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Renal Transplantation

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Chimerism After Whole Organ Transplantation: An Explanation of Renal and Other Organ Acceptance

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Chapter 2 described the evolution of a generic therapeutic principle on which the clinical fields of renal and other kinds of whole organ transplantation are based. In kidney recipients who were treated with azathioprine and prednisone in 1962 and 1963, a characteristic cycle was recognized in which rejection in the first postoperative days or weeks could be reversed with increased doses of steroids.[1] Following this critical period, in successful instances it was frequently possible to reduce (Fig. 9–1) or rarely, as it turned out much later, even to stop immunosuppression.

The reproducibility of these events after renal and subsequently other kinds of organ transplantation led to the therapeutic practice that can be observed in every transplantation clinic today. It calls for daily baseline treatment with a maintenance drug (or drugs) plus trial and error intervention with the highly dose-maneuverable adrenocortical steroids to whatever level is required to maintain stable graft function. This can be accomplished without (double-drug therapy) or with the adjuvant use of antilymphoid or other agents in triple or quadruple drug cocktails. Throughout the years, this framework of treatment has accommodated increasingly potent new agents[2–9]. The most commonly used drugs are summarized in Table 9–1.

A great appeal of this empirical therapeutic approach was its simplicity and the consequent ease with which it could be taught or modified. Anyone with reasonable clinical judgment could become an expert after experience with a few patients. Of equal practical importance, the algorithmic steps were not drug specific except for the use of prednisone as a common constituent. Although the other individual drugs

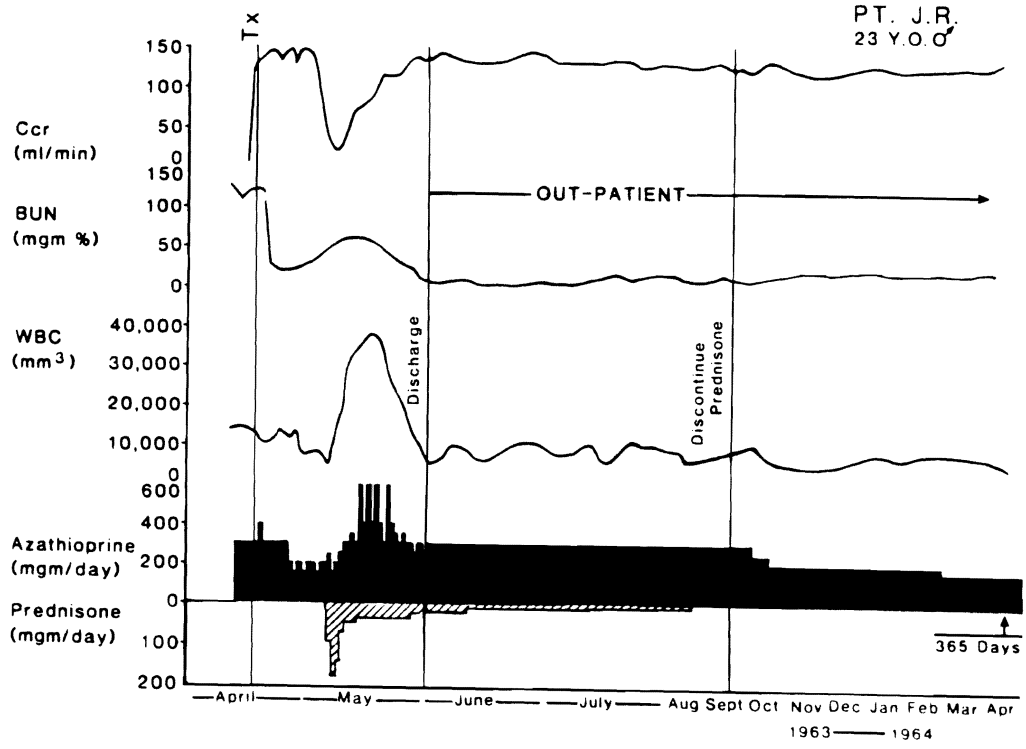

Figure 9–1. The characteristic cycle of immunologic confrontation and recovery after organ transplantation (in this case a kidney) that has dictated the use of immunosuppressive drugs for the last 30 years. After a period of good postoperative renal function, rejection 2 1/2 weeks after transplantation (Tx) was correlated with a fall in creatinine clearance (Ccr), a rise in blood urea nitrogen (BUN), and an increase in circulating white blood cells (WBC). The adverse findings were reversed with prednisone, which was later discontinued along with a reduction of maintenance azathioprine. The kidney allograft in this 23-year-old man functioned continuously from April 1963 until 1990, when the patient died of myocardial infarction. *(From Starzl TE, et al: Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation.* Immunol Today *14:326–332, 1993.)*

in these cocktails were increasingly characterized in terms of their cellular or molecular site of disruption of the alloactivated T-cell response,[10,11] the cycle of posttransplant recovery was always much the same, differing only in the ease and reliability with which it could be negotiated.

The events of convalescence suggested all along that the drugs or other treatment modalities were affecting a common pathway by facilitating some kind of natural immunologic change in the host, the graft, or both.[12,13] This concept was consistent with a number of early experimental observations in our laboratory. In 1962–1964, drug-free dogs that had survived with kidney or liver allografts for prolonged periods or permanently after a 4-month course of azathioprine had inexplicable waxing and self-resolving rejections.[12,13] In discussing the altered immunologic

TABLE 9–1 ▲ DRUG COCKTAILS WITH EMPHASIS ON DOSE MANEUVERABILITY OF ADRENAL CORTICAL STEROIDS[a]

Agents	Year Described and Reported	Place	Deficiencies	Reference
Azathioprine, steroids	1963	Denver	Suboptimal	1
Antilymphocyte globulin (ALG) as adjunct[b]	1966	Denver	Suboptimal	2
Cyclophosphamide substitute for azathioprine	1970	Denver	No advantage except for patients with azathioprine toxicity	3
Total lymphoid irradiation	1979	Palo Alto, Minneapolis	Dangerous, extensive preparation, not quickly reversible	4, 5
Cyclosporine alone or with cytotoxic drugs[c]	1978–1979	Cambridge, England	Suboptimal	6
Cyclosporine, steroids[d]	1980	Denver	Nephrotoxicity; rejection not always controlled	7
FK 506, steroids[d]	1989	Pittsburgh	Nephrotoxicity; rejection not always controlled	8

