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The mystique of transplantation: biologic and psychiatric considerations

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Most major advances in medicine spring from discoveries in basic science and are therefore predictable, or at least logical. Organ transplantation was the supreme exception to the rule. Although the potential benefit of whole-organ replacement in the absence of an immune barrier was dramatically demonstrated with the identical-twin kidney transplantation performed, in December 1954, by Joseph E. Murray (Nobel Laureate, 1990; Merrill et al. 1956), this achievement only confirmed what already was known to be possible with identical-twin skin grafts (Padgett 1932; Brown 1937). In 1961, two months after receiving the 1960 Nobel Prize for research in immunology, Macfarland Burnet wrote in the *New England Journal of Medicine* that "much thought has been given to ways by which tissues or organs not genetically and antigenetically identical 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..." (Burnet 1961).

This grim prospect, only a third of a century ago, faced the pioneer organ recipients whose courage in offering themselves up for human experimentation made it possible to crack the immunologic barrier (Starzl 1992). For three decades after this was done, there was no explanation for what had been accomplished. The resulting mystique of transplantation as well as the unpredictable outcome of these procedures created a fertile emotional soil for psychiatric complications. Consequently, when the light of understanding was finally switched on in 1992, it was as much a spiritual as a scientific awakening. The acquisition of this insight exposed a seminal principle of allograft acceptance that is the same with all kinds of whole organs.

The immunologic barrier: the one-way paradigm

What was the immunologic barrier described in such pessimistic terms by Burnet, who saw no way that a transplanted histoincompatible organ could pass through the seemingly inviolate barrier of immunologic reactivity? In the identical-twin kidney transplantations, the problem was by-passed, but in all other cases, it was necessary to reckon with two potential complications,

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either separately or together: rejection and graft-versus-host disease (GVHD).

Rejection

Although the details remain incomplete today, there was little mystery about the general meaning of rejection, following its elucidation in 1944 by Medawar (Nobel co-Laureate with Burnet 1960) as an immunologic event (Medawar 1944). This great contribution created the indelible image that transplantation involved a one-way immune reaction. Thus, a tissue (or organ) allograft was a defenseless island in a hostile recipient sea (Figure 1.1(a)).

Tolerance

In this context, an unsolved mystery of biology for nearly 40 years was how allografts could escape rejection without the recipient being crippled with immunosuppression. The description of acquired tolerance by Billingham, Brent, and Medawar (1953, 1956) did not provide an answer. In their experimental model, immunocompetent adult spleen cells were injected in utero or perinatally into mice that had not yet evolved the necessary immunologic maturity to reject them. The engrafted cells flourished and were thought to have in effect endowed the recipient with the donor immune system (donor leukocyte chimerism) (Figure 1.1(b)).

Thereafter, the mice failed to recognize donor strain skin grafts or other tissues as alien (i.e. they had acquired tolerance). The switch in immunologic apparatus was consistent with the definition of transplantation immunology in terms of a unidirectional immune reaction (the "one-way paradigm"). Main and Prehn (1955) demonstrated the same tolerance outcome as Billingham et al. in irradiated adult mice, whose cytoablated hematolymphopoietic cells were reconstituted with bone marrow instead of spleen cells. Thousands of subsequent tolerance induction experiments in animals, and eventually clinical bone marrow transplantation, seemingly depended upon a similar natural, or iatrogenically imposed, defenseless recipient state (Figure 1.1(b)).

Graft-versus-host disease

It was recognized as early as 1957 in mouse (Billingham and Brent 1957) and chicken (Simonsen 1957) models that an immunologically active graft had a genetically controlled repertoire of immune reactivity comparable to that of the recipient, and could therefore turn the tables and reject the recipient. This was called graft-versus-host disease (GVHD), or alternatively "runt disease". The risk if the host was immunologically defenseless was roughly propor-

tional to the extent of the major histocompatibility complex (MHC) difference between donor and recipient. Such disparities became measurable serologically in humans after identification of the human leukocyte antigens (HLAs) by Dausset (1990; Nobel Laureate 1980), Terasaki, and others whose reminiscences have been collected (Terasaki 1990). The complication of GVHD in rodent (Trentin 1957) and large animal irradiation chimera models (Mannick et al. 1959; Hume et al. 1960; Rapaport et al. 1979; Thomas 1991) forestalled for many years the clinical use of HLA mismatched bone marrow cells or other mature immunocytes, either for immunologic reconstitution with purely hematologic objectives or as a means of facilitating whole-organ graft acceptance.

