Anesthesia and Transplantation

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Chapter 1

History of Organ Transplantation via the Two-Way Paradigm*

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Chapter Plan

The story of how whole-organ transplantation came to be a clinical discipline has been told elsewhere by many of those who were directly involved.[1] The kidney dominated events through 1959,[2] but in the late 1950s, canine transplant models were developed to study intra-abdominal and thoracic organs. Pig and rodent models came later.

Each organ-defined specialty has had its historians, but they have been preoccupied with a succession of events rather than with the poorly understood biological principles by which all organs can escape rejection. This conventional approach is characterized by noting the first successful allotransplantation of the kidney,[3] liver,[4] heart,[5] lung,[6] pancreas,[7] intestine,[8] multiple abdominal viscera,[9] and bone marrow.[10–12] Such milestones are important, but the concern here is the steps by which organ transplantation was developed empirically and the understanding of what had been accomplished that came only later. Such generic information may be of use to anesthesiologists who care for various organ transplant recipients.

The Immunologic Barrier

In December 1954, Joseph E. Murray unequivocally demonstrated the potential benefit of human whole-organ replacement with an identical twin kidney donor. His achievement was symbolic only, showing with an identical twin organ what was already known to be possible with skin grafts. Seven years later, the father of modern immunology, Macfarland Burnet, wrote in the *New England Journal of Medicine*, "much thought has been given to ways by which tissues or organs not genetically and antigenetically identi-

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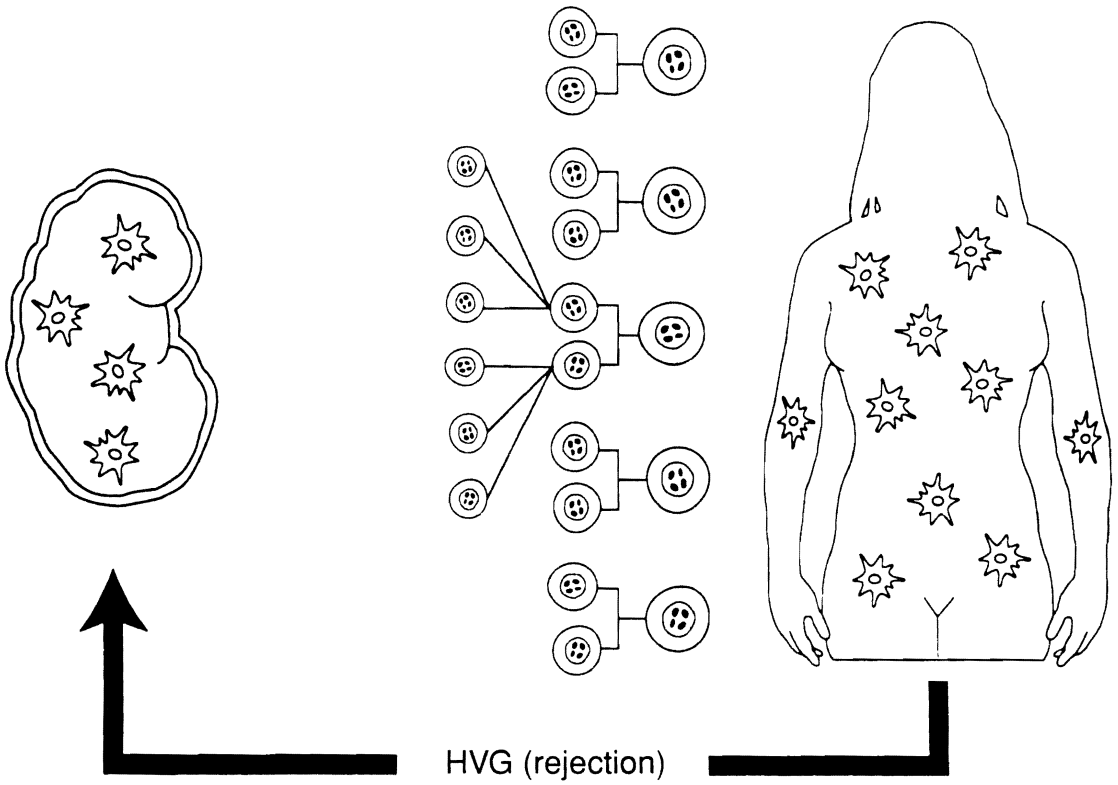


A

Figure 1-1. The one-way paradigm: Transplantation is conceived as involving a unidirectional immune reaction: host-versus-graft (HVG) reaction with whole organs (**A**) and graft-versus-host (GVH) reaction with bone marrow or other lymphopoietic transplants (**B**).

cal with the patient might be made to survive and function in the alien environment. On the whole, the present outlook is highly unfavorable to success."[13]