[a]Until 1966, these cocktails were developed for kidney transplantation and applied to liver transplantation. From 1966 onward, the liver increasingly became a dominant test organ.
[b]Original ALGs were polyclonal; monoclonal ALG (OKT_3) introduced in 1981.[9]
[c]Steroids were given to some of these patients, but the therapeutic objective was avoidance of steroids.
[d]Compatible with azathioprine or antilymphoid drugs as third or third and fourth agents.

environment following transplantation in 1964, it was suggested, as it turned out correctly, that:

> Prevention of rejection is not entirely dependent upon immunosuppressive agents; a key factor involves a dynamic biologic process in which the immunologic relationship between the host and graft changes rapidly. . . . Through pharmacologic or other means it may become possible to augment the biologic changes. . . . An example of this last possibility is the current research in many laboratories which is directed toward achieving enhancement by inoculating the recipient with [donor] spleen, liver or peripheral white cells. . . .[12]

This strategy, in which bone marrow was the ideal source of augmenting donor antigen, was to become the dominant theme for more than two decades in Monaco's research laboratories[14–16] and elsewhere. However, it was assumed that survival of these cells was limited to a few weeks.

It was soon learned that the postulated "dynamic biologic process" sometimes occurred without immunosuppression, as was demonstrated after liver transplanta-

tion in pigs by Garnier et al,[17] Peacock and Terblanche,[18] Calne et al[19] and us.[20] Porter,[21] who had access to tissues from the different research groups, summarized the histopathologic proof that these "non-rejecting" pig recipients were actually going through subclinical rejection and spontaneous remission. Corry et al[22] and Russell et al[23] showed the same thing after heart and kidney transplantation between certain strains of mouse recipients.

Hypotheses to explain graft acceptance under immunosuppression or sometimes without it invoked multiple mechanisms, including graft adaptation, clonal deletion, antibody enhancement, defective macrophage function, and the development of suppressor or veto cells.[12,13,24–29] However, we suggested that the proposed elements of these "concatenation" hypotheses are epiphenomena of the key events of cell migration, repopulation, and systemic chimerism[30–36] on which the ultimate acceptance of kidney and other whole organ grafts depends. In this context, the immunosuppressive drugs used clinically are facilitators that allow the bidirectional host-graft leukocyte migration that leads in successful cases to mixed chimerism in the recipient as well as the transplanted organ (Fig. 9–2). In addition to explaining how allografts are accepted, this concept can be used to improve future therapeutic strategies.

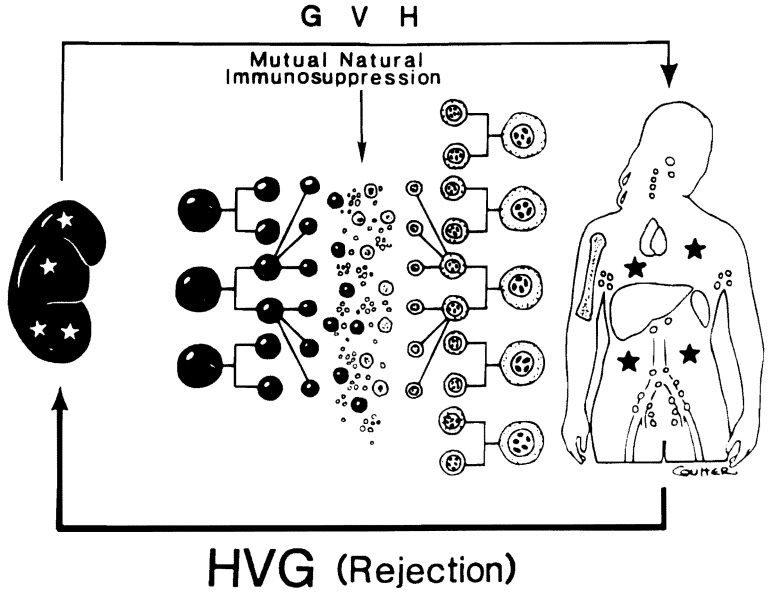

Figure 9–2. The mutual engagement of migratory immunocytes from the graft and the recipient after organ transplantation under potent pharmacologic immunosuppression. The stars represent the migrated cells. Ubiquitous chimerism was inferred from the more extensive studies in recipients of liver, kidneys, and other organs. GVH, graft versus host; HVG, host versus graft.

▲ CHIMERISM IN KIDNEY RECIPIENTS

Historical Neglected Clues

Graft Adaptation

A much discussed hypothesis in the early 1960s was that kidney grafts underwent a diminution in allogenicity after transplantation. Such a change had been noted a decade before by Woodruff in thyroid tissue during a period of privileged sanctuary in the anterior chamber of the guinea pig eye. When Woodruff's "conditioned" endocrine grafts were retransplanted to a subcutaneous location in the same recipient, they were not rejected as normally would have occurred.[37,38] The inability to explain logically how genetically proscribed transplant antigens could change their character caused this "graft adaptation" concept to lose favor. Later, it was shown in skin graft experiments that the antigenicity change was due to depletion of the donor interstitial leukocytes in the graft and their replacement with a recipient population.[39] The kidney transplants became genetic composites.[33,40]

Adoptive Transfer

The results of exhaustive skin test studies (tuberculin, histoplasmin, blastomycin, coccidioidin, mumps, *Candida*, and *Trichophyton)* in our early Colorado kidney recipients and their donors provided an additional lead[41] but one that was not considered plausible at the time. Seventy-seven percent of the skin reactions that were positive preoperatively in the donors but not in the patients crossed over to the previously negative recipients in successful cases, along with the transplanted kidney (Fig. 9–3). Wilson and Kirkpatrick, the immunology fellows who performed these studies under the supervision of David Talmage, speculated that the secondary acquisition of the positive skin tests was "caused by adoptive transfer of donor cellular immunity by leukocytes in the renal graft vasculature and hilar lymphoid tissue."[41]