Clinical bone marrow transplantation

The strategy that made possible the first successful clinical bone marrow transplantations in 1968 was an extension of the rodent experiments, with similar histocompatibility-imposed restrictions (Mathe et al. 1963; Bach 1968; Gatti et al. 1968). After recipient cytoablation with total body irradiation (TBI) or cytotoxic drugs (Figure 1.1(b)), stable chimerism could be induced in humans by the infusion of donor bone marrow, but only if there was a good HLA match. Otherwise there was an intolerable incidence of lethal GVHD. Maintenance immunosuppression could frequently be stopped in these patients, mimicking the kind of acquired immunologic tolerance originally described by Billingham et al. (1953, 1956), and by Main and Prehn (1955).

Clinical organ transplantation

Ironically, surgeons and physicians had recorded thousands of successful human whole-organ transplantations (mostly kidneys) by the time bone marrow transplantation was finally accomplished. However, the conditions were dramatically different. First, continuous immunosuppression was needed, presumably for life. Second, success did not depend on HLA matching. Third, the complication of GVHD was rare. Most immunologists were dumbfounded by these results. The inability to explain them detached workers in the whole-organ field from the scientific base enjoyed by bone marrow transplanters. Thus, further steps in the improvement of whole-organ transplantation were largely the product of trial and error. To comprehend how this occurred requires a historical perspective.

With total body irradiation
Host preconditioning played an important role in the first six successful renal

(a) One-Way Paradigm (Organ)

HVG (Rejection)

(c) Two-way Paradigm (Organ)