The One-Way Paradigm

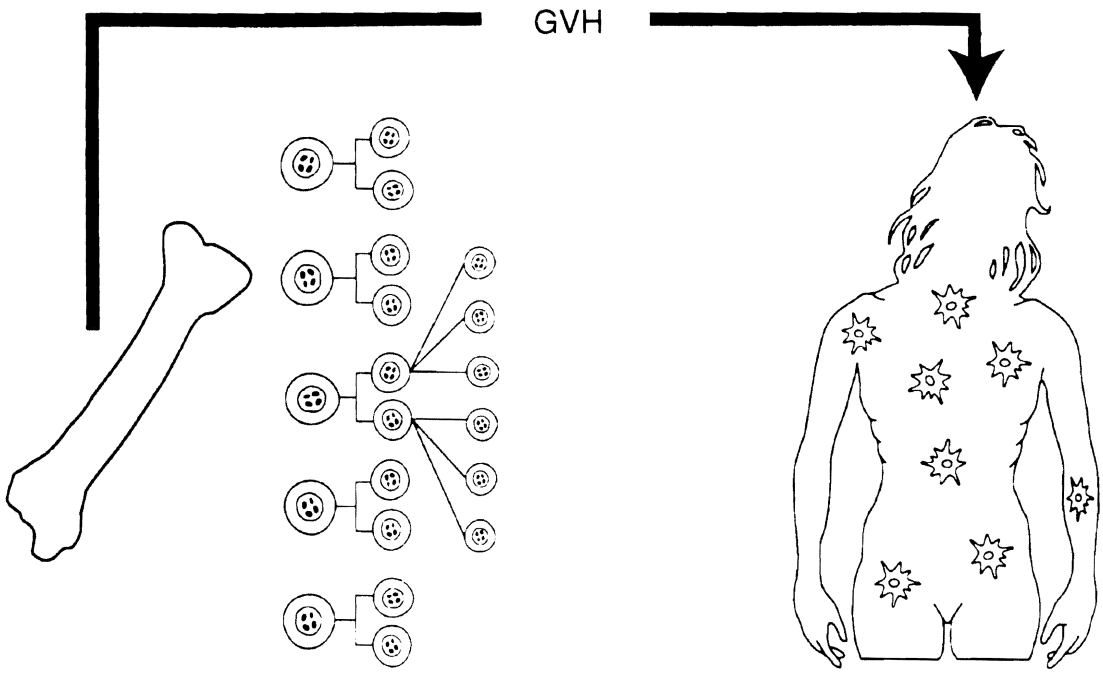
Rejection

What was the genetically determined barrier? Although details are obscure, there was little mystery after 1944 about the general meaning of transplant rejection, after its elucidation by Peter Medawar as an immunologic event.[14] Medawar's contribution created the image of a tissue (or organ) allograft as an island in a hostile recipient sea (Figure 1-1A).

Tolerance

In contrast, how allografts or xenografts can escape rejection with or without the aid of immunosuppression has been one of the most arcane subjects in biology since Billingham, Brent, and Medawar described acquired tolerance in 1953.[15, 16] A simple explanation for the tolerance in their special model was at first beguiling. Immunocompetent adult spleen cells were injected in utero or perinatally into mice that had not yet evolved the immunologic equipment to reject them. The engrafted cells flourished, perpetuated themselves, and, in effect, endowed the recipient with the donor immune system. Thereafter, the chimeric mice failed to recognize donor-strain skin or other donor tissues as alien.

In this second landmark contribution from Medawar's laboratory, tolerance was explained as a



