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The Two-Way Paradigm of Transplantation Immunology

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The events following organ transplantation require a reciprocal cell interaction which includes both the conventional host-versus-graft reaction and a graft-versus-host component. With all successful transplantation, both graft and recipient become genetic composites. Where donors were available, chimerism has been confirmed in 30-year kidney-recipient survivors, as well as in several liver and lung recipients. A majority of liver recipients have been able to acquire an immunosuppressant-free state after 10-year survival. Animal models suggest that donor-derived cells may exert a tolerogenic effect. © 1996 Academic Press, Inc.

INTRODUCTION

A transplanted organ was typically perceived, until recently, as an island in a hostile recipient sea (Fig. 1). We have referred to this hypothesis as "the one-way paradigm." A different perspective then emerged which elucidated the mechanisms responsible for organ-graft acceptance. This was based on the novel observations that donor leukocytes migrated from these transplanted organs and survived for long periods throughout the body of the recipient (1–6). This discovery necessitated the development of a new paradigm (7).

With the new paradigm, the events following renal (or other organ) transplantation were seen as a two-way cell interaction which includes both a conventional host-versus-graft reaction and a graft-versus-host component (Fig. 2). Passenger leukocytes from the graft were multilineage and of bone marrow origin, which made the placement of the organ allograft the equivalent of a mini-bone marrow transplantation. At the vast interface between the coexisting cell populations, we suggested that changes occurred in the way each cell cohort viewed the other and that these changes elicited the development of suppressor and veto cells, enhancing antibodies, and observed changes in the cytokine profile.

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Cell migration and chimerism are observed more dramatically after liver transplantation, which may explain why the liver is a more tolerogenic organ than the kidney. In fact, with all successful transplantations, regardless of which organ is involved, both graft and recipient become genetic composites, bearing the cells of both individuals. With all transplanted organs, the interaction of the two cell populations can be envisioned as a teeter-totter in which each side can abrogate the immunologic effect of the other in what we have called mutual natural immunosuppression (1, 4, 6). This reciprocal interaction effectively "blindfolds" the major histocompatibility complex (MHC) effect and explains why tissue matching, which is crucial for successful clinical bone marrow transplantation, does not accurately predict the outcome after the grafting of a whole organ.

The relationship of this concept to that of acquired tolerance, as described by Billingham et al. (8, 9), is easy to understand. Their experimental model was imbalanced because the immune system of their recipient was immature (Fig. 3). Imbalance can also be achieved genetically in the F_1 hybrid parent-to-offspring animal model and iatrogenically by recipient-cytoablation with irradiation or cytotoxic drugs. Then, as with bone marrow transplantation, if the immunocytes in the transplanted organ are sufficiently numerous, as in the intestine, one of the censoring limbs is absent and graft-versus-host disease (GVHD) follows (10). If neither the recipient nor the graft is leukocyte-depleted, however, it is possible to perform intestinal or even multivisceral transplantation without an exorbitant risk of GVHD (11, 12). Thus, the difference between bone marrow and whole-organ transplantation is merely a reflection of the therapeutic strategies used. Bone marrow transplantation (Fig. 3) is conceptually derived from the original Billingham et al. model in that one of the reaction limbs is dysfunctional or disabled. This leaves the recipient GVHD-prone and requires MHC matching for survival. Success, in this model, is called tolerance. In contrast, the treatment strategy for whole-organ transplantation leaves both cell populations intact (Fig. 2), thereby eliminating MHC matching as a prerequisite for success and largely eradicating the threat of GVHD. This is commonly referred to as "graft acceptance."
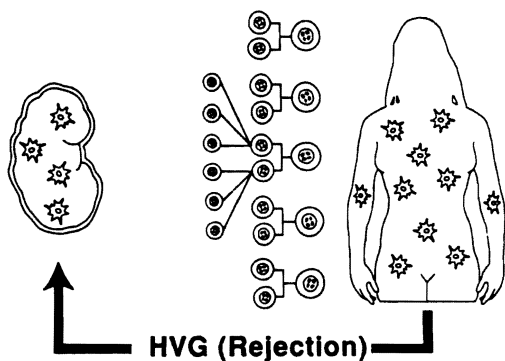
FIG. 1. One-way paradigm (organ). In nonconditioned recipients, transplanted whole organs were generally considered as defenseless islands in the hostile environment of the host's alloreactive cells. This unidirectional immune reactivity led to unrestrained HVG reaction (rejection).