Immunosuppression

GVH

Mutual Natural
Immunosuppression

Not Quite
Defenseless Graft

Veto/Suppressor Cells
Cytokine Profile Changes
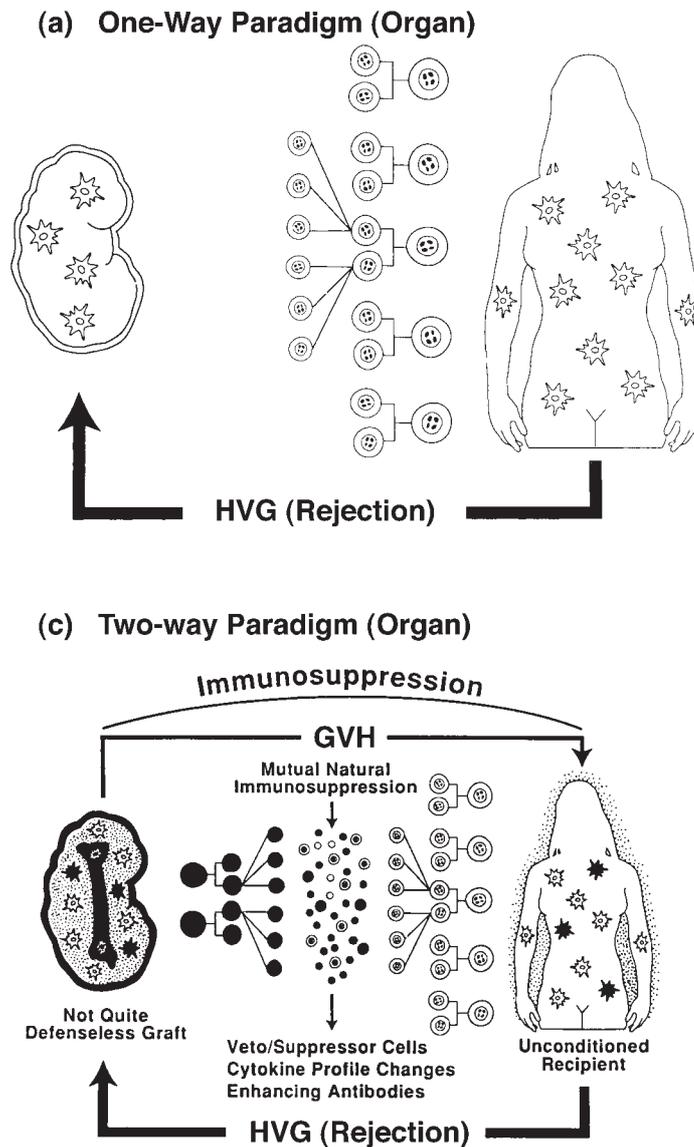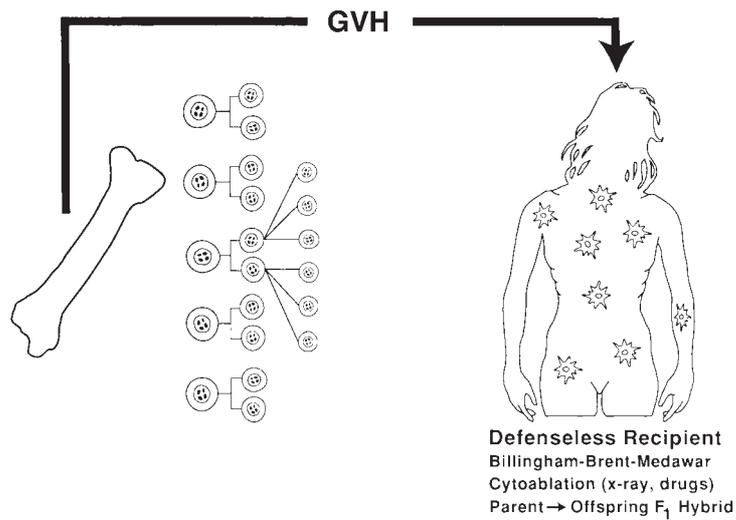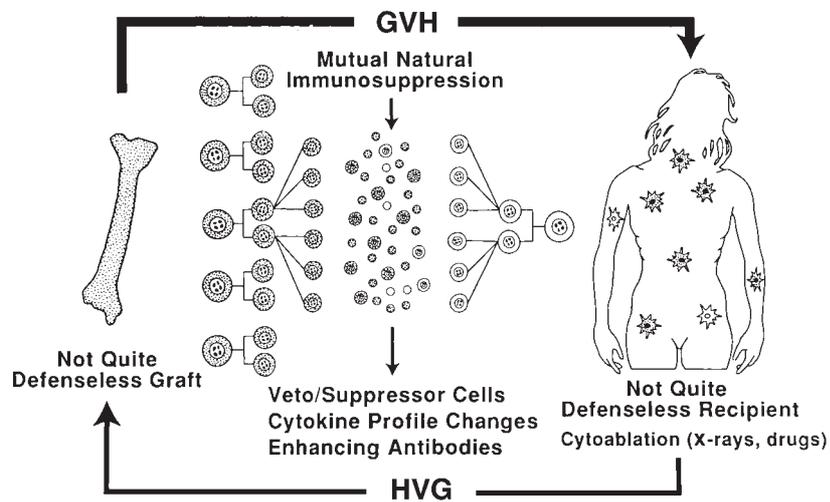Enhancing Antibodies

Unconditioned
Recipient

HVG (Rejection)

Figure 1.1. Upper panels: One-way paradigm in which transplantation is conceived as involving a unidirectional immune reaction: host-versus-graft (HVG) with whole organs (a) and graft-versus-host (GVH) with bone marrow or other lymphopoietic transplants (b). Lower panels: Two-way paradigm with which transplantation is seen as a bidirectional and mutually cancelling immune reaction that is predominantly HVG with whole-organ grafts (c), and predominantly GVH with bone marrow grafts and (d).

(b)   One-Way Paradigm (Bone Marrow)

Defenseless Recipient
Billingham-Brent-Medawar
Cytoablation (x-ray, drugs)
Parent → Offspring F_1 Hybrid

(d)   Two-way Paradigm (Bone Marrow)

transplantations (defined as survival to more than one year) between 1959 and 1962, one in Boston (Merrill et al. 1960) and five in France (Hamburger et al. 1962; Kuss et al. 1962). The recipients were prepared for operation with sublethal TBI, but without donor bone marrow; their own bone marrow recovered and one of these patients (in Paris) survived for 26 years. However, these were isolated successes in a sea of failures, and pessimism set in worldwide about the prospects of moving forward.