B

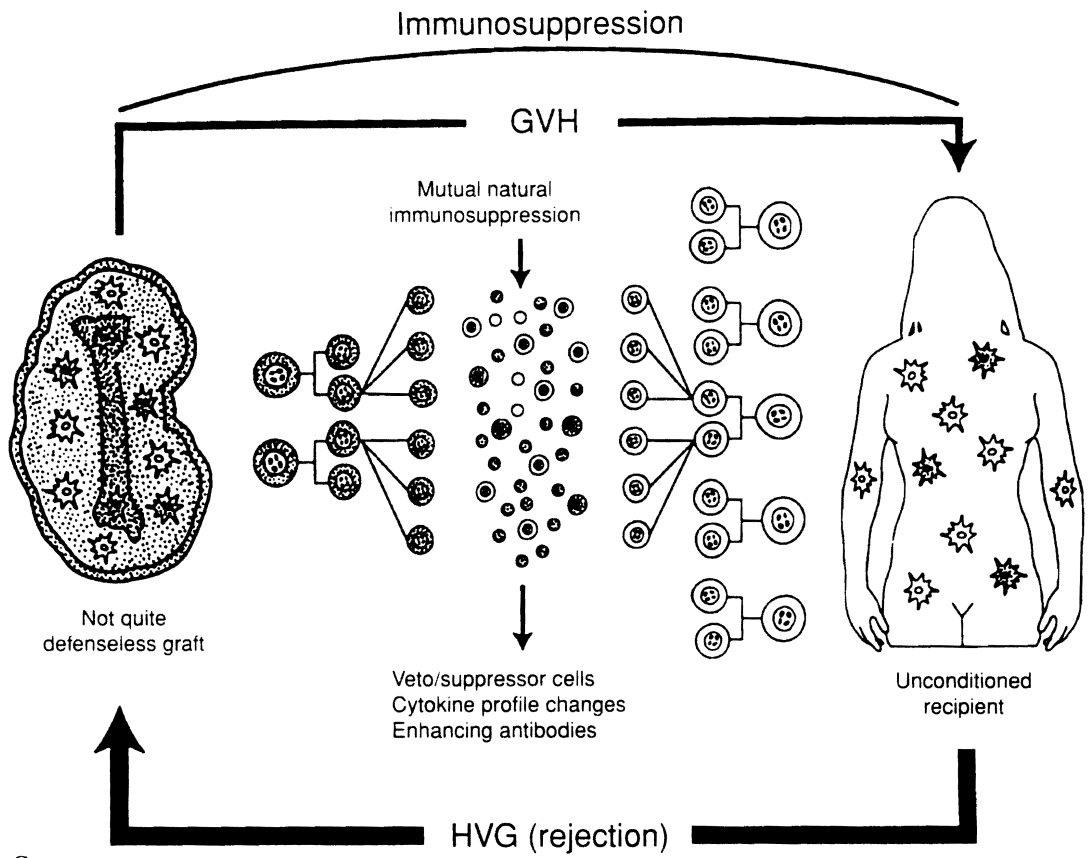
switch in immunologic apparatus. It was consistent with the definition of transplantation immunology as a unidirectional immune reaction (the "one-way paradigm"). Main and Prehn[17] strengthened this view by demonstrating the same tolerance outcome as that of Billingham, Brent, and Medawar in irradiated adult mice. Main and Prehn reconstituted the hematolymphopoietic cells of their cytoablated mice with bone marrow. Hundreds of subsequent tolerance-induction experiments in animals, and eventually clinical bone marrow transplantation, seemed to depend on a similar natural, or iatrogenically imposed, defenseless recipient state (Figure 1-1B).

Graft-versus-Host Disease

The anticipated clinical application of this kind of tolerance induction was temporarily derailed in 1957, when it was realized that an immunologically active graft could turn the tables and reject the recipient (graft-versus-host disease [GVHD]). Billingham and Brent showed in their mouse model[18] and Simonsen in chickens[19] that this risk (also called *runt disease*) was roughly proportional to the extent of the major histocompatibility complex (MHC) barrier. Such disparities became measurable in humans after identification of the HLA by Dausset,[20] Terasaki, and others.[21] For many years, the complication of GVHD in rodent[22] and large animal irradiation chimera models[23–26] forestalled the clinical use of HLA-mismatched bone marrow cells or other mature immunocytes, either for immunologic reconstitution for purely hematologic purposes or as a means of facilitating whole-organ graft acceptance.

Clinical Bone Marrow Transplantation

Nevertheless, a strategy for clinical bone marrow transplantation eventually was assembled directly



C

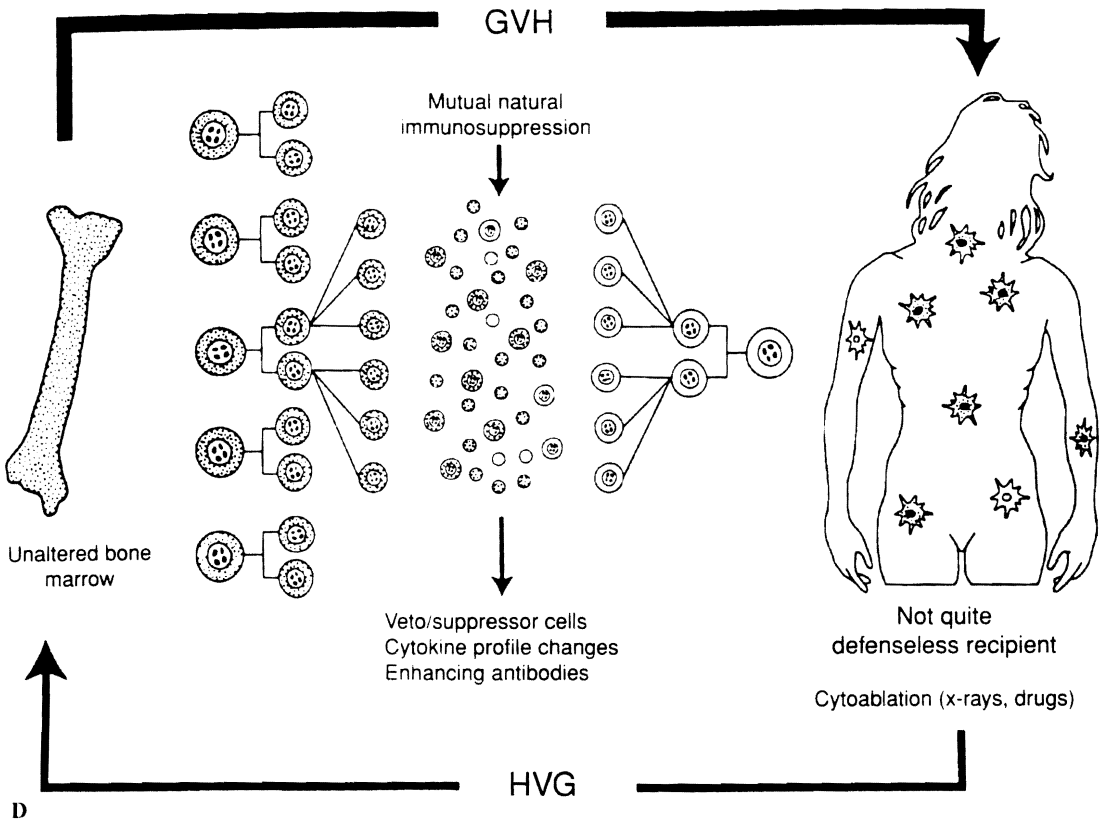
Figure 1-1. *Continued.* The two-way paradigm: Transplantation is seen as a bidirectional and mutually canceling immune reaction that is predominantly HVG with whole-organ grafts (**C**) and predominantly GVH with bone marrow grafts (**D**).

