraft to produce mixed chimerism, resulting in superior immunocompetence and resistance to GVHD (95, 146–150).

a clinical trial by Barber and associates based on Monaco's animal protocols, donor bone marrow was stored and given 3 weeks after cadaver kidney transplantation (159). From what has been learned of cell traffic, this may be too late for an optimal effect because the early events of alloreactivity would have been initiated. In our own human trial of induction of mixed allogeneic chimerism, bone marrow will be given at the same time as the solid organ transplantation under FK506-based immunosuppression, either omitting conditioning of the recipients or limiting this to low dose TLI.

Because solid organ transplantation already has reached such a high level of utility (greater than 90%
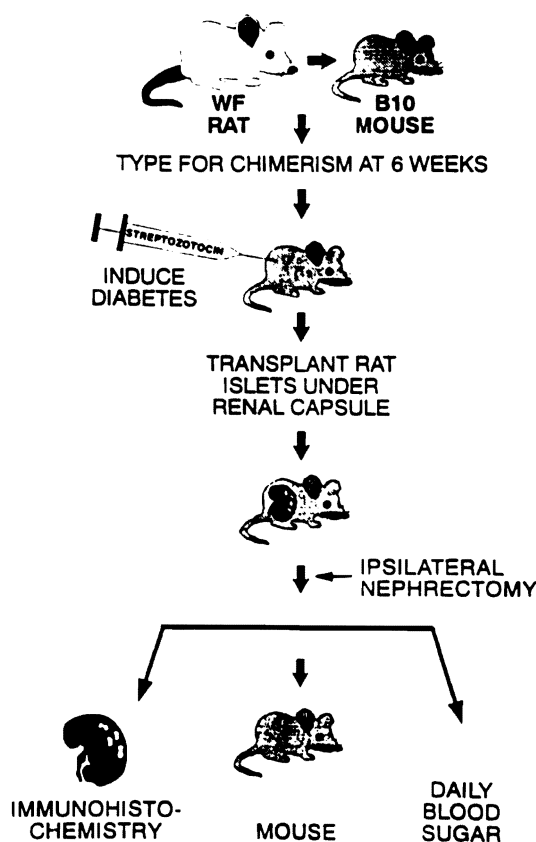

Fig. 39.4 Long-term rejection-free survival of donor-specific pancreatic islet xenografts occurred in xenogeneic chimeras. Chimeras were prepared and typed for chimerism at 6 weeks. Diabetes was induced using streptozotocin, and donor-specific islets were placed beneath the renal subcapsular space. In all recipients, normoglycemia occurred within 3 days following placement of the donor-specific islet xenografts. To demonstrate that the islet xenografts were functional to support the normoglycemic state, a transplant nephrectomy was performed at 9 months following graft placement. In all chimeras, the diabetic state recurred, demonstrating that glucose homeostasis was supported by the islet xenografts (153).

success with most kinds of grafts) without the necessity for attention to histocompatibility matching or recipient pretreatment, the acceptance of techniques for facilitation of mixed allogeneic (or even xenogeneic) chimerism will depend on their convenience and expense. In contrast, cell transplantation would be revolutionized if the systematic production of mixed allogeneic chimerism proves to be feasible, no matter how complex and costly the recipient preparation. Rejection remains the major factor limiting the clinical applicability of cell transplant procedures such as pancreatic islets (160,161).

Apart from its use alone to treat hematologic diseases and inborn errors, bone marrow as the means to transplant pancreatic islets or other cells is breathtaking in its ramifications. This already has been accomplished in animal allograft and xenograft models


Fig. 39.5 Anti-insulin peroxidase stain of a representative pancreatic islet xenograft 9 months after placement under renal subcapsular space in xenogeneic (rat-to-mouse) chimeras. Note the absence of mononuclear cell infiltrates, which would indicate chronic rejection, and the healthy-appearing red-staining tissue for presence of insulin.
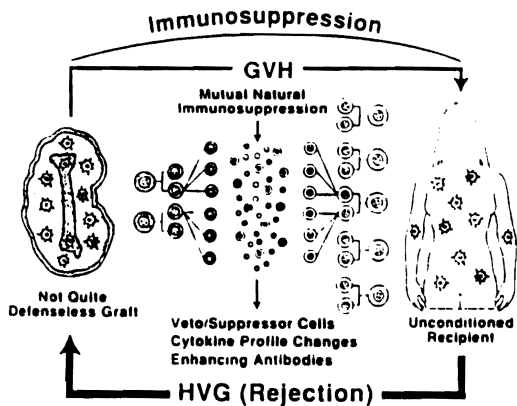
One-Way Paradigm (Bone Marrow)
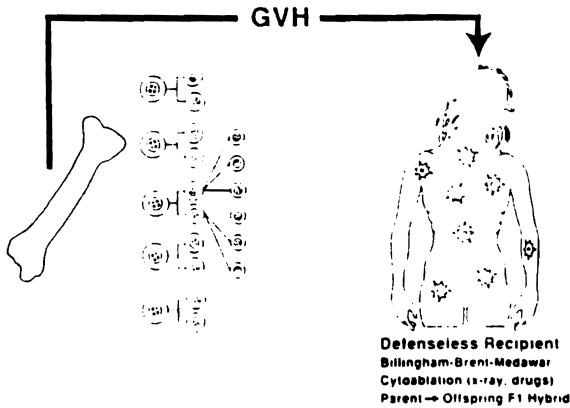
One-Way Paradigm (Organ)
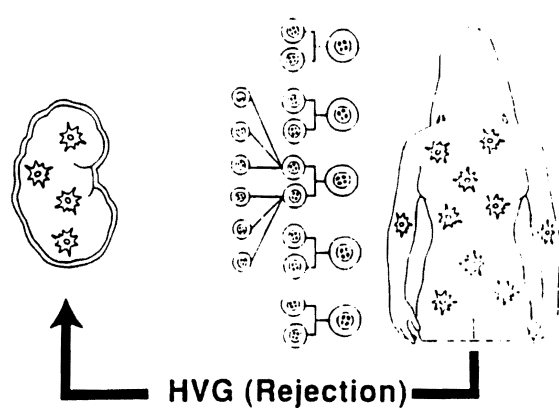
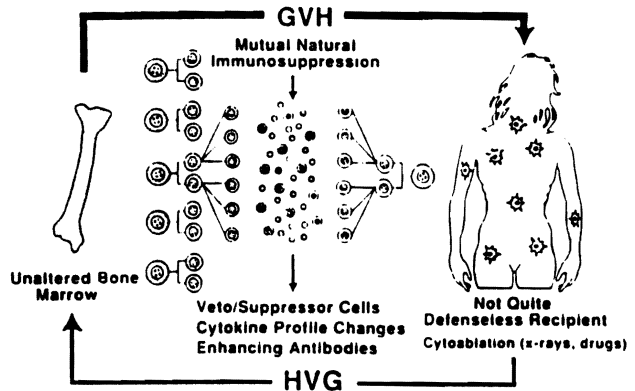## Two-Way Paradigm (Organ)