Chemical immunosuppression

The introduction, for human renal transplantation, of 6-mercaptopurine (6-MP) and its analogue azathioprine did not at first relieve the frustration. The clinical use of these drugs followed the demonstration of their immunosuppressive effects in extensive experimental studies, first with rodent skin transplantation (Meeker et al. 1959; Schwartz and Dameshek, 1960) and then with the canine kidney transplant models (Calne 1960, 1961; Zukoski, Lee, and Hume 1960; Murray et al. 1962). The drugs had been developed originally for their antileukemic effect by Elion and Hitchings (Nobel Laureates, 1988; Elion, Bieber, and Hitchings 1955) and were first demonstrated to be immunosuppressive by Schwartz and Dameshek (1959). Although the kidney of the sixth patient treated by Murray with one of these myelotoxic drugs had the function of a non-related renal allograft for 17 months, the clinical results with chemical immunosuppression were generally poor at first (Murray et al. 1962, 1963), similar to those with TBI.

The drug cocktail breakthrough

The tidal wave of whole-organ cases began in earnest in 1962–3, when a characteristic cycle of convalescence was identified in which kidney rejection could be reversed surprisingly easily when prednisone was added to azathioprine (double-drug therapy) (Starzl, Marchioro, and Waddell 1963). More importantly, the need later on for maintenance immunosuppression often declined as if the immune barrier had been lowered (Figure 1.2), and in occasional cases therapy could be stopped. Since then, the same sequence has been seen with all other organs transplanted and with all of the two-drug and more complex multiple agent immunosuppressive regimens. Drugs introduced later were more potent and reliable in chaperoning the desired chain of events: antilymphocyte globulin (ALG) (Starzl et al. 1967), cyclosporine (Calne et al. 1979), and FK506 (Starzl et al. 1989). Notwithstanding their diversity, all seemed in a fundamentally similar way to have allowed something to change in the host, the graft or both. But what?

Deficiencies of the one-way paradigm

Although the one-way paradigm did not provide the answer, this false
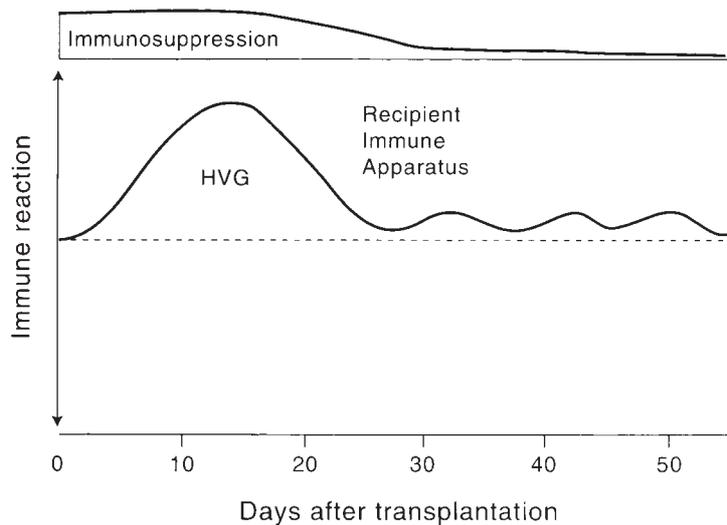
Figure 1.2. Pattern of postoperative events with whole-organ allograft acceptance, in the framework of the one-way paradigm. HVG, host-versus-graft.

conceptualization of graft acceptance as a product of a unidirectional reaction was reinforced with the introduction in 1963 of the one-way mixed lymphocyte reaction (Bach and Hirschhorn 1964; Bain, Vas, and Lowenstein 1964). Although these and other in vitro techniques (the so-called minitransplant models) generated increasingly sophisticated cellular and ultimately molecular studies of one-way immunologic reactions, the resulting plethora of new information resembled an exponentially expanding telephone directory. 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. The strategy always included host preconditioning in preparation for a variety of donor leukocyte preparations.

