Transplantation of the Liver

Ronald W. Busuttil, M.D., Ph.D.
Professor of Surgery
Director, Liver Transplantation Program
University of California, Los Angeles
School of Medicine
Los Angeles, California

Goran B. Klintmalm, M.D., Ph.D.
Professor of Surgery
Director of Transplantation
Department of Surgery
Baylor University Medical Center
Dallas, Texas
Cell Migration, Chimerism, and Graft Acceptance, With Particular Reference to the Liver

Thomas E. Starzl, MD, PhD, Anthony J. Demetris, MD, Massimo Trucco, MD, Noriko Murase, MD, Camillo Ricordi, MD

Improvements in the prevention or control of rejection of the kidney and liver are largely interchangeable and then applicable with little modification to thoracic and other organs (see Chapter 50). The mechanism by which antirejection treatment permits any of these grafts to be accepted, however, has been an immunological enigma. We have proposed that the exchange of migratory leukocytes between the transplant and the recipient with consequent long-term cellular chimerism in both is the basis for acceptance of all whole organ allografts and xenografts (Fig 27-1). Although such chimerism was first demonstrated as recently as mid-1992, the observations have increased our insight into transplantation immunology and have encouraged the development of alternative therapeutic strategies.

LOCAL (GRAFT) CHIMERISM

The Liver

The first unequivocal evidence that whole organ grafts in humans become genetic composites (local chimerism) was obtained in 1969 with karyotyping techniques in female recipients of livers obtained from male cadaveric donors. These studies were done by K.A. Porter of St. Mary’s Hospital and Medical School in London on allografts from the first long-surviving patients in the University of Colorado liver series. Postoperatively, both the hepatocytes and the endothelium of the major blood vessels of the grafts retained their donor sex, whereas the entire macrophage system, including the Kupffer cells, was replaced with recipient female cells (identified by their characteristic Barr bodies) within 100 days (Fig 27-2. middle). The assumption for many years was that the composite (chimeric) structure of the hepatic allograft was a special feature of this organ.

Other Organs

The illusion of the uniqueness of the hepatic graft was dispelled in 1991 with the demonstration, first in rat models and then in human intestinal grafts, that the epithelium and vascular endothelium remained donor specific, whereas lymphoid, dendritic, and other leukocytes were replaced by recipient cells in the lamina propria, Peyser’s patches, and mesenteric nodes. As with the liver, the chimerism of the intestinal graft was made easy to demonstrate because of its large constituency of lymphoreticular cells.

The suspicion that this transformation must be occurring with other kinds of whole organ grafts was promptly confirmed with the kidney and with the thoracic organs.

DISCOVERY OF SYSTEMIC CHIMERISM

Circumstantial Evidence

Twenty-two years passed between the discovery of the chimerism of the transplanted liver and that of the intestine; during this period, several clinical reports noted the local chimerism in long-surviving human kidney allografts and in subhuman primates. Throughout this time, the tacit or explicit assumption was that the cells departing the liver or kidney had been destroyed. With the evidence that the local chimeric changes occurred in all kinds of grafts, the burning question became what happened to the donor cells that had disappeared. Much earlier circumstantial evidence existed that donor leukocytes migrating from the engrafted organs were still present in the body; however, this evidence had been largely ignored, forgotten, or misinterpreted.

Kidney Transplantation. The results of exhaustive skin test studies (tuberculin, histoplasmin, blastomycin, and
cell migration, chimerism, and graft acceptance, with particular reference to the liver

Figure 27-1. The mutual exchange of migratory immunocytes from the graft and the recipient after organ transplantation under potent pharmacological immunosuppression. GVH = graft versus host; HVG = host versus graft. (Reprinted from Immunology Today, 14, Starzl TE, Demetris AJ, Murase N, et al, Donor cell chimerism permitted by immunosuppressive drugs: A new view of organ transplantation, 326–332, Copyright 1993, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK.)