from the rodent experiments, but with similar histocompatibility-imposed restrictions.[23] After recipient cytoablation with total body irradiation (TBI) or cytotoxic drugs, stable chimerism could be induced in humans by the infusion of donor bone marrow if there was a good HLA match. Otherwise, the incidence of GVHD was intolerable. After successful engraftment, maintenance immunosuppression frequently was not needed, mimicking the kind of acquired immunologic tolerance originally described by Billingham, Brent, and Medawar[15, 16] and then by Main and Prehn.[17] The eventual success of clinical bone marrow transplantation[10–12] was a straight-line extension from these rodent models, as Donnel Thomas (1990) has observed.[23]

Clinical Organ Transplantation

Total Body Irradiation

The achievement of clinical bone marrow transplantation effectively detached the surgeons from a scientific base because there was no explanation for successful engraftment. Nevertheless, by the time of the first successful bone marrow transplantation, surgeons had already recorded many successful human whole-organ transplantations (mostly kidneys) under continuous immunosuppression, without dependence on HLA matching or the complication of GVHD, and as it turned out, without host preconditioning. In fact, preconditioning with sublethal TBI was used in the first



successful renal allotransplantation, described by Merrill et al. in 1960.[3] The kidney recipient, however, whose donor was his fraternal (dizygotic) twin brother, was not given bone marrow, which was a significant departure from the Billingham-Brent-Medawar framework. The recipient's own bone marrow recovered, and the transplanted kidney and patient survived for 20 years. Six additional examples of protracted kidney graft survival (longer than 1 year) after recipient irradiation without marrow were recorded in Paris over the next 36 months.[27, 28] Five of the six donors were more distant relations than a fraternal twin, and two were genetically unrelated.[28] However, these were isolated successes in a sea of failures.

Chemical Immunosuppression

The frustration continued after Murray et al.[29] introduced 6-mercaptopurine and its analogue, azathioprine, for human renal transplantation. This followed extensive experimental studies, first with rodent skin transplantation[30, 31] and then with canine kidney transplant models.[29, 32-34] The drugs were originally developed as antileukemic agents by Elion et al.[35] and were first demonstrated to be immunosuppressive by Schwartz and Dameshek.[36] Although the sixth patient treated by Murray with one or the other of these myelotoxic drugs had function of a nonrelated renal allograft for 17 months, the clinical results were poor at first,[29, 37] similar to those with TBI.

The Double-Drug Breakthrough

The tidal wave of whole-organ transplant cases began in 1962, when azathioprine was combined with prednisone to reverse rejection.[38] More important, the subsequent need for maintenance immunosuppression frequently declined, and in occasional

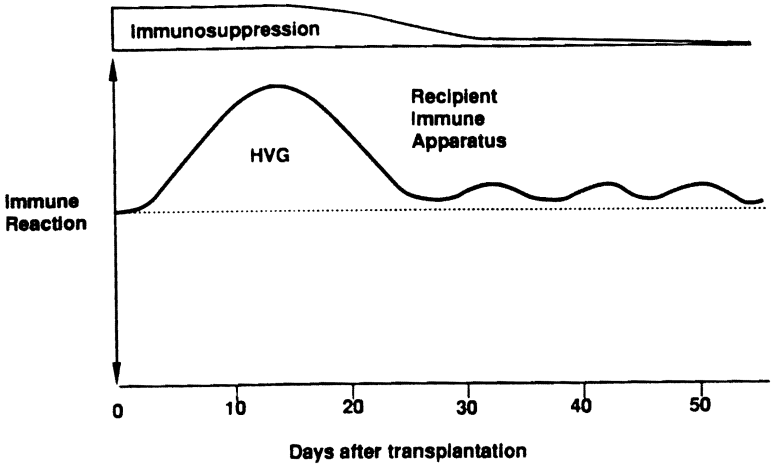


Figure 1-2. Pattern of postoperative events with whole-organ allograft acceptance in the framework of the one-way paradigm. (HVG = host-versus-graft [reaction].)