FIG. 2. Two-way paradigm (organ). Bidirectional mechanism of whole-organ graft acceptance involves a GVH component mediated by the bone marrow-derived donor leukocytes from the graft and the response of the host's immune cells referred to as HVG reaction. For standard whole-organ clinical transplantation, the recipient is not preconditioned.

IMMUNOSUPPRESSIVE REGIMENS FOR WHOLE ORGANS

The two-way paradigm explains the success of the empirically derived therapeutic dogma upon which the field of whole-organ transplantation is based. The dogma calls for baseline therapy with one or two immunosuppressant drugs, secondary adjustments as needed with steroids, followed by individualized reduction to maintenance levels which are required to sustain stable graft function. Although the baseline therapeutic agents, beginning with azathioprine (subsequently cyclosporine and tacrolimus), have improved through the years, the strategy has remained the same over the past three decades.

Many aspects of immune recognition and response were subsequently clarified. These have included the role of antigen-presenting cells, the necessity for a costimulatory molecule, the role of accessory molecules, and the way cytokines controlled clonal expansion of the helper and cytotoxic T cells which mediate allograft destruction. Moreover, the diversity of agents with which long-term or permanent graft survival could be induced in experimental animals with a short course of therapy has been surprising no matter what the level of intervention in the immune process, i.e., from the most proximal inhibition by $CTLA_4Ig$ to the distal interdiction of the action of normally formed cytokines by rapamycin. These nonspecific drugs became specific only by virtue of the presence of donor antigen (13).

An Early Clue of Spontaneous Chimerism

A clue to what was happening was obtained from studies of a group of patients who underwent successful renal transplantation at the University of Colorado in 1962 and 1963. Both the donors and these pioneer recipients underwent preoperative skin tests to deter-
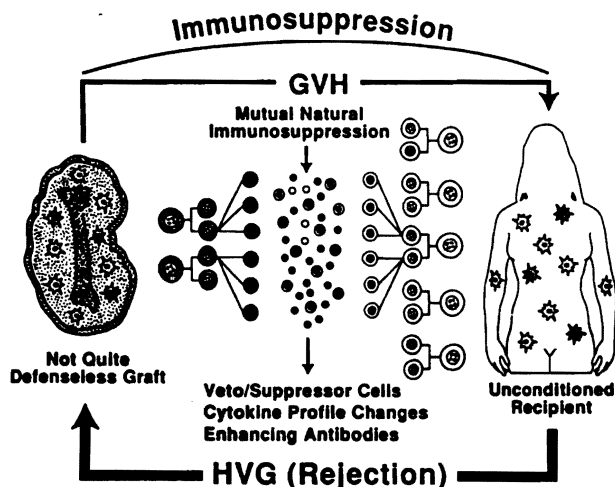
mine delayed hypersensitivity reaction to tuberculin, histoplasmin, and other antigens. Recipients who were negative to these antigens preoperatively, but whose donors were positive, acquired the positive skin tests only if the kidney transplantation was successful, but not if it failed (14, 15). The adoptive transfer of immunity became evident and implied that the donor leukocytes had survived and were functional. At the time, however, the kidney was considered to be a leukocyte-
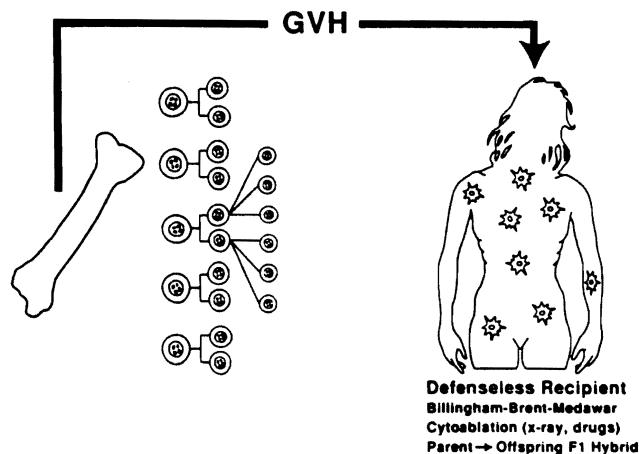