The implication that cells from the graft had migrated into recipient tissues was considered untenable nearly 30 years ago because the kidney then was thought to be a leukocyte-poor organ. Instead, the transfer factor of Lawrence[42] was suggested as an alternative explanation. Ironically, several of these original patients were actually found to have chimerism nearly three decades later.[33]

Modern Technology of Chimerism Detection

The search in April–July 1992 for chimeric cells in transplanted kidney and other kinds of whole organs and in recipients was made feasible by the distinctive features of two chromosomes.[30–35] In female recipients of organs from male donors, the presence of cells with the Y chromosome in recipient tissues (or blood) was considered unequivocal evidence of systemic chimerism. Alternatively, probes were used that detected human leukocyte antigen (HLA) alleles of chromosome 6. For study of ei-

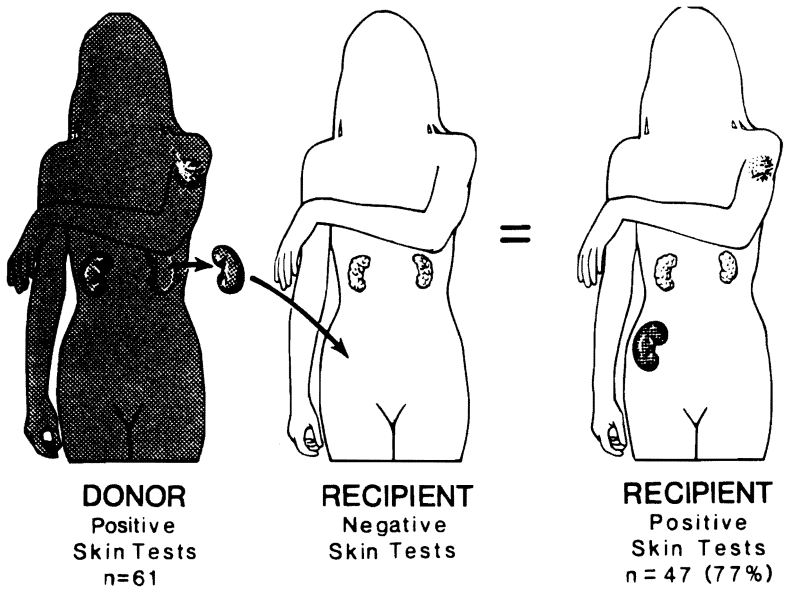

Figure 9–3. Transfer of positive skin test results from kidney donors to recipients at the University of Colorado from 1962 to 1964.[41] Although inexplicable at the time, these observations reflected adoptive transfer after cell migration, repopulation, and chimerism. *(From Starzl TE, et al: Cell migration and chimerism after whole organ transplantation: the basis of graft acceptance.* Hepatology *17:1127–1152, 1993.)*

ther the Y chromosome or chromosome 6, one or the other of two techniques, and usually both, were used. Cytostaining allows the location and morphologic characterization of phenotypically distinct donor and recipient cells. The polymerase chain reaction (PCR) is used to differentiate donor from recipient DNA.

Cytostaining

Staining for the Y probe was performed with a fluorescence method after in situ hybridization. Immunostaining for the HLA markers was with monoclonal antibodies to class I and class II antigen phenotypes present in the donor but not the recipient. Staining with irrelevant anti-HLA antibodies and omission of the primary antibody provided negative controls (See Color Insert, Part C). Tissues with known HLA antigens along with the allograft biopsy and skin sections of the recipient stained with matching anti-HLA monoclonal antibodies were used as positive specificity controls.

Polymerase Chain Reaction

In the PCR search for the Y chromosome, oligonucleotides specific for the satellite region of the Y chromosome centromere Y-A[43] and for the sex-determining region of the Y chromosome[44] were used as primers to determine the presence of male DNA

in the female recipient tissues.[31] After Southern blotting, the amplified material was hybridized to radioactively labeled Y-specific probes. PCR tests for donor- and recipient-specific HLA alleles of chromosome 6 were performed according to the reference protocol of the XIth International Histocompatibility Workshop.[45] With this protocol, preliminary generic amplification of the DRB gene is performed followed by allele-specific amplification and testing.

Direct Evidence of Chimerism

One of the five kidney recipients who were studied 27–29 years after transplantation had stopped immunosuppression 12 years earlier. The others were still taking azathioprine with or without prednisone. These five patients were selected for the chimerism study[33] in preference to other surviving kidney recipients from the 1963 era for three reasons. First, four of the five donors still were alive, allowing HLA retyping and use of their lymphocytes for studies of immunologic competence of the recipient. Second, known HLA incompatibilities between these four donors (three parents, one aunt) and their recipients allowed immunocytochemical and PCR differentiation of donor from recipient cells in the biopsy tissues, as described in the preceding section. In the fifth case, in which the donor in a father-to-daughter transplantation had died, chimerism in tissues could be determined by sex typing.

In each of four kidney recipients whose donor was alive, leukocytes with the appearance of dendritic cells that had migrated from the renal allografts were found in recipient skin and lymph node biopsies. Although they were few in number, these HLA mismatched donor cells were unmistakable with the immunostaining techniques (See Color Insert, Part B). Chimerism was confirmed with PCR in the recipient tissues of all four of these patients and in the blood of two of them (Fig. 9–4). In the fifth patient, the woman who had been given her now-deceased father's kidney 29 years previously, cells with the Y chromosome were found in her skin with fluorescent in situ hybridization studies and confirmed with PCR.

In all five cases, biopsies of the kidney showed that the vascular endothelial cells remained donor. The tubular epithelial cells, which did not express HLA antigens well, were proved to be donor with sex karyotyping. In contrast, the leukocytes departing the allograft had been replaced by similar cells from the recipient (See Color Insert, Part A). Thus in addition to showing systemic chimerism, it was established that the kidney grafts were chimeras—composed of cells with two different genomes[33] (Fig. 9–2).