coccidioidin; and tests for mumps, candidiasis, and trichophytosis) in our early Colorado kidney recipients and their donors provided a clue to the fate of the replaced donor cells, but one that was considered implausible at the time. Preoperatively, skin test reactions of recipients were negative and those of donors were positive; postoperatively, 77% of the recipients had positive skin test results (Fig 27-3). When this did not occur (in the other 23%), it meant that the kidney transplantation had failed. It was speculated that the secondary acquisition of the positive skin test results was "caused by adoptive transfer of donor cellular immunity by leukocytes in the renal graft vasculature and hilar lymphoid tissue." However, the implication that cells from the graft had migrated to recipient tissues was considered untenable nearly 30 years ago because the kidney was then thought to be a leukocyte-poor organ. Consequently, the flash of potential insight faded, in spite of the fact that the "transfer factor" of Lawrence was suggested as a possible amplification device for the ostensibly small number of donor cells.

Liver Transplantation. Clues to the development of systemic chimerism were also ignored after liver replacement and were correctly interpreted only later. When the composite structure of liver allografts was discovered in 1969, the continued viable presence outside the liver of the de-
parted donor cells was signaled by the appearance and maintenance in the recipient blood of new donor-specific immunoglobulin (Gm) types.\textsuperscript{17, 30} Fifteen years later, emigrant cells from the graft were proposed to be the source of anti–red blood cell antibodies that developed in patients who were the recipients of livers from donors with ABO nonidentity.\textsuperscript{31} Subsequently, Davies et al\textsuperscript{32} showed the appearance in liver recipients of new circulating, donor-specific class I antigens, and they attributed these antigens to synthesis by the graft hepatocytes rather than to migratory passenger leukocytes. However, because these molecules were known to come from bone marrow–derived macrophages, dendritic cells, or both,\textsuperscript{33-35} they presumably had the same donor origin as the additional Gm types and anti–red blood cell antibodies after the completion of cell migration to extrahepatic sites.

Direct Evidence of Chimerism

In 1993, Murase et al\textsuperscript{36,37} showed with flow cytometry that the stromal leukocytes leaving the small bowel allografts in rat recipients treated with a short course of FK506 migrated in large numbers through vascular routes to widely distributed host lymphoid tissues. This created a state of systemic mixed allogeneic chimerism for at least 45 days that was free of lethal or even detectable graft-versus-host disease (GVHD), except in strain combinations in which the Brown Norway (BN) rat was the recipient.\textsuperscript{36-38} Because the circulating cells detectable with flow cytometry were not studied for more than 45 days and because their number diminished throughout this time, the ultimate fate of these cells remained in doubt until all of 1991.

The next, and as it turned out decisive, step was to look for evidence of chimerism in human recipients of kidneys, livers, and other organs whose successful transplantation had been performed many months or years earlier. This required more sophisticated and sensitive techniques than had been previously used for this purpose. The search from April through July 1992 for chimeric cells in the tissues of these whole organ recipients was made feasible by the distinctive features of two chromosomes. In females who had been given an organ from a male donor, the presence in recipient tissues (or blood) of cells with the Y chromosome was considered unequivocal evidence of systemic chimerism. Alternatively, probes were used that detected human leukocyte antigen (HLA) alleles of chromosome 6. For study of either the Y chromosome or chromosome 6, one or the other of two technologies, and usually both, were exploited.\textsuperscript{9-14}

One of these technologies was cytostaining, which allows the location and morphological characterization of phenotypically distinct donor and recipient cells. The cytostaining for the Y probe was accomplished by a fluorescence method after in situ hybridization (Fig 27–4). The immunostaining for the HLA markers was done with indirect immunofluorescence, an avidin-biotin-complex immunoperoxidase method, or both and used monoclonal antibodies to class I and class II antigen phenotypes present in the donor but not the recipient.

The other technology was polymerase chain reaction, which distinguishes donor from recipient DNA.\textsuperscript{9-14} In the polymerase chain reaction search for the Y chromosome, oligonucleotides specific for the satellite region of the Y chromosome centromere Y-A and for the sex-determining region of the Y chromosome were used as primers to determine the presence of male DNA in the female recipient tissues. The polymerase chain reaction tests for donor- and recipient-specific HLA alleles of chromosome 6 were performed by preliminary generic amplification of the
DRB gene, followed by allele-specific amplification and testing (Fig 27-5).