Cell-mediated immunity

The inability of clinicians to explain what was going on with their patients did not dissuade them from developing their own voluminous literature which, was largely phenomenologic. An increasing number of transplant surgeons and physicians began to regard basic immunology as an interesting hobby, but one that was irrelevant to their practice. Meanwhile, most virologists and the majority of basic immunologists trying to understand rejection had shifted their efforts by the early 1970s from whole-animal studies to T
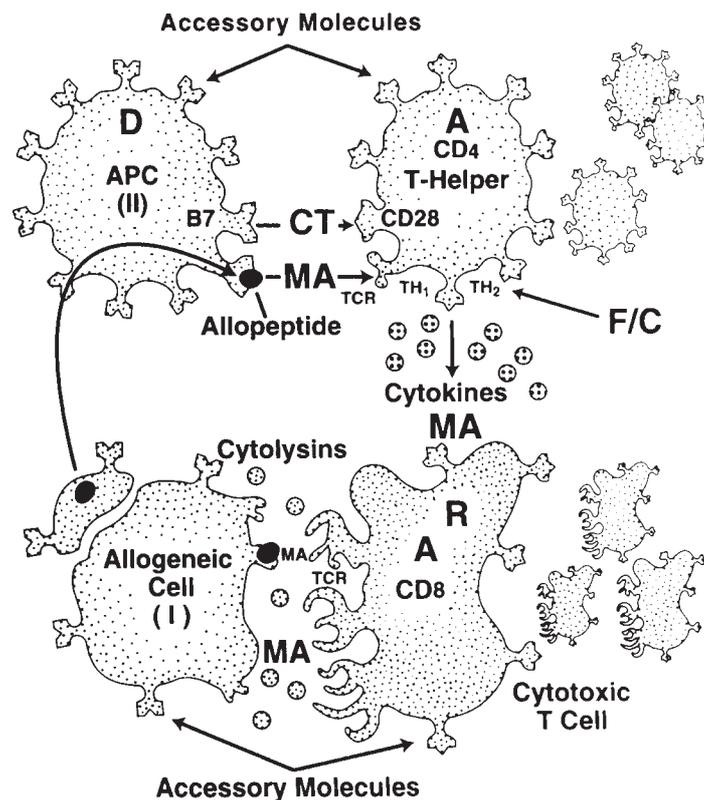
Figure 1.3. A schematic representation of the antiallograft immune response showing the cell surface proteins that participate in antigen recognition and signal transduction, the contribution of cytokines and the sites of action of the diverse agents that prolong graft survival. Antigen (allopeptide) recognition via the T cell receptor (TCR) and the role of accessory molecules can be blocked by monoclonal antibodies (MA), as can cytokine receptor expression. Deoxyspergualin (D) is believed to inhibit the function of antigen-presenting cells (APC). FK506 (F, now tacrolimus) and cyclosporine (C) inhibit cytokine gene expression within T helper (TH) cells, whereas rapamycin (R) blocks the responses of T cells to interleukin (IL)-2. By inhibiting DNA synthesis, the antimetabolite drugs (A) act later than F, C or R to block lymphocyte proliferation. CTLA4-Ig (CT) is a new agent that blocks transmission of second signal (the B7-CD28) pathway essential for T cell activation. F/C, FK506/CsA (cyclosporin A). (I) and (II) indicate major histocompatibility complex (MHC) antigens classes I and II, respectively.

lymphocyte-oriented cell culture (in vitro) systems. These labors were re-warded by a Nobel Prize (to Baruj Benacerraf, 1980) and the Lasker Prize in Basic Science of 1995, which was shared by four Americans and one Swiss (Doherty 1995; Unanue 1995; Zinkernagel 1995). The conceptual model that

emerged provided an explanation of cell-mediated immunity (Figure 1.3). In the context of the one-way paradigm, the details of the putative allogeneic reaction (rejection) included its dependence on antigen-presenting cells, the necessity for a co-stimulatory molecule(s) (the two-signal concept of self/non self discrimination), an important role of accessory molecules, and cytokine control of clonal expansion of T helper lymphocytes as well as of the cytotoxic T cells that are the agents of allograft destruction. The bewildering mass of details to which thousands of investigators had contributed over three decades (Janeway and Travers 1994) had long since overwhelmed most clinicians interested in applying the new information.