Two of the four recipients of kidneys from the still-living donors had no donor-specific reactivity by mixed lymphocyte reaction (MLR), whereas the other two had greatly reduced antidonor reactivity. With cell-mediated lymphocytotoxicity testing, all four were nonresponsive to donor lymphocytes. Three of the four patients were otherwise fully immunocompetent with responsiveness to third-party lymphocytes and mitogens.[33]

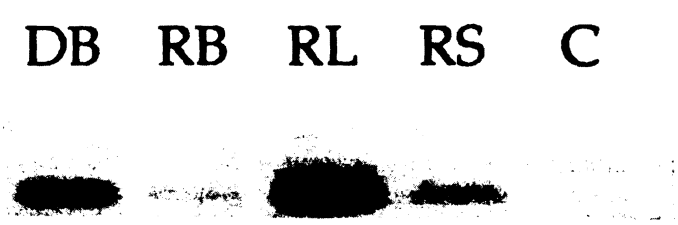

Figure 9–4. LD 90:PCR demonstration with Southern blot technique of chimerism in blood (RB), lymph node (RL), and skin (RS) of the recipient as detected with DR7 (donor) specific oligonucleotide probes. DB, donor blood DNA diluted 100 times to avoid overwhelming the other bands. *(From Starzl TE, et al: Chimerism and donor specific nonreactivity 27 to 29 years after kidney allotransplantation.* Transplantation *55:1272–1277, 1993.)*

▲ LIVER AND OTHER ORGANS

Graft Chimerism

Another underappreciated clue to cell migration was the demonstration in 1969 that the Kupffer cells and other interstitial monocytes and macrophages of a transplanted human liver were replaced within 100 days by cells of recipient phenotype.[46] A composite (chimeric) structure was shown in 1991 also to be a feature of long-surviving transplanted intestine in rats[47] and humans.[48] The suspicion that this process must be generic in all successfully engrafted organs soon was confirmed.[35]

Systemic Chimerism

Like the kidney recipients, most of a cohort of 25 liver recipients studied for chimerism were clinically well and fully immunocompetent according to conventional in vitro testing 2–22 years after transplantation under azathioprine- or cyclosporine-based immunosuppression.[35] Donor cell chimerism was found with cytochemical or PCR techniques in all 25 patients in locations that included skin, lymph nodes, heart, lungs, spleen, intestine, kidneys, bone marrow, and thymus. Chimeric cells were present in larger numbers at any given site than in the contemporaneously studied long-surviving kidney recipients.

▲ CELL TRAFFIC AND SITES OF DONOR-RECIPIENT IMMUNOLOGIC INTERACTIONS

The early events leading to the chimeric state have been studied in rats[36] and mice,[19] including the pathways of passenger leukocyte dissemination. Within minutes or hours, these cells leave the graft and home to the central lymphoid organs (spleen, lymph nodes, thymus, and probably bone marrow), and are destroyed by rejection in most animals except mice. However, with temporary immunosuppression in rats (2 weeks of daily FK 506), these mononuclear cells pause for about 2 weeks in the

lymphoid organs but then break out and move secondarily to all recipient tissues[36] (Fig. 9–5). Rat liver recipients treated this way (LEW to ACI) survive indefinitely without further treatment and retain their graft and systemic chimerism.

Interestingly, cell migration and chimerism, with permanent survival of the engrafted livers, occur without any immunosuppression in some rat strain combinations (Brown Norway to Lewis for example), and occur without treatment in virtually all mouse strain combinations. The liver recipients in either species can accept skin, kidney, or a heart from the original donor strain but no other (donor-specific nonreactivity).[36,49] This kind of evidence has indicated that the heavy endowment of the liver with potentially migratory white cells is the basis for the well-known but previously inexplicable phenomenon of "hepatic tolerogenicity." This term has been used to define the ability of the liver to induce its own acceptance or that of companion organs more readily than other kinds of allografts and in some experimental models without immunosuppression.[13,17–20,47,50–53]

By the end of 1992, it was appreciated that all whole organs undergo the same process of potential tolerance induction as the kidney and liver,[34,35] the dynamics being particularly easy to study with the leukocyte-rich intestine.[54,55] The same

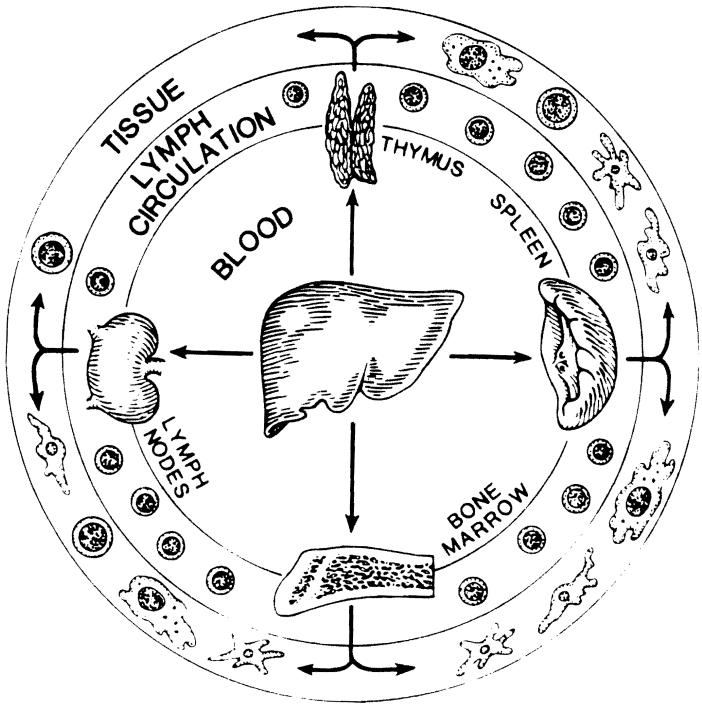

Figure 9–5. The dissemination of passenger leukocytes from the graft (rat liver in these experiments) to the central lymphoid organs and then ubiquitously after a brief pause. The events are similar to those after successful bone marrow transplantation.[36]

kind of traffic in the context of alloactivation and rejection rather than tolerization had been well worked out earlier with the so-called lymphoid-poor organs, including the kidney. Studies with untreated animals have shown that the alloreaction starts in two general sites, peripherally in the graft and centrally in the recipient lymphoid tissues.