Human Kidney Recipients. These patients included some who had participated in the skin test studies nearly 3 decades before. By this time, they had become part of an elite group of forerunners bearing the longest continuously surviving kidney grafts in the world. Of the five who were studied 27-29 years after transplantation, one had stopped immunosuppression 12 years earlier and the others were still taking azathioprine with or without prednisone. The distinction of donor from recipient cells was feasible because all five patients had received HLA-incompatible kidneys; in two cases, the kidneys had come from donors of the other sex.

Low-level chimerism was found in the host tissues of all five of these kidney recipients. Biopsy specimens of the allografts showed that the cells that departed from the transplants were replaced by similar cells from the host. Thus, in addition to systemic chimerism, these patients were shown to have chimeric kidney grafts composed of cells with two different genomes. 12,20

Human Liver Recipients. As with the kidney recipients, most of the patients in a cohort of 25 liver recipients studied for chimerism were clinically well and fully immunocompetent by conventional in vitro testing when they were studied between 2 and 22 years after transplantation under azathioprine- or cyclosporine-based immunosuppression. 14

Donor cell chimerism was found with cytochemical or polymerase chain reaction techniques in all 25 patients in locations that included the skin, lymph nodes, heart, lungs, spleen, intestine, kidneys, bone marrow, and thymus. Chimeric cells were present in larger numbers at any given site than they were in the long-surviving kidney recipients being studied at the same time.
Cell Migration, Chimerism, and Graft Acceptance, With Particular Reference to the Liver

Control
Small Bowel
Skin
Bone Marrow
Blood
Liver

Figure 27-5 Detection of chimerism by molecular human leukocyte antigen (HLA) class II typing in various tissues after liver transplantation in a patient with type I Gaucher's disease. The illustration shows southern blot analysis of DR1-specific amplification of the DNA extracted from small bowel, skin, bone marrow, blood, and liver. The denatured DNA present on the nylon membrane was hybridized to a radioactively labeled DR1 (donor)–specific oligonucleotide probe (7001). In the case of the liver, only 1/10 of amplification product was used. C = negative control of the polymerase chain reaction amplification. (From Starzl TE, Demetris AJ, Trucco M, et al. Cell migration and chimerism after whole organ transplantation: The basis of graft acceptance. Hepatology 17:1127-1152, 1993.)

Figure 27-6 The dissemination of passenger leukocytes from the graft (rat liver in these experiments) to the central lymphoid organs; these leukocytes become ubiquitous after a brief pause. The events are similar to those after successful bone marrow transplantation.

CELL TRAFFIC AND SITES OF DONOR-RECIPIENT IMMUNOLOGICAL INTERACTIONS

The early events leading to the chimeric state after liver transplantation have been studied in rats and mice, including the pathways of passenger leukocyte dissemination. Within minutes or hours, these cells leave the liver and migrate to the spleen, lymph nodes, thymus, and probably bone marrow, where they are destroyed by rejection in most animal models except those that use mice as subjects. However, under temporary immunosuppression in rats (FK506 daily for 2 weeks), these mononuclear cells pause for about 2 weeks in the lymphoid organs but then break out and move secondarily to all recipient tissues (Fig 27-6). Rat liver recipients treated in this way (for example, Lewis [LEW] to BN) survive indefinitely without further treatment and retain their graft and systemic chimerism.

Interestingly, cell migration and chimerism with permanent survival of the engrafted liver occur without any immunosuppression in some rat strain combinations; of these combinations, BN to LEW has been most completely studied, but graft survival occurs without treatment in virtually all mouse strain combinations no matter how severe the histoincompatibility. These nonrejecting liver recipients in either species and those whose liver acceptance is induced with immunosuppression can receive skin, a kidney, or a heart from the original donor strain but no other strain (donor-specific nonreactivity). This kind of evidence has indicated that the heavy endowment of the liver with potentially migratory leukocytes is the basis for the well-known but previously inexplicable phenomenon of hepatic tolerogenicity.