cases, treatment could be stopped. The same sequence has been shown with all other organs transplanted and with all the immunosuppressive regimens (Figure 1-2). Agents introduced later were more potent and reliable in chaperoning the desired chain of events: antilymphocyte globulin,[39] cyclosporine,[40] and tacrolimus (FK506).[41] Despite their diversity, all these drugs seemed, in a fundamentally similar way, to have allowed something to change in the host, the graft, or both. But what was that something?

The one-way paradigm of transplantation immunology that had gained ascendency nearly a half-century before did not provide answers to that question. The false conception of a unidirectional reaction was never seriously challenged after it was seemingly supported by studies with the one-way mixed lymphocyte reaction introduced in 1963 by Bach and Hirschhorn[42] and Bain et al.[43] These in vitro techniques (so-called minitransplant models) generated thousands of increasingly sophisticated cellular and ultimately molecular studies of unidirectional immunologic reactions. Ironically, the resulting plethora of new information sometimes resembled an exponentially expanding phone book filled with wrong numbers. Most seriously, the flawed context lured successive generations of investigators into the trap of believing that tolerance induction for whole-organ recipients (the "holy grail") lay in variations on the HLA-limiting strategy used for bone marrow transplantation, which included host preconditioning in preparation for a variety of donor leukocyte preparations.

The Two-Way Paradigm

Whole-Organ Transplantation

A plausible explanation for the success of the empirically developed whole-organ transplantation procedures did not emerge until 1992. Then, a study of the surviving pioneer kidney and liver recipients from the earliest clinical trials revealed that donor leukocytes of bone marrow origin, which are part of the structure of all complex grafts (passenger leukocytes[44, 45]), had migrated from the organs to ubiquitous sites in the recipient and survived for up to 30 years.[46, 47] Thus, organ allograft acceptance was associated with the cryptic survival of a small fragment of extramedullary donor marrow, including stem cells (depicted as a bone silhouette encased by the kidney in Figure 1-1C), which was disseminated throughout the recipient after the transplantation and assimilated into the much larger immunologic network of the host. In the meantime, the cells that left the graft were replaced by recipient immune cells moving in the opposite direction. The end result was a small number of residual donor leukocytes (microchimerism) in both graft and host.

From this information, a revision of transplantation immunology was possible. In the new view, the immunologic confrontation after whole-organ transplantation could be seen as bidirectional (GVH as well as HVG) and mutually canceling (Figure 1-3), provided that the participants in the David-and-Goliath mismatch could survive the initial onslaught. In a clinical context, but not in several

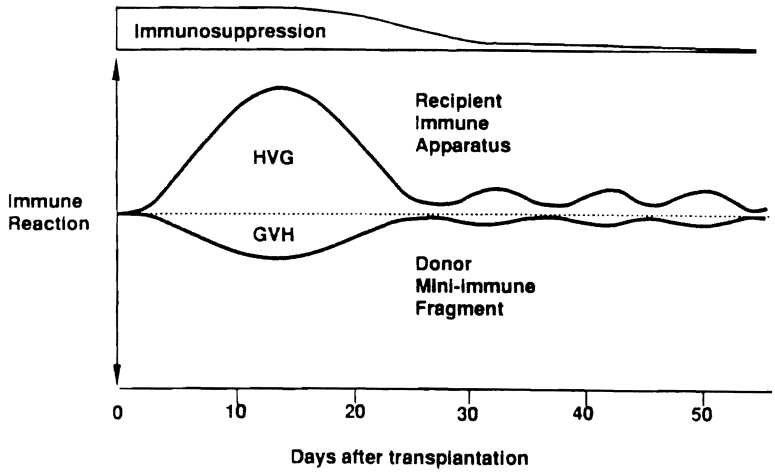


Figure 1-3. The pattern of convalescence after organ or bone marrow transplantation in the framework of the two-way paradigm. With bone marrow transplantation, the dominant immune reaction usually is graft-versus-host (GVH) reaction. (HVG = host-versus-graft [reaction].)

animal models, this survival requires an umbrella of immunosuppression that protects both cell populations equally (see Figure 1-1C). Current research aims at understanding the amplification device that enables a small number of cells to affect so profoundly the immunology of the vast cellular army of the host. Although the chimeric leukocytes are multilineage,[46–49] the antigen-presenting dendritic cells of Steinman and Cohn[50, 51] are thought to be critical because they can modify the expression of cell interaction, MHC, and adhesion molecules, all of which determine how T cells heed antigen signals.[51]

Historic Enigmas

With the two-way paradigm, virtually every previously unexplained experimental or clinical observation after whole-organ transplantation was understood or at least susceptible to experimental inquiry.[46, 47] It was clear why organ grafts are inherently tolerogenic, why HLA matching is so poorly predictive of outcome, and why GVHD does not develop after the transplantation of immunologically active grafts, such as the liver and intestine.