In a remarkably complete study in 1981 of untreated rat kidney recipients, Nemlander et al[56] demonstrated the same kind of leukocyte migration as we noted with rat liver allografts 12 years later.[36] If Nemlander et al had given one or two doses of cyclosporine to their recipient animals (an "easy" strain combination) and had followed them further, they almost certainly would have uncovered the events of cell migration and repopulation that awaited another 12 years for exposure with the liver.[36] Larsen et al[57] found that donor dendritic cells from heterotopic cardiac allografts are released into the circulation, where they eventually home into the T-cell areas of the recipient spleen. In the spleen, the donor cells initiate proliferation of recipient cells and vice versa.[56–60] This reaction might be considered to be an in vivo MLR and epitomizes central allosensitization.

Allosensitization (and tolerization) presumably also occur within the graft. Forbes et al[59] were the first to show that clustering of recipient lymphocytes occurs around donor dendritic cells in the interstitium of cardiac grafts within a few days after transplantation. The recipient lymphoid cells undergo blastogenesis and proliferate within these clusters. We have described analogous events in the rejection of rat livers.[58]

In human recipients of kidney grafts under cyclosporine-prednisone immunosuppression, Hayry and Willebrand[61,62] noted what appeared to be a bidirectional MLR in needle aspiration biopsies. When studied with the *Staphylococcus aureus* assay and alloantibodies to nonshared donor and recipient allelic specificities, most of the collected blast cells in some cases were derived from the donor or else the response was split, "resembling a bidirectional mixed lymphocyte reaction in vitro."[61]

▲ FUNCTIONAL CONSEQUENCES OF MICROCHIMERISM

Cause and Effect?

Questions have continued to be raised whether the low-level chimerism found in our long-surviving patients and experimental animals[30–35] was an irrelevant histopathologic curiosity or a condition with immunologic significance. This has been surprising in view of Russell's elegant formal proof of the association of chimerism with acquired tolerance as well as runt disease.[63] The skepticism arose because the chimeric donor cells were so sparse in recipient tissues of the patients and the animals of our studies. The term *microchimerism* (as opposed to *macrochimerism*) to describe a small proportion of chimeric cells in recipient blood was introduced by Liegeois et al[64] in 1974 in a report of experiments with the mouse model used earlier by Monaco et al.[65] Far from being insignificant, there is much evidence that the cumulative effect of these microchimeric cells is substantial, especially after liver transplantation, in which they are most easily demonstrated.

Metabolic Implications

The small population of chimeric cells has been shown to affect total body metabolism in patients treated with liver transplantation for the enzyme deficiencies of type 4 glycogen storage disease and Gaucher's disease in which the consequences of the missing enzymes are widespread storage of amylopectin and B glucocerebroside, respectively.[32] These disorders were thought to be treatable only by bone marrow transplantation because the enzyme deficiency is of all cells. Two to 8 years after liver replacement, however, there was a dramatic resorption of both kinds of storage material from host tissues. As an explanation for the metabolic amelioration, chimeric donor cells were found ubiquitously in recipient tissues, including heart, lymph nodes, bone marrow, intestine, and skin. There apparently had been a coculture effect of a small number of chimeric donor cells on the contiguous overwhelming numbers of enzyme-deficient recipient cells.[32]

If, as we have concluded,[30–36] chimerism differing only in degree with different kinds of organ allografts is an integral feature of all successful engraftments, any such procedure can result in such a gain of systemic metabolic function, as suggested for different reasons by Groth et al[66] in 1979. Although the chimerism in our long-surviving kidney recipients was only one fifth or less of that in the liver recipients, this may have been enough to explain an enigmatic observation made in 1972 by Desnick et al.[67] After successful renal allotransplantation, this group at the University of Minnesota described recovery of function in the patient's diseased kidneys, which had been shut down by the glycosphingolipid accumulations of Fabry's storage disease. A reasonable hypothesis is that donor chimeric cells had settled there (Fig. 9–6).

The Immunologic Interface

The foregoing metabolic observations raise important questions about potential analogous cell-to-cell effects of other molecules directly involved in immunologic processes, including those subserving tolerance induction. Much needs to be learned about how the chimeric donor cells, many of which resemble dendritic cells, are perpetuated for as long as three decades after transplantation. The dendritic and other leukocytes could be spawned by small numbers of pluripotent progenitor cells coming from the allograft interstitium. Dendritic cell precursors have been grown from mouse blood, bone marrow, or whole organs by means of granulocyte-macrophage colony-stimulating factor (GM-CSF)–enriched media.[68] Alternatively, tissue leukocytes in the organ may not have reached terminal differentiation as previously assumed. We have suggested that subsequent survival and renewal of these cells depends on chronic mutual stimulation of the donor and recipient cell populations[31,36] in a process of tolerization that has many of the cellular characteristics associated with immunity.[69] However, there has been no direct way to test how this small population of donor dendritic and other cells can have an impact far exceeding its numbers.

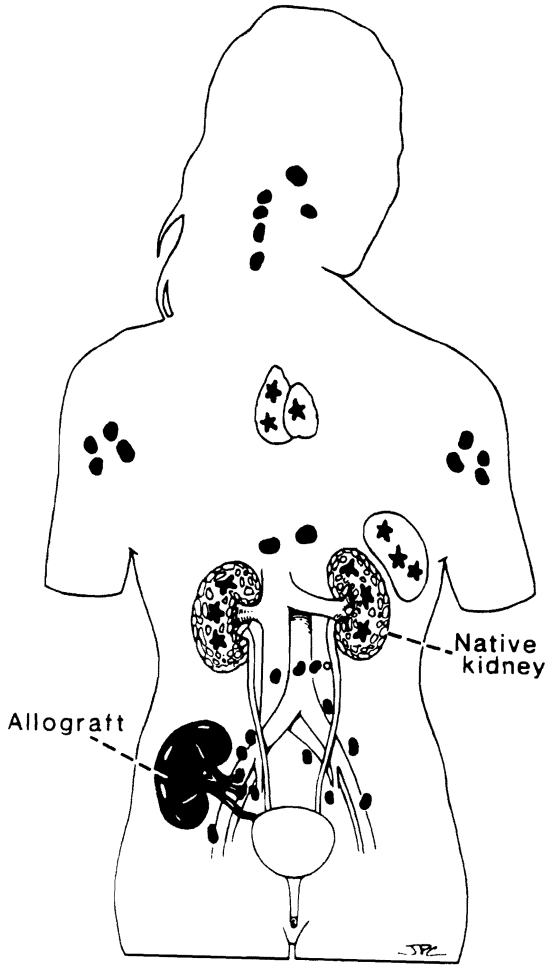

Figure 9–6. Explanation for 1972 observation by Desnick et al[67] of recovery of native kidneys after successful renal transplantation for Fabry disease. The migration of passenger leukocytes from the allograft to the host kidneys and elsewhere is shown with black stars.