Hepatic Tolerogenicity

Our hypothesis is that cell migration and repopulation is the central mechanism of acceptance of all whole organ grafts. Although this is a generic process, there are quantitative differences between organs in the density of the potentially migratory dendritic cells, macrophages, and lymphoid collections (Fig 27-7). The heavy endowment of the liver with the foregoing leukocyte lineages (including Kupffer cells) is a particularly striking feature that invites further speculation about the role of these cells in the well-known tolerogenicity of this organ.

The immunological advantage of the liver relative to other organs includes a greater ease of inducing the acceptance of liver allografts or xenografts either after a limited course of immunosuppression or, in swine and some rat strain combinations, with no treatment at all. In addition, the transplanted liver graft is relatively resistant to the preformed antigraft antibodies that cause hyperacute rejection of the kidney and heart. Another quality is the liver's unusual ability to induce a state of unresponsiveness to other tissues and organs transplanted...
concomitantly or subsequently from the donor or donor strain\textsuperscript{46, 49, 52} and even to shield these organs from the hyperacute rejection caused by preformed allospecific\textsuperscript{51} or xenospecific\textsuperscript{53} antidonor antibodies. In all of these circumstances, the liver can quickly transform the recipient environment to one more favorable to all donor tissues, including itself. All of these qualities of the liver are evident in practically every mouse strain combination, no matter what the degree of histoincompatibility.\textsuperscript{41}

The foregoing observations have been attributed to hepatic tolerogenicity incorrectly, we believe, because the term implies that the hepatocytes are responsible. We have proposed that the crucial variable distinguishing the tolerogenicity of one organ graft from that of another under effective immunosuppression (or, in some animal models, with no treatment) is its leukocyte, not its parenchymal component.\textsuperscript{9-15, 41} This is a reversal of the immunogenic role described classically for the passenger leukocytes.\textsuperscript{54-67}

Thus, because of the dense constituency of these migratory leukocytes, the liver is high on the favorable tolerogenic list, with the lung and intestine following and the kidney and heart bringing up the rear (see Fig 27-7). Experimental studies showing the less striking tolerogenicity of the lymphoreticular-rich spleen,\textsuperscript{68-70} the intestine,\textsuperscript{18} and the lung\textsuperscript{71, 72} are compatible with this generalization.

**Tolerogenicity of Leukocyte-Poor Organs**

By the end of 1992, it was appreciated that all whole organs underwent the same process of potential tolerance induction as did the liver, although the dynamics of organs other than the leukocyte-rich intestine were not as easy to study.\textsuperscript{18, 19, 36, 37} However, the same 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, which included the kidney. Studies in 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 complete study of untreated rat kidney recipients performed in 1981, Nemlander and associates\textsuperscript{73} demonstrated extensive leukocyte migration. If the investigators had given one or two doses of cyclosporine in their kidney transplantation experiments (which were with an "easy" strain combination) and had followed the animals further, they almost certainly would have uncovered the events of cell migration and long-term repopulation that waited another dozen years for exposure with the liver.\textsuperscript{15} Larsen et al\textsuperscript{74} found that donor dendritic cells from heterotopic cardiac allografts were released into the circulation, where they eventually home into the T cell areas of the recipient spleen. In the spleen, the donor cells were found to initiate the proliferation of recipient cells, and vice versa.\textsuperscript{73-77} This reaction might be thought of as an in vivo mixed lymphocyte response; it epitomizes central allosensitization with potential tolerization.

Allosensitization and tolerization presumably also occur within the graft. Forbes et al\textsuperscript{75} showed that clustering of recipient lymphocytes occurs around donor dendritic cells in the interstitium of heart grafts, within a few days after transplantation. The recipient lymphoid cells undergo blastogenesis and proliferate within these clusters.

Demetris et al have described analogous events in rat livers being rejected.\textsuperscript{75}

In human recipients of kidney grafts\textsuperscript{78, 79} under cyclosporine plus prednisone immunosuppression, Hayry and von Willebrand noted what appeared to be a bidirectional mixed lymphocyte response in needle aspiration biopsy specimens. When collected blast cells were studied with the *Staphylococcus aureus* assay and alloantibodies to nonshared donor and recipient allelic specificities, most of the blast cells in some cases were derived from the donor; alternatively, the response was split, "resembling a bidirectional mixed lymphocyte reaction in vitro."\textsuperscript{78}

In these models, the difference from the experiments with liver transplantation appears to be quantitative rather than fundamental. With the smaller number of passenger leukocytes, there is a greater tendency to allosensitization and less tendency to tolerogenicity. Nevertheless, Corry et al\textsuperscript{80} and Russell et al\textsuperscript{81} showed that tolerance without drug induction could be induced by heart and kidney transplantation between weakly major histocompatibility complex-incompatible strains of mouse recipients.