Mechanisms of drug action

However, the foregoing information allowed precise documentation of the surprising diversity of drugs with which long-term or permanent graft survival could be induced, no matter what the level of intervention in the immune reaction (Thomson and Starzl 1994). This is summarized in Figure 1.3. Deoxyspergualin was said to alter the function of antigen-presenting cells, of which dendritic cells have been conceded to be the most important. The anti metabolite drugs (including azathioprine) prevented clonal expansion of lymphocytes by inhibition of DNA synthesis. Cyclosporine and tacrolimus (FK506) disrupt signals from T cell receptor sites to the nucleus. Monoclonal antibodies (MAs) interrupt the immune reaction at the various specific targets (Figure 1.3), and rapamycin interdicts the effector events even after the secretion of the cytokine interleukin 2 (formerly called the "T cell growth factor"). The new immunosuppressive fusion protein CTLA4-Ig blocks the transmission of a "second signal" (the B7-CD28 pathway). All appear to be permissive of a natural event that became specific only by virtue of the presence of donor antigens.

The immunologic barrier: the two-way paradigm

Whole-organ transplantation

Insight into what had happened to the pioneer organ recipients was obtained in retrospect by studies, at the University of Pittsburgh nearly 30 years later, of a group of kidney and liver recipients from the earliest clinical trials at the University of Colorado, who still had good function of their original grafts. Donor leukocytes of bone marrow origin that are part of the structure of all complex grafts ("passenger leukocytes" (Snell 1957; Steinmuller 1967)) were found in 1992 to have migrated from the organs and survived ubiquitously in the patients for up to three decades (Starzl et al. 1992, 1993). Thus, organ allograft acceptance was associated with the cryptic persistence of a small

fragment of extramedullary donor marrow, including stem cells (depicted as a bone silhouette in Figure 1.1(c)). These cells had been assimilated into the overwhelmingly larger immunologic network of the host. The leukocyte movement was in both directions, with small numbers of residual donor leukocytes (microchimerism) in both the graft and host.

The discovery was instinctively understood by most patients to whom it was explained, and it had a surprisingly great emotional impact. The point was not lost that the physical intimacy of the donor to the recipient was greater than anyone had ever imagined. It was closer and more lasting than that of a gestational fetus with its mother. The woman applying lipstick in the morning was touching that unknown cadaveric male donor of 30 years ago whose live cells were everywhere in her own tissue. She was not the recipient of an organ only. The realization was usually moving, and it was invariably sobering.

Scientifically, a revision of transplantation immunology was mandated in which the immunologic confrontation following whole-organ transplantation could be seen as a bidirectional and mutually cancelling (graft-versus-host (GVH) as well as host-versus-graft (HVG)) interaction (Figure 1.4), provided the two participants in the David (donor)/Goliath (recipient) mismatch could survive the initial confrontation. Clinically, but not in several animal models, this outcome requires an umbrella of immunosuppression that protects both cell populations equally (Figure 1.1(c)).

Understanding the amplification device by which the small number of donor cells can so profoundly affect the immunologic vision of, and eventually be assimilated by, the vast recipient army against which it is arrayed is of intense scientific interest. The chimeric leukocytes are multilineage (Starzl et al. 1992, 1993; Demetris et al. 1993; Qian et al. 1994). However, the antigen-presenting dendritic cells (DCs) of Steinman and Cohn (1973; Steinman 1991) are thought to be the key to the reciprocal tolerogenic process because they can modify in both cell populations the expression of cell interaction, MHC, and adhesion molecules. All of these interactions determine how antigen signals are responded to by T cells (Steinman 1991). Evidence confirming the original observations, allowing a more complete understanding of the tolerogenic mechanisms, has been summarized recently (Starzl and Zinkernagel 1998).

Historical enigmas

With the two-way paradigm, the reason for virtually every previously unexplained experimental or clinical observation after whole-organ transplantation became either transparent, or at least susceptible to experimental inquiry (Starzl et al. 1992, 1993). It could be understood why organ grafts are inherently tolerogenic and therefore "accepted" by the recipient. With the