Changed Host and Graft Interactions

There are many indications that the coexisting immunocyte populations in successful transplantations come to regard each other in a revised light. The evidence on one hand is the fading of the threat of clinical rejection concomitant with development of decreasing donor-specific reactivity in spite of lightened treatment and on the other the waning specter of graft versus host disease (GVHD). It is quite natural to expect that the threat of GVHD would decline at the same time as the threat of rejection, because both of the cell populations are receiving the same protective immunosuppression in an organ recipient. Appreciation of the intercellular relation-

ship and the need *not* to alter it by ablating one side or other was the crucial conceptual advance allowing the successful engraftment of leukocyte-rich organs such as the liver, intestine, both together, or all of the intra-abdominal organs (multivisceral transplantation).[70] Once it was proved that low-level mixed allogenic chimerism invariably was found after successful whole organ transplantation, the reason seemed clear why GVHD was not common among recipients of liver, intestinal, or multivisceral transplants. The explanation was that mixed chimerism was being produced in the same way as in the classic GVHD-free mouse bone marrow mixed chimerism models of Slavin et al[71] and Ildstad and Sachs.[72]

The Critical Dendritic Cell

Generation of an immune response that leads under normal circumstances to graft destruction or GVHD requires effective antigen presentation and recognition in the initial phase followed by a second costimulatory signal and the response of T cells to the combined signal.[73] Both of these signals are normally delivered to T cells by professional antigen-presenting cells (APC). Of these APCs, the dendritic cell[74,75] (the most prominent chimeric cell according to morphologic criteria) is critical because it can modify the expression of cell interaction, major histocompatibility (MHC) locus, and adhesion molecules, all of which determine how antigen signals are heeded by T cells.[76] Thus the dendritic leukocyte is the prime candidate in this tolerogenicity scenario, even though other lineages may also be essential for the successful outcome of such an immunologic transaction.

Impact on Tissue Matching

In the directions of both host versus graft (rejection) and graft versus host, cellular interactions resulting in "mutual natural immunosuppression" are envisioned as occurring on a sliding scale with each further level of histoincompatibility (Fig. 9–7). With protection by modern-day immunosuppression, the alloreactions caused by the mutual cell engagement usually can be mitigated enough to allow the tolerogenic changes to occur and a rapprochement to be reached between the coexisting immunocytes. The anticipated histocompatibility influence on both rejection and the severity of GVHD are then expected to dwindle. We have postulated that this explains the poor correlation of HLA matching with outcome after cadaveric transplantation of whole organs, including the kidney.[30,34,35]

▲ RELATION OF CELL MIGRATION TO TOLERANCE

The inadequacy of thymic clonal deletion to explain acquired transplantation tolerance has been emphasized in recent reviews.[77] Although a discussion of the meaning

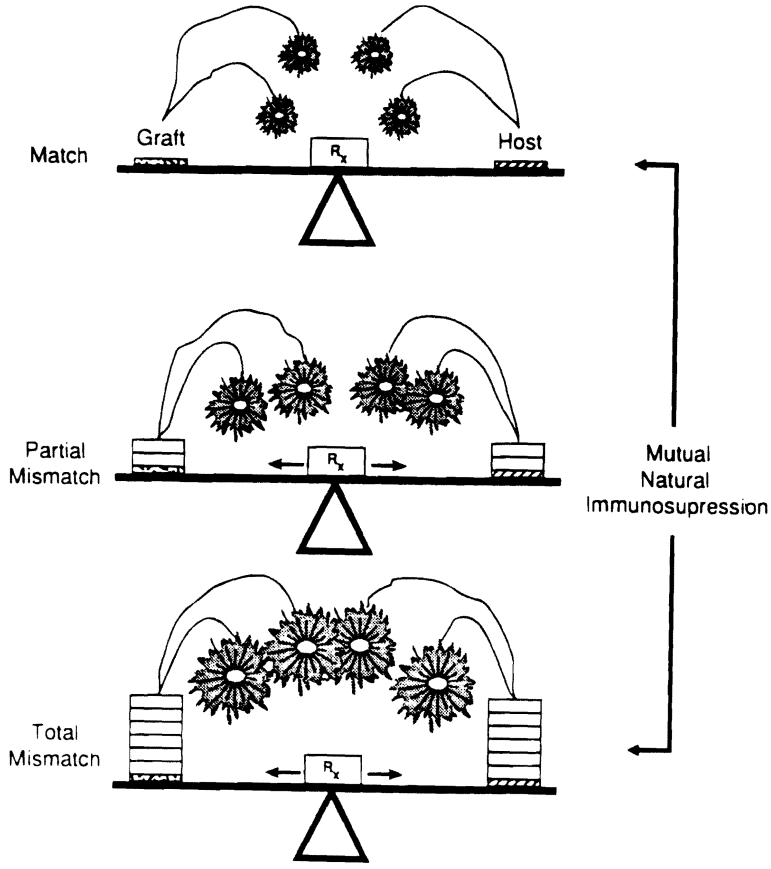

Figure 9–7. The donor-recipient leukocyte interaction is a buffer against rejection on one hand and graft versus host disease on the other. Veto and suppressor cells are postulated to be the result of the interaction shown at the cell population interface. R_x, iatrogenic immunosuppression. *(From Starzl TE, et al: Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation.* Immunol Today *14:326-332, 1993.)*

of tolerance is beyond our intention, it should be noted that all of the hypotheses to explain clonal "silencing," including peripheral clonal deletion and anergy, could mesh with the discovery of the enduring graft-host intimacy that is inherent with chimerism.