**FUNCTIONAL CONSEQUENCES OF MICROCHIMERISM**

Questions have been raised regarding whether the low-level chimerism found in our long-surviving patients and experimental animals is an irrelevant histopathological curiosity or a condition with immunological significance. Such questions appear naive in view of Russell's elegant formal proof of the association of chimerism with ac-
quired tolerance as well as runt disease. However, the skepticism was generated by the small numbers of chimeric donor cells in the recipient tissues of the patients and animals in our studies. We have called this condition microchimerism, a term introduced in the literature in 1974 by Liegeois et al. to describe a small proportion of chimeric cells in the recipient blood. The presence of microchimerism is far from insignificant; there is much evidence that the cumulative effect of microchimeric cells is substantial, especially after liver transplantation, when they are most easily demonstrated.

**Metabolic Effects**

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 IV glycogen storage disease and Gaucher's disease. In these diseases, the consequences of the missing enzymes are widespread storage of amylopectin and glucocerebroside, respectively. The disorders were previously thought to be treatable only by bone marrow transplantation because the enzyme deficiency affects all cells; however, 2–8 years after liver replacement, there was a dramatic resorption of both kinds of storage material from host tissues (Fig 27–8). As an explanation for the metabolic amelioration, chimeric donor cells were found to be ubiquitous in recipient tissues, including those of the heart, lymph nodes, bone marrow, intestine, and skin (see Fig 27–5). 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.

**The Immunological Interface**

The foregoing metabolic observations raised important questions about the potential cell-to-cell effects of other molecules directly involved in immunological rather than metabolic processes, including those subserving tolerance induction (see Fig 27–1). Perhaps such questions could be answered if it were known how the chimeric donor cells, many of which resemble dendritic cells, are perpetuated for as long as 3 decades after transplantation. The dendritic cells 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 using media enriched with granulocyte/macrophage colony-stimulating factor. The products of these stem cells should reach terminal differentiation, however, unless there is a reason for their continued proliferation. We have suggested that the subsequent survival and renewal of these cells depend on continuous mutual stimulation of the donor and recipient cell populations in a process of tolerization that shares many of the cellular characteristics associated with immunity.

**CHANGED HOST AND GRAFT INTERACTIONS**

There are indirect ways to show that the coexisting immunocyte populations in successful cases (see Fig 27–1) come to regard each other in a revised light. The evidence, on the one hand, is the fading of the threat of clinical rejection concomitant with development of donor-specific nonreactivity in spite of lightened treatment (or, in some animal models, without any treatment); on the other hand, the evidence is the waning specter of GVHD. It is quite natural to expect that the threat of GVHD and rejection would decline contemporaneously in an organ recipient because both of the cell populations are receiving the same protective immunosuppression. Appreciation of the two-cell population relationship and the need not to alter it by ablating one side or the other was the crucial advance made empirically that permitted the successful engraftment of leukocyte-rich organs such as the liver, intestine, both together, or all of the intra-abdominal organs (multivisceral transplantation). Once the cardinal principle was understood that low-level mixed allogeneic chimer-
ism was invariably found after the successful transplantation of any whole organ, the reason GVHD was not common in liver, intestinal, or multivisceral recipients seemed obvious. Mixed chimerism was being produced empirically in the way that had been documented in the classic GVHD-free mouse bone marrow mixed chimerism models of Slavin et al and Ildstad and Sachs.