FIG. 3. One-way paradigm (bone marrow). The unidirectional paradigm of bone marrow transplantation, where the recipient is rendered defenseless by cytoablative conditioning, leads to a GVH reaction. A similar outcome was also witnessed in the Billingham *et al.* experimental model following bone marrow transplantation into immunologically incompetent (neonate) animals.

poor organ, and the implication that donor cells must have migrated was ignored.

THE DISCOVERY

In 1992, 5 of these original kidney recipients, whose grafts still functioned normally, were studied for chimerism in their blood, skin, and lymph nodes. Their donors, who still were alive, cooperated. All five of the recipients were found to be chimeras (5). The identity of the donor and recipient cells was established by either cytostaining, allowing the donor cells to be visualized in the tissues, or by polymerase chain reaction, which identified donor DNA. Markers used were donor-specific HLA alleles on chromosome 6 and the SRY region of the Y chromosome in female recipients of male organs. Using donor-specific HLA alleles as markers, chimerism was also found in all 22 liver recipients who were studied from $10\frac{1}{2}$ to 21 years after liver replacement, many with more comprehensive sampling (including biopsies of heart, bone marrow, and intestine) than that in the kidney recipients (4). Additionally, in 9 of these 22 patients who were female recipients of male livers, chimerism was also confirmed by the presence of Y chromosomes (2).

The presence of chimerism was subsequently established in recipients of thoracic organs (4, 16). In an important study, Keenan et al. (16) stratified 15 lung-transplant recipients followed 1 to 5 years into a favorable group of 8 with no bronchiolitis obliterans and 7 who had the ominous finding of chronic rejection. The patients without bronchiolitis obliterans had dense chimerism, which was positive in 8/8 lymph nodes, 7/8 skin biopsies, and 6/8 blood samples. Furthermore, lower levels of donor cell chimerism were variably demonstrable in the less favored group. Using irradiated cryopreserved splenocytes as stimulators, donor-specific nonreactivity was demonstrated in all but 1 of the densely chimeric recipients, but in only 2 of those in the less favored group.

Can Chimeric Organ Recipients Be Drug-Free?

Envisioning the engraftment of donor cells after whole-organ transplantation in the same context as that of a mini-bone marrow infusion prompted the question of whether some of these patients could have their immunosuppression stopped altogether. At the time of the liver chimerism study in early 1992, there were 43 patients who had survived for 12 to 23 years posttransplantation. Six of them had long since discontinued therapy (4), and subsequently 4 additional patients have been added to this list. Presently, 10 of the remaining 43 long-term survivors have been drug-free for 2 to 16 years. As the longest survivors in the world, this cohort of patients has taught us important lessons

about discontinuation of immunosuppression. Armed with the foregoing information, a deliberate drug weaning program was begun in other patients who were 5 to 10 years postliver transplantation (17). The results suggest that the majority of liver recipients surviving at least a decade can eventually acquire a drug-free state (18).