With the two-way mutual cancellation implicit in this concept, the loss or blunting of an HLA matching effect is easy to understand. With each further level of histoincompatibility, the reciprocal effect is postulated to escalate both ways, providing the process is chaperoned with an effective immunosuppressive umbrella (Figure 1-4). The consequent dwindling of the matching effect as donor-specific and recipient-specific nonreactivity evolves accounts for blindfolding of the expected HLA effect. In addition to explaining why the HLA matching effect is blindfolded, this bidirectional canceling effect of the two cell populations explains why GVHD does not develop after liver, intestinal, multivisceral, and heart-lung transplantation, despite the heavy lymphoid content of those organs.

Augmentation of Spontaneous Chimerism

Historic efforts to give extra donor antigen in the form of bone marrow[52, 53] or donor blood transfusions[54–56] were hampered in design or execution by the assumption that the infused cells would be destroyed without recipient preconditioning, by the justifiable anxiety about GVHD if the host was preconditioned, and by a lack of information about the appropriate timing of the infusions. The new information that chimerism is a naturally occurring event after whole-organ transplantation[46, 47] exposed a perioperative window of opportunity. In this window, unaltered HLA-incompatible bone marrow or donor-specific blood transfusion was predicted to be safe without recipient preparation or any deviation from the generic practices of immunosuppression for whole-organ transplantation, which had evolved from the original azathioprine-prednisone formula.[38]

The validity of this strategy was verified in unpreconditioned recipients of cadaveric kidneys, livers, hearts, and lungs who were given $3–5 \times 10^8$

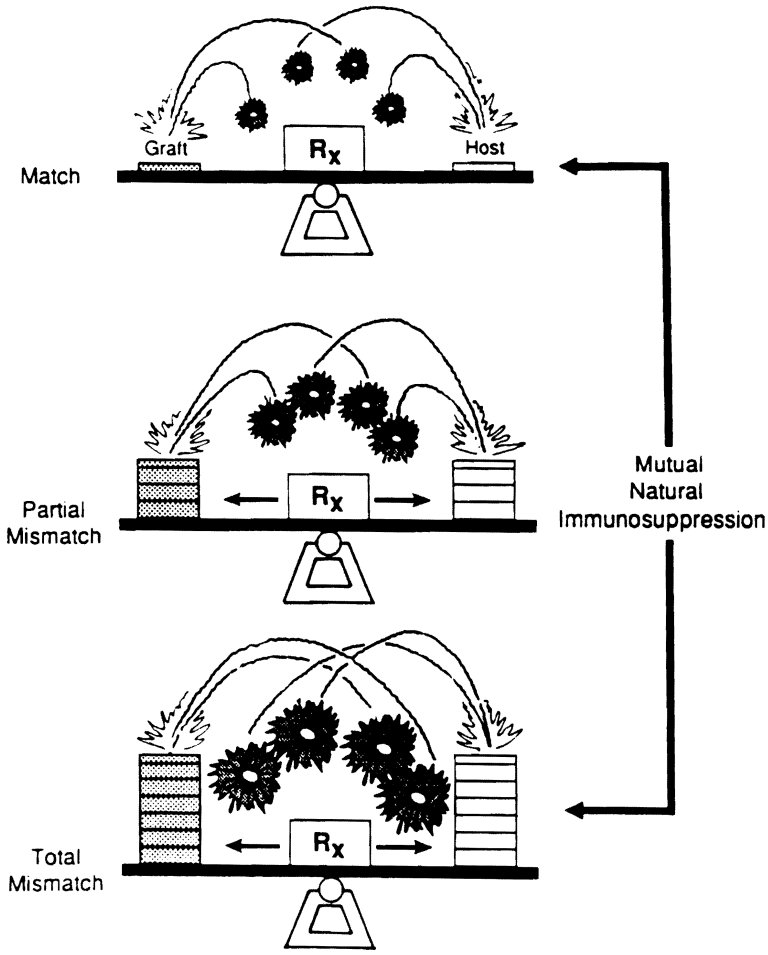


Figure 1-4. Explanation for the loss of an HLA-matching effect with whole-organ transplantation. (Rx = immunosuppression.)

donor bone marrow cells per kg recipient body weight at the same time as organ transplantation under standard FK506-prednisone treatment (Figure 1-5).[57] Chimerism estimated at more than 1,000 times that occurring in conventional whole-organ recipients was reliably and safely produced and sustained. Persistent blood chimerism (usually greater than 1%), a trend toward donor-specific nonreactivity, and a high rate of patient and graft survival has marked these bone marrow–augmented recipients as an advantaged cohort. They are the first patients to undergo HLA-mismatched cadaveric organ transplantation with the reasonable prospect of eventually being drug free. The process of tolerance induction and drug weaning is expected to take 5–10 years in most patients who are given mismatched organs. In some patients, the drug-free state may never be attained.