THE CRITICAL DENDRITIC CELL

The generation of an immune response leading, under normal circumstances, to graft destruction, GVHD, or both, requires effective antigen presentation and recognition in its initial phase; this signal is followed by a second, costimulatory signal and the response of T cells to the combined signal. Both of these signals are normally delivered to T cells by professional antigen-presenting cells. Of these cells, the dendritic cell (the most prominent chimeric cell by morphological criteria) is critical because it can modify the expression of cell interaction, major histocompatibility complex, and adhesion molecules, all of which determine how antigen signals are heeded by T cells. The dendritic leukocyte is thus the prime candidate in this tolerogenic scenario, even though other lineages may also be essential for a successful outcome.

IMPACT ON TISSUE MATCHING

In the directions of both host-versus-graft (HVG) and graft-versus-host (GVH) rejection, cellular interactions resulting in mutual natural immunosuppression are envisioned as occurring on a sliding scale with each further level of histoincompatibility (Fig 27-9). With effective immunosuppression, it has become increasingly possible throughout the last 3 decades to mitigate the alloreactions enough to allow the tolerogenic changes to occur after mutual cell engagement and to allow a rapprochement to be reached between the coexisting immunocytes. The anticipated histocompatibility influence on both rejection and the severity of GVHD is then expected to dwindle. We have postulated that this dwindling influence explains the poor correlation of HLA matching with outcome after the cadaveric transplantation of whole organs, including the kidney. With regard to liver transplantation, two large centers have actually reported an inverse relationship between the quality of HLA matching and the clinical outcome.

RELATION OF CELL MIGRATION TO TOLERANCE

The inadequacy of thymic clonal deletion to explain acquired transplantation tolerance has been emphasized in a

---

Figure 27-9 The donor-recipient leukocyte interaction is a buffer against rejection on the 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, = iatrogenic immunosuppression. (Reprinted from Immunology Today, 14, Starzl TE, Demetris AJ, Murase N, et al, Donor cell chimerism permitted by immunosuppressive drugs: A new view of organ transplantation. 326–332. Copyright 1993, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, UK.)
Although a discussion of the meaning of tolerance is beyond our intention, it should be noted that all of the mechanisms that explain clonal silencing, including peripheral (nonthymic) clonal deletion and anergy, could mesh with the discovery of the enduring graft-host intimacy inherent with chimerism. The production of suppressor cells, veto cells, or both could be an epiphenomenological consequence. Evidence of the long-term vitality and turnover of donor leukocytes in recipient tissues is particularly supportive of the opinions of Bandeira et al., Coutinho,96 and Cohen,99 who have defined acquired tolerance as a high (not anergic) level of sustained immune activity in immunological networks. These networks presumably interact in a more complex way than do the idioype systems originally postulated by Jerne.98

Apart from explaining why the events of convalescence follow the same pattern after all transplantations, no matter what the organ, the cell migration–chimerism concept shows how donor-specific nonreactivity can be achieved with a common mechanism, regardless of the site of action of the immunosuppressive drugs administered, or, in some experimental models, without drugs at all. It has been proposed on the basis of observations in drug-free models of tolerance induction that T cell receptor occupancy leads to the production of negative regulators of interleukin (IL)-2 production (anergy proteins).89,99 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 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 27–10), for instance, by the absence of a costimulatory signal in drug-free models.

The same effect could be induced iatrogenically by pharmacological interdiction of IL-2 gene transcription (cyclosporine and FK506) or by the administration of a DNA synthesis inhibitor (azathioprine, cyclophosphamide, and numerous others). The use of monoclonal antibodies that do not deplete T cells, such as those directed against the cell surface CD4 antigen, or monoclonal antibodies that are directed against adhesion molecules, including intercellular adhesion molecule 1 and lymphocyte function related antigen 1 (LFA-1),100 can also be envisaged.