It is far more dangerous to attempt drug discontinuance in kidney recipients, but it is well known to be feasible in isolated cases. Our 10 patients with the most prolonged follow-up include the recipient of the longest continuously functioning kidney allograft in the world and constitute about two-thirds of those from the world experience through January 1964 (12) who are still surviving. When tested in 1992, all had donor-specific non-reactivity which was absolute in 8 and pronounced in the other 2. Of these 10 patients, all now more than 31 years posttransplantation, 5 are off immunosuppression and have been for 2, $2\frac{2}{3}$, 15, 29, and 31 years, respectively (19–21). Three of these 5 were HLA-mismatched with their donors.

DONOR LEUKOCYTE AUGMENTATION

If our hypothesis of the mechanism of graft acceptance is correct, it should be possible to safely facilitate this process by adding unaltered donor bone marrow perioperatively to the minimal dose of the so-called passenger leukocytes contained in a whole-organ allograft. Such a trial is well underway in Pittsburgh (22) and now includes 89 patients entered between December 1992 and February 1995. Donor bone marrow cells, obtained from cadaveric vertebral bodies, were not T-cell depleted or modified prior to infusion. Subsequent to organ placement, $3–5 \times 10^8$ cells/kg were infused into nonconditioned recipients who were then maintained on routine immunosuppression with tacrolimus and prednisone. No complication of bone marrow infusion was observed in any of the 89 primary whole-organ transplant recipients and their convalescence was rapid. The cumulative risk of rejection was similar in both bone marrow-augmented and nonaugmented recipients and there was no incidence of clinical GVHD. The results of this study are summarized in Table 1.

EXPERIMENTAL VERIFICATION

Phenomenology

The clinical aspect of these developments has supported the establishment of the two-way paradigm. Animal experimentation has provided much additional information. In immunosuppressed rats, Demetris et al. (23) have shown how the migratory cells begin to home to the central lymphoid organs within minutes after whole-organ transplantation where they pause for 2 or

TABLE 1

Function and Actuarial 1-Year Graft Survival of Bone Marrow-Augmented
and Nonaugmented Whole-Organ Transplant Recipients

Organs transplanted	n	Graft survival[a] (%)	Function ($\bar{x} \pm$ SD)			
			Bilirubin (mg/dl)	Creatinine (mg/dl)	Cardiac output (liters/min)	FEV$_1$ (liters)
Bone marrow-augmented						
Liver	34[b]	31 (91)	0.8 ± 0.6	—	—	—
Kidney	40[c]	39 (98)	—	1.7 ± 0.6	—	—
Heart	10	08 (80)	—	—	6.0 ± 1.7	—
Lungs	05	05 (100)	—	—	—	2.0 ± 2.0
Controls						
Liver	33	26 (79)	0.7 ± 0.3	—	—	—
Kidney	21[d]	18 (86)	—	2.2 ± 1.5	—	—
Heart	04	06 (100)	—	—	6.3 ± 3.2	—
Lung	01	0	—	—	—	—

[a] Actuarial 1-year graft survival.
[b] One type I diabetic also received pancreatic islets, not insulin-free.
[c] Nineteen type I diabetics also received either whole-organ pancreas ($n = 13$) or isolated pancreatic islets ($n = 6$); 11 patients (all recipients of pancreata) are insulin-free.
[d] four type I diabetics also received whole-organ pancreas transplants; 2 are currently off insulin.

3 weeks before becoming generalized. The migration is multilineage, following the same routes as that of syngeneic cells, i.e., B cells to B-cell follicles in lymph nodes, T cells to the T-cell areas, and the dendritic cells and macrophages to their normal areas of localization. The same events have also been documented in mice (24). A bonus in the mouse experiments, however, was that permanent survival of liver allografts occurred without the need for any treatment and across all MHC disparities. Similar to our observations in rats, the chimeric mouse liver recipients could then accept donor-strain skin and heart, but not grafts from third-party animals.