Whole-Organ Transplantation versus Bone Marrow Transplantation

With the discovery that whole-organ transplantation caused spontaneous chimerism, it was realized that the apparently vast gap between the bone marrow and whole-organ transplantation fields merely reflected entrenched differences of treatment strategy (Figure 1-6). The mutually censoring immunologic limbs were being left intact with organ transplantation, whereas the recipient limb was deliberately removed (cytoablation) in preparation for bone marrow grafting procedures. It is doubtful that it is ever possible (much less desirable) to completely eliminate the entire recipient immune system with the cytoablation techniques of bone marrow transplantation. Although this was long assumed to have occurred in successful cases (see Figure 1-1B),

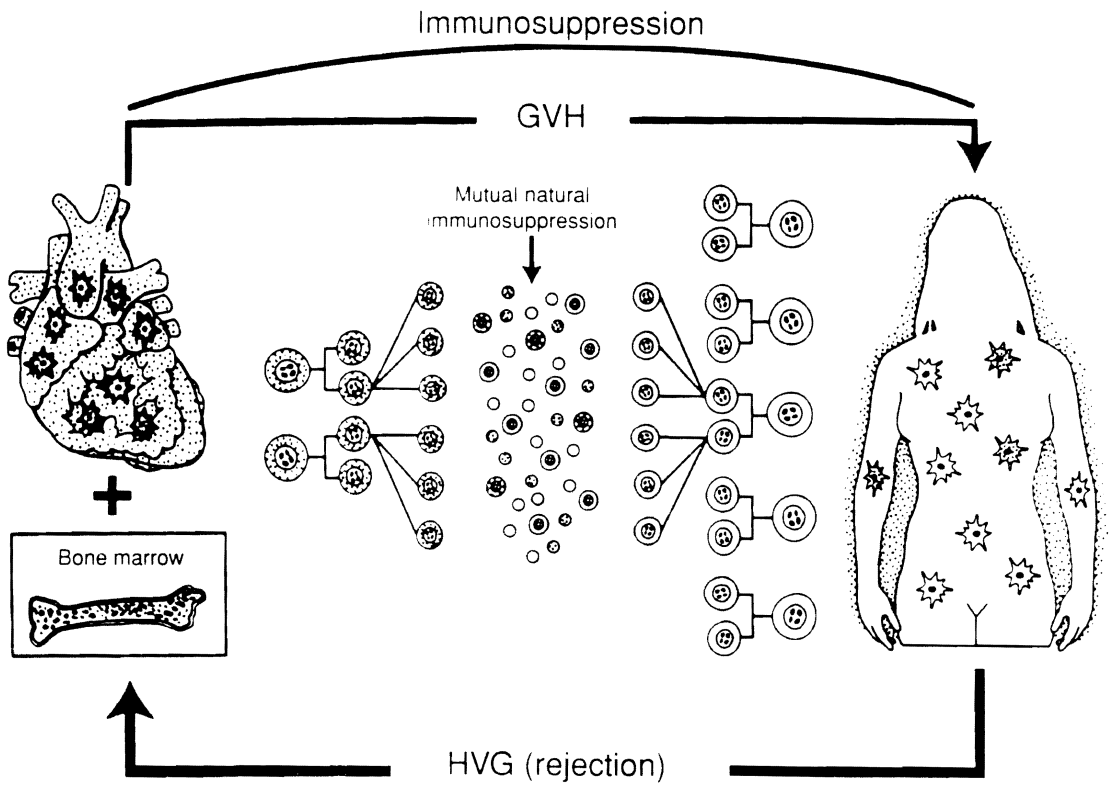


Figure 1-5. Iatrogenic augmentation of the graft-versus-host (GVH) component of the two-way paradigm by infusing 3–6 × 10^8 unaltered donor bone marrow cells per kg recipient body weight at the same time as heart or other whole-organ transplantation. When the recipient is not cytoablated, there is essentially no risk of GVH disease. (HVG = host-versus-graft [reaction].)

a trace population of recipient leukocytes has almost invariably been detected with sensitive techniques in patients previously thought to have complete bone marrow replacement.[58, 59] These bone marrow recipients were mirror images of successfully treated whole-organ recipients, the difference being that their own, rather than donor leukocytes, constituted the trace population. In either kind of recipient (whole-organ or bone marrow), the appearance of MHC-restricted veto and suppressor cells, enhancing antibodies, and changes in cytokine profile could be construed as a by-product of and accessory to the seminal event of mixed chimerism and resulting reciprocal clonal exhaustion and deletion (see Figure 1-1C and D).[46, 47, 60]