However it occurs, the reciprocal educational process of donor and recipient leukocytes and its perpetuation resemble, in the direction of either rejection or GVHD (see Fig 27–10), the “infectious” transplantation tolerance of Waldmann and Cobbold, which can be passed on to naive lymphocytes and be self-sustaining in some circumstances.101 It is postulated that in fully successful cases, the mini-immune system of the graft is incorporated into the existing recipient immunological network,13,15 a concept compatible with the hypothesis of Coutinho.96

**UNSTABLE MIXED CHIMERISM**

Cell migration conceptually reunites bone marrow transplantation and whole organ transplantation. We believe that there are not different mechanisms involved in successful engraftment and that these two seemingly dis-

![Model of dendritic cell antigen-presenting-cell–T cell interaction, showing 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 (e.g., 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 envisaged as promoting anergy. In some instances, tolerance can be broken, for example, by administration of exogenous IL-2. HVG = host versus graft response (allograft rejection). (From Thomson, AW, Demetris AJ, Murase N, Starzl TE. Promotion of cell chimerism by immunosuppression drugs: A possible basis for tolerance induction following organ transplantation. In Thomson AW, Starzl TE, eds. Immunosuppressive Drugs: Developments in Anti-Rejection Therapy. London, Edward Arnold, 1994, p 226. Reproduced by permission of Edward Arnold [Publishers] Limited.)**

parate clinical disciplines merely reflect contrasting treatment dogmas (Fig 27–11). 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 of solid organ transplantation encourages, or at least permits, these consequences of mutual cell engagement and thereby liberates the patient from the restrictions of HLA matching and from an overwhelming threat of GVHD.

Failure of the chimeric and recipient immunocytes to reach an immunological truce (see Fig 27–1) leads to re-
The division of transplantation into two separate disciplines by divergent therapeutic dogmas that created one-way versus two-way in vivo mixed lymphocyte response analogues. The policies used in bone marrow transplantation inhibited or precluded bidirectional cell migration, whereas this phenomenon formed the fundamental basis of graft acceptance in whole organ transplantation. (From Starzl TE, Demetris AJ, Trucco M, et al. Cell migration and chimerism after whole organ transplantation: The basis of graft acceptance. Hepatology 17:1127-1152, 1993.)

Figure 27-11

The potential adverse consequences of deliberately unbalancing the donor-recipient interface were illustrated by the case of a 56-year-old man with gastric leiomyosarcoma and liver metastases who underwent a liver transplantation after an upper abdominal exenteration. Just before the operation, the recipient was prepared with a single dose of 550-rad thoracoabdominal lymphoid irradiation followed by an intravenous dose of $19 \times 10^9$ nonpurged bone marrow cells. Several weeks postoperatively, he developed progressive and grave GVHD with more than 80% skin involvement that did not respond to increased or decreased immunosuppression. On days 42 and 43 after surgery, respectively, $1.23 \times 10^8$ and $1.6 \times 10^8$ unpurged bone marrow cells per kilogram (collected and stored from before the preoperative total lymphoid irradiation) were infused intravenously. The rash dramatically resolved over the next 2 weeks, coincident with a fall in mixed lin-
eage donor cells in the blood from approximately 25% of total to 3% by flow cytometry, without the advent of rejection.

The ability of the stored autologous marrow cells to turn off the potentially lethal GVHD may be a clue to the changes that occur during the mutual cellular engagement of mixed chimerism. Although the cells used for rescue from GVHD were thought to be smaller in number than those in the circulation of the patient, they had not been exposed to donor marrow. Their therapeutic effect resembled the ability of virgin donor strain immunocytes to “break tolerance,” as originally described by Billingham et al.112

Two clinically relevant lessons from this traumatic experience have been reported elsewhere.111 The first was the inadvisability of trying to make space for the augmenting marrow cells with total lymphoid irradiation. The second lesson was the potential value of naive autologous bone marrow, which can be stored as a safety net should GVHD occur. These lessons have guided further attempts at bone marrow augmentation for the induction of tolerance in whole organ recipients.

CLINICAL TRIALS OF BONE MARROW AUGMENTATION

Kidney Alone or Kidney Plus Pancreatic Islets

The concept developed in this chapter is that the content (and perhaps the specific lineages) of migratory cells, which are particularly numerous in the liver, confers potential immunological advantages. These passenger leukocytes, which are of bone marrow origin, have been considered an immunological liability for transplantation54–67; however, under the appropriate conditions, they may be tolerogenic, as exemplified by the liver. A corollary, therefore, is that organs such as the kidney and heart, which have a smaller leukocyte component, could be brought to the liver’s tolerogenic potential. In fact, the frequently advanced strategy of intravenously infusing donor bone marrow, donor blood (donor-specific transfusion), or other hematolymphoid cells at the same time or shortly after the transplantation of whole organs83, 87, 88, 113–116 is merely an augmentation of the normal post-transplantation cell migration. To mimic the natural process, these cells should be given perioperatively, not in advance or afterward, as has usually been done with the Monaco experimental model.114–116