Fundamental Mechanisms

How the migratory leukocytes induce tolerance is the question currently being investigated by the cellular immunologists in Pittsburgh. The initial observations indicate that a subset of donor-derived dendritic cells may play a key role in the induction of donor-specific tolerance. The mouse liver was selected as the source of nonparenchymal leukocytes (NPCs). After discarding the hepatocytes and duct cells, about 10^7 NPCs could be obtained from each liver (25). These NPCs were cultured in GM-CSF-enriched medium which gives a selective growth advantage to leukocytes of myeloid lineage (26). After 4–5 days of culture, 2×10^6 cells with dendritic morphology and surface phenotype could be identified (25). A subpopulation of these cells

formed clusters on the bottom of the culture wells. Loosely adherent cells were harvested and further cultured and studied according to the methods described by Inaba et al. (26). Although they had the phenotype and function of precursor dendritic cells, it was difficult at first to prove their dendritic leukocyte origin because it was impossible to drive them to maturation, even with the addition of interferon-γ and tumor necrosis factor. They had poor allostimulatory function, expressed low levels of MHC class II antigen, and were avidly phagocytic (25).

This impasse was broken when the culture wells were coated with Type I collagen, thus simulating the natural microenvironment of liver where mature dendritic cells are normally known to reside. Under these conditions, the precursor cells in the wells promptly assumed the properties of mature dendritic cells, now expressed high levels of MHC class II antigen, and acquired potent allostimulatory activity. The question of whether these unusual cells would mature and express class II antigen *in vivo* was investigated by injecting the purified precursor cells from B10.BR livers into the footpad of fully allogeneic B10 mouse recipients. The cells migrated promptly to the T-cell areas of the central lymphoid organs where they were easily phenotyped as donor and shown to express high levels of class II antigen (25).

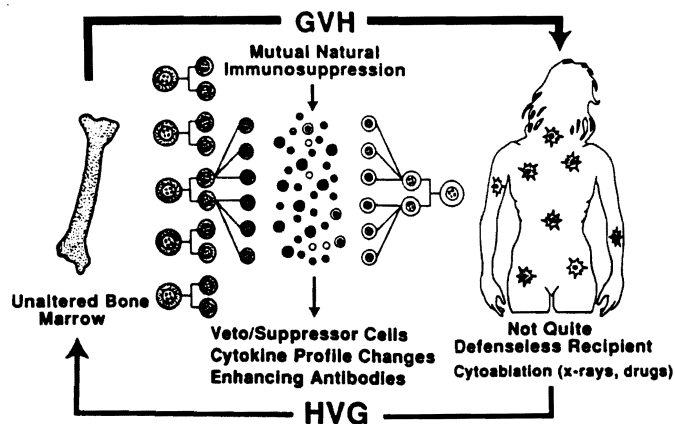It has been demonstrated recently how these putatively tolerogenic immature dendritic cells, after they

FIG. 4. Two-way paradigm (bone marrow). Bidirectional paradigm in bone marrow transplantation where cytoablation is used to precondition the recipient. However, complete elimination of the recipient leukocyte population is almost never possible (see text). Note that the result in the recipient is a mirror-image version of whole-organ transplantation.

migrate from transplanted organs, establish residence in multiple active niduses within the recipient tissues creating widespread and persistent cellular oases (27, 28). These remarkable findings suggest a mechanism for perpetuation of the migratory dendritic cells and the means by which these cells may exert a tolerogenic effect.

THE TWO-WAY PARADIGM: BONE MARROW

Although the vast gap between the fields of bone marrow and whole-organ transplantation was bridged, one clarifying detail was missing before the linkage could be considered seamless. Complete donor chimerism was long assumed to be the objective of bone marrow transplantation, and if this were true, the one-way paradigm would still apply. However, Thomas and others have recently shown that a trace population of recipient leukocytes can be detected with sensitive molecular techniques in recipients who have received bone marrow from opposite sex donors (29, 30). Thus, the veto and other accessory events at the cellular interface are the same, in principle, with bone marrow as with organ transplantation, differing only in their role in the David/Goliath mismatch (Fig. 4). With this final piece of information, the story was complete.

ACKNOWLEDGMENT

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