The evidence of long-term vitality and turnover of donor leukocytes in recipient tissues is particularly supportive of the opinions of Coutinho et al[69,78] and Cohen,[79] who defined *acquired tolerance* as a high (not anergic) level of sustained immune activity in networks. These networks presumably interact in a more complex way than the idiotype systems originally postulated by Jerne.[80] Suppressor or veto cells or both could be epiphenomena of this kind of activity.

Such a hypothesis would explain why cell migration and chimerism are a common mechanism of donor-specific nonreactivity no matter what the site of action of immunosuppressive drugs or in some experimental models without drugs. It has

been proposed from observations in drug-free models of tolerance induction that T-cell receptor occupancy leads to production of negative regulators of interleukin-2 (IL-2) production (anergy proteins).[73,81] According to this hypothesis, during the course of a normal T-cell response (to alloantigens) these negative regulators of IL-2 production have an inconsequential effect because they are diluted out by vigorous cell replication driven by IL-2. However, these negative regulators would accumulate with consequent anergy if clonal expansion were prevented at any level (Fig. 9–8), for instance, by the absence of a costimulatory signal in drug-free models.[73]

The same effect could be induced iatrogenically by pharmacologic interdiction of IL-2 gene transcription (cyclosporine and FK 506) or administration of a DNA syn-

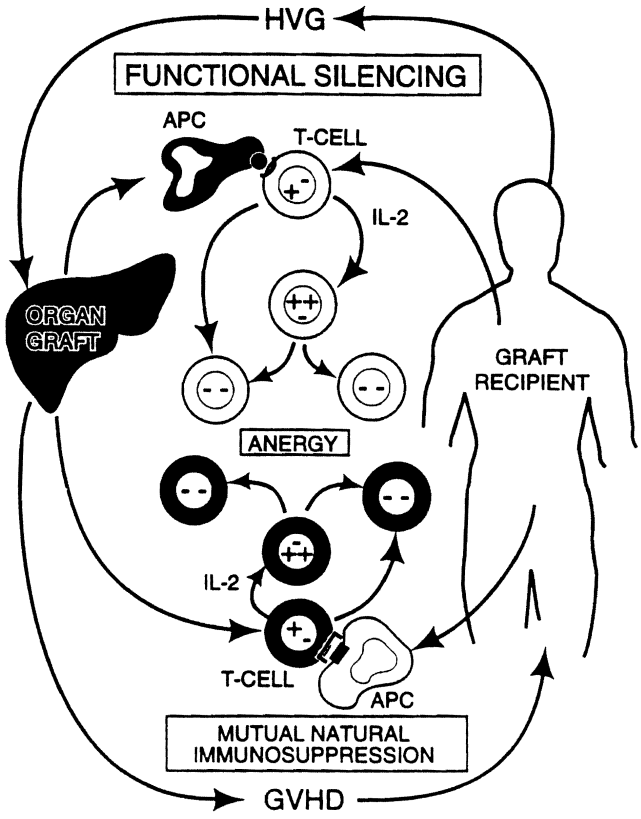

Figure 9–8. Model of dendritic cell (APC)–T_{H1}–cell interaction shows the production within the nucleus of positive (+) and negative (−) regulators (anergy proteins) of interleukin-2 (IL-2) gene transcription. In this model, anergy relates only to the IL-2 gene, and other cytokines (eg, interferon-γ) may be secreted, albeit at suboptimal levels. In the absence of persistent costimulatory signals (or under the umbrella of immunosuppressive drugs), cell division does not proceed, and negative nuclear regulators accumulate, resulting in T-cell anergy. In addition to the action of immunosuppressive agents, chronic antigen stimulation is also envisioned as promoting anergy. In some instances, tolerance can be broken, as by administration of exogenous IL-2. HVG, host versus graft response (allograft rejection); GVHD, graft versus host disease. (*From Thomson AW, Starzl TE (eds): Immunosuppressive Drugs: London, Edward Arnold, 1993.*)

thesis inhibitor (azathioprine, cyclophosphamide, and numerous others). The use of non–T-cell depleting monoclonal antibodies, such as those directed against the cell surface CD4 antigen or monoclonal antibodies against adhesion molecules, including ICAM-1 and LFA-1,[82] can also be envisioned.

However it occurs, the reciprocal educational process of donor and recipient leukocytes and its perpetuation resembles in either the direction of rejection or GVHD (Fig. 9–8) the "infectious" transplantation tolerance that can be passed on to naive lymphocytes and be self sustaining in some circumstances.[83] It is postulated that in fully successful transplantations, the mini-immune system of the graft is incorporated into the existing recipient immunologic network,[34,36] compatible with the hypothesis of Coutinho.[78]

▲ UNSTABLE MIXED CHIMERISM

Cell migration conceptually reunites bone marrow transplantation with transplantation of whole organs. Far from involving different mechanisms for successful engraftment, we believe that these two seemingly disparate fields merely reflect contrasting treatment dogmas. For bone marrow transplantation, the conventional treatment strategy of recipient cytoablation eliminates mutual immunocyte engagement and thus necessitates heavy reliance on HLA matching to prevent GVHD in the unbalanced system. The treatment for solid organ transplantation encourages, or at least allows, these consequences of mutual cell engagement, thereby liberating the patient from the restrictions of HLA matching and an overwhelming threat of GVHD.

Failure of clinical organ transplantation implies the inability to achieve mixed allogeneic chimerism despite the best available immunosuppression—almost invariably after kidney transplantation because of an imbalance that leads to rejection. However, because an incipient GVH reaction is a necessary condition for success (this is our fundamental premise), clinical GVHD is a possibility after every transplantation, although the threat varies from organ to organ. We now know that about 5% of all liver recipients experience a bout of clinical GVHD, which in the past usually was attributed to an allergic skin reaction.[35] The incidence after kidney transplantation is not known because it is not considered.