Two-Way Paradigm (Bone Marrow)

Fig. 39.6 (Upper panels) *One-way paradigm in which transplantation involves a unidirectional immune reaction: graft-versus-host (GVH) with bone marrow or other lymphopoietic transplants* (left) *and host-versus-graft (HVG) with transplanted whole organs* (right). (Lower panels) *Two-way paradigm with a bidirectional and mutually cancelling immune reaction that is predominantly GVH with bone marrow grafts* (left) *and HVG with whole organ grafts* (right).

(Figures 39.4 and 39.5) (153). Diseases in addition to diabetes mellitus that are potentially treatable include muscular dystrophy (myoblasts), hepatic enzymatic defects (hepatocytes), hormonal deficiencies (adrenal medulla, adrenal cortex), coagulation defects (vascular endothelial cells) and parkinsonism (adrenal). Many of these are the same disorders that would be amenable to gene therapy. It is likely that the two fields will merge and become complementary, allowing targeted therapy of previously untreatable disease states.

SUMMARY AND CONCLUSIONS

Historically, an allograft has been envisioned as an alien island in a hostile recipient sea. The corollary assumption was that the defenseless organ's attractiveness as a target for immunologic attack could be predicted by disparity between its major histocompatibility complex and that of the recipient. This one-way paradigm defined transplantation immunology in terms of a unidirectional immune reaction (Fig. 39.6, top panels). We propose that the interaction of two coexisting donor and recipient leukocyte populations, each to the other, is the fundamental explanation of both bone marrow and organ allograft acceptance and of transplantation tolerance generally. Therapeutic exploitation of the mechanisms of this "two-way (bidirectional) paradigm" (Fig. 39.6, bottom panels) is predicted to be the basis of the next phase of evolution in the transplantation field, which will include xenotransplantation.

ADDENDUM

January 1, 1995—During the Festschrift at Harvard honoring Paul Russell's retirement in late November 1990, Norman Shumway told one of us (T.E.S.) of his *Thoracic Transplantation* textbook for which he wanted one chapter on classic immunologic tolerance, and another on the ostensibly different mechanisms of whole organ allograft acceptance under chronic immunosuppression. On learning that we thought the subjects were of the same in principle, Dr. Shumway assigned us a chapter in which we could defend this opinion.

The preparation of the manuscript was not begun until a year later, but then it consumed nearly 5 months of intense and continuous effort. There had been no mention of a two-way immunologic reaction to explain organ acceptance in the voluminous literature of transplantation during the preceding four decades, and only a few long-ignored clues suggesting that migratory leukocytes from whole organs persisted and were mechanistically important. Consequently, the chapter was entirely hypothetical, and for that matter heretical, at the time it was submitted to the editors of *Thoracic Transplantation* in April 1992.

The overwhelming evidence showing persistent low-level chimerism after successful transplantation was compiled later. This was obtained first from investigation of long-surviving liver, kidney, and other organ recipients (100,162–166) and then from detailed animal studies (167–170). The observations conformed perfectly with what had been predicted. Consequently, the original description of the two-way paradigm written for Dr. Shumway has been preserved without any additions (except for the new summary and typographical corrections) or emendations.

The now widely accepted concept that transplantation is a bidirectional immune reaction (see Fig. 39.6) has permitted the historic milestones in clinical transplantation to be seen in a truer light (171). However, beacons of understanding shine forward as well as back. Thus, the principle of the two-way paradigm is being systematically applied in clinical trials of donor leukocyte augmentation for whole organ recipients (172) and has generated research initiatives.

ACKNOWLEDGMENT

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