Beyond an adjuvant role for whole-organ transplantation, an important question is whether HLA-mismatched bone marrow without an accompanying organ can be engrafted in patients whose disease can be corrected with a minimally chimeric or even microchimeric state, using the same immunosuppression as for marrow-augmented kidney, liver, and heart recipients. The potential list of indications in which complete marrow replacement is unnecessary is a long one, exemplified by the lysosomal enzyme deficiencies.[61] Another look into the future has been provided by the demonstration that xenograft transplantation is followed by the same cell migration process seen with allografts.[62]

Importance of History

The legendary immunologist Melvin Cohn (father of the two-signal concept of self-nonself discrimination) wrote in 1994, "In its recent history, immunology has advanced largely by volume [of

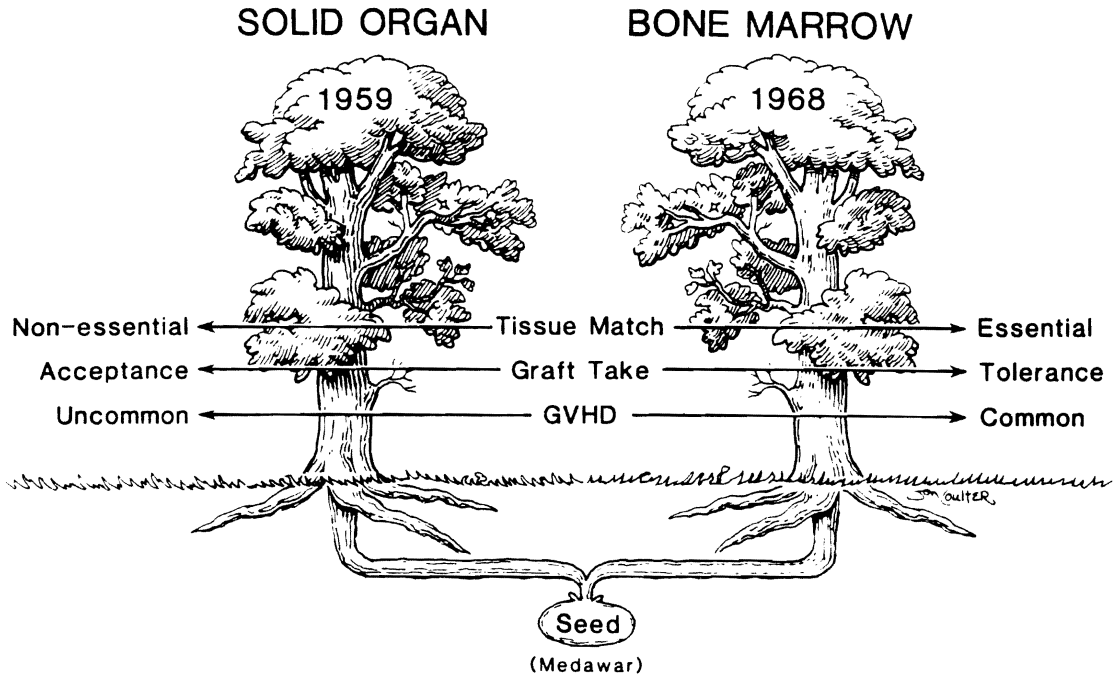


Figure 1-6. The growth of separate disciplines of bone marrow and whole-organ transplantation from the seed planted by Peter Medawar during World War II. It was recognized in 1992 that these seemingly disparate disciplines were mirror images caused by different treatment strategies. (GVHD = graft-versus-host disease.)

publications], complete with waste."[63] In Cohn's opinion, the reason for the slow conceptual advancement in this branch of science has been the immunologists' preference for small theories that explain one or only a few facts (articulated by Mitchison[64]) over the development of generalized principles that explain all facts (coherence of context). It would be hard to find a better way to illustrate the consequences of a small theory than those derived from the durable one-way paradigm, which was blindly accepted despite its failure to explain what was seen daily in every transplantation clinic and laboratory. Virtually no hint of the two-way paradigm can be found in the literature before the description in June 1992 of microchimerism in organ recipients. If the spontaneous development of chimerism after organ transplantation had been recognized 30 years ago, it would have been possible to correctly interpret observations in splenocyte and bone marrow transplant experiments reported in 1960–1962 by Simonsen[65, 66] and Michie, Woodruff, and Zeiss.[67] The hypothesis of these earlier workers—that acquired tolerance must result from a two-way (donor-recipient) immune reaction—resembled the hypothesis that was later used to explain organ graft acceptance. Their great idea was abandoned because it could not be proved, thereby delaying a true understanding of transplantation immunology for a third of a century.

Beacons of understanding shine forward as well as backward. Understanding the history of transplantation in terms of the two-way paradigm provides the intellectual means to devise better treatment strategies, including the achievement of drug-free tolerance and, ultimately, xenotransplantation.

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