▲ CLINICAL TRIALS OF BONE MARROW AUGMENTATION

The concept developed in this chapter is that the content (and perhaps the specific lineages) of migratory cells that are particularly numerous in the liver confer potential immunologic advantages. These passenger leukocytes, which are of bone marrow origin, have been considered an immunologic liability for transplantation.[84] Under the appropriate conditions, however, they may be tolerogenic, as exemplified by the liver. A corollary, therefore, is that organs, such as the kidney and heart, with a smaller leukocyte component must have similar inherent, although less tolerogenic, potential. The frequently advanced strategy of intravenous infusion of donor

bone marrow, donor blood (donor-specific transfusion), or other hematolymphoid cells at the same time or soon after transplantation of whole organs[14–16,64,65,71,72,85–87] is merely an augmentation of the normal posttransplant cell migration. To mimic the natural process, these cells should be given perioperatively, not before or after the operation, as usually has been done with the so-called Monaco model.

This strategy is currently under clinical evaluation, using bone marrow for the leukocyte augmentation. Since December 1992, over 70 patients with end-stage renal disease have received a simultaneous kidney–bone marrow allograft from the same cadaveric donor at our center. Additional renal transplant patients with diabetes (Type I) also have received a pancreas or pancreatic islets from their kidney–bone marrow donor. As a precaution, autologous bone marrow was harvested from the first 10 recipients immediately before kidney transplantation and cryopreserved for potential future use in case of GVHD.[88]

The donor bone marrow was obtained from vertebral bodies at the end of multiorgan procurement: 3–5 $\times$ 10^8 untreated bone marrow cells/kg were administered by means of intravenous infusion immediately after the kidney transplant. The patients with diabetes received the pancreas or pancreatic islets; the latter were infused intraportally. Intravenous bone marrow infusion was performed at the conclusion of the procedure. All patients were treated with FK 506 and steroids. Chimerism was assessed in the recipients by flow cytometry, PCR, and immunostaining from peripheral blood lymphocytes. Immunologic monitoring was by MLR and cell-mediated lympholysis.

Detectable levels of donor cells were present in the peripheral blood lymphocytes of 90% of the recipients. Donor cells were also detected in two patients who underwent lymph node biopsy 2 and 4 months after transplantation. One-year actuarial patient and graft survival have been 100% and 98%, respectively.

None of the recipients manifested GVHD. The only example we observed of such a potential bone marrow–related complication was in a patient who underwent 550 cGy total lymphoid irradiation before a combined liver–bone marrow allograft.[88] The unwise decision to produce an iatrogenic imbalance in the donor-recipient interface by means of total lymphoid irradiation was based on the dogma that "making space" would facilitate engraftment of the marrow. This concept has been eroded with direct experimentation.[89,90]

Further evaluation of passenger leukocyte augmentation (with bone marrow and other leukocyte sources) will be of particular interest for pancreatic islet transplantation. This means of treating diabetes has consistently failed because of the high rate of rejection.[91] If it is possible to increase islet allograft survival with minimum ultimate immunosuppression by concomitant donor bone marrow infusion, this will be an approach opposite to the numerous attempts to reduce islet immunogenicity by selective destruction of the bone marrow–derived antigen presenting cells that are normally contained in islet preparations.[92] Then donor bone marrow would become a dose-maneuverable component of any organ or cellular transplantation for facilitation of graft acceptance and induction of donor-specific nonreactivity.

As discussed earlier, liver transplantation can be envisioned as a mini–bone marrow engraftment. Among a group of 44 human liver recipients who had survived 11–23 years, six (14%) had stopped all immunosuppression 1–11 years postopera-

tively with subsequent, clinically stable, drug-free intervals of 5–13 years. Another 15 patients with shorter follow-up periods all were drug-free and in stable condition.[35,93] The most extreme example of early successful drug discontinuation in a liver recipient was after 6 months, with a subsequent follow-up period of 3 years. A trial of drug weaning has been started in liver recipients with a rejection-free course exceeding 5 years. Liver graft rejection (if it occurs) can be so effectively treated with FK 506[8] that the benefits of discontinuation of drugs appear to outweigh the risks for selected patients.

The liver experience in which the hepatic graft brings its own intrinsic quota of bone marrow–derived leukocytes should provide insight about what can be achieved with use of bone marrow augmentation for recipients of other organs or cells such as the kidney or pancreatic islets. Even if this strategy to obtain drug independence is successful, there is no way to know when a drug-free state has arrived. It seems clear that if the clinical experience with the tolerogenic livers is taken seriously, renal or heart transplantation with bone marrow augmentation also will require protracted immunosuppression before this treatment can be stopped altogether and then only with precautions. Such conclusions also have been reached by Barber et al[86] using delayed supplementary bone marrow for cadaveric kidney transplantation as advocated by Monaco.

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Although this chapter was written in the spring and early summer of 1993, further work in our laboratories and elsewhere have strengthened the hypothesis it contains. The work and progress of the last 3 years has been summarized in the following three recent reviews:

Thomson AW, Lu L, Murase N, Demetris AJ, Rao AS, Starzl TE: Microchimerism, dendritic cell progenitors and transplantation tolerance. *Stem Cells* 13:622–639, 1995.

Starzl TE, Demetris AJ: Transplantation milestones: Viewed with one- and two-way paradigms of tolerance. *JAMA* 273:876–879, 1995.

Starzl TE, Demetris AJ, Murase N, Trucco M, Thomson AW, Rao AS: The lost chord: Microchimerism and allograft survival. *Immunol Today*, 17(12):577–84, 1996.

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Kidney allograft. (A, × 450) and recipient lymph node (B, × 100) stained with anti-HLA B 7,40 mAb using an avidin-biotin complex method. The rust-colored endothelial cells in the kidney and scattered cells in the lymph node are of donor origin. (C, × 100) The negative control in which anti-A2. 28 (irrelevant class-matched antibody) was used as the primary antibody. (From Starzl TE, et al: Chimerism and donor specific nonreactivity 27 to 29 years after kidney allotransplantation. *Transplantation* 55:1272–1277, 1993.)

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