CLINICAL EXPERIENCE

Since December 1992 at our center, four patients with end-stage renal disease have received a simultaneous kidney–bone marrow allograft from the same cadaveric donor. In addition, two diabetic (type I) renal patients were given pancreatic islets from their kidney–bone marrow donor. As a precaution, autologous bone marrow was harvested from all the recipients immediately before the kidney transplantation and cryopreserved for potential future use in case of GVHD,111 as described in the preceding section.

The donor bone marrow was obtained from vertebral bodies at the end of multiorgan procurement, and 3 × 10⁸ untreated bone marrow cells were infused intravenously immediately after the kidney transplantation. The two diabetic patients received the additional pancreatic islet infusion intraperitoneally at the time of the intravenous bone marrow infusion. All six patients were treated with FK506 and prednisone. Chimerism was assessed in the recipients by flow cytometry, polymerase chain reaction, and immunostaining with peripheral blood lymphocytes. Immunological monitoring was done by mixed lymphocyte response and cell-mediated lympholysis.

None of these patients exhibited GVHD. Detectable levels of donor cells were present in the peripheral blood lymphocytes of all six recipients during the 1- to 6-month follow-up. Donor cells were also detected in two patients whose lymph nodes were biopsied 2 and 4 months after transplantation. All six patients and their renal grafts are doing well. The two patients who were also given pancreatic islets have been treated too recently to evaluate their insulin status accurately.

Further evaluation of passenger leukocyte augmentation (with bone marrow and other leukocyte sources) is of particular interest for pancreatic islet transplantation, which has consistently failed as a means of treating diabetes because of the high rate of rejection.117 If it is possible to increase islet allograft survival with minimal 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 antigen-presenting cells derived from bone marrow that are normally contained in islet preparations.118 Donor bone marrow would then become a dose-manageable component of any organ or cellular transplantation for the facilitation of graft acceptance and for the induction of donor-specific nonreactivity.

A LIVER TRANSPLANTATION ROAD MAP FOR DRUG WEANING

As discussed earlier, past experience with conventional liver transplantation can be envisioned as a mini–bone marrow engraftment. In one reported group of 44 human liver recipients who had survived from 11 to 23 years, 6 (14%) had stopped all immunosuppression between 1 and 11 years postoperatively and had subsequently experienced clinically stable, drug-free intervals of 5–13 years; another 15 patients were drug free and stable after a shorter follow-up period.14, 119 The most extreme example of early successful drug discontinuance 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 FK506120 that the benefits of drug weaning appear to us to outweigh the risks in selected patients.

This liver experience should provide insight regarding the use of bone marrow augmentation to achieve freedom from immunosuppression in recipients of organs and cells such as the kidney or pancreatic islets. Even when drug weaning has been decided upon, there is presently no way to know when a potential drug-free state has arrived. It seems clear that, if the clinical experience with the tolerogenic livers is taken seriously, kidney or heart transplantation plus bone marrow augmentation will also require
protracted immunosuppression before the treatment can be stopped altogether and that, even then, it can be stopped only with precautions. Such conclusions have also been reached by Barber et al., who used delayed supplementary bone marrow for cadaveric kidney transplantation as advocated by Monaco et al, 114, 115

Liver Transplantation

CLINICAL EXPERIENCE

Two liver recipients have received the same doses of perioperative bone marrow as patients in the kidney series, and 500–550 Gy total lymphoid irradiation was added preoperatively. The second patient developed nearly fatal hepatitis, but there were also signs of vascular rejection. Blood chimerism was no longer detectable immediately before retransplantation, even by polymerase chain reaction.

The unwise decision made regarding this patient and the second one to unbalance the donor-recipient interface iatrogenically with total lymphoid irradiation was based on the dogma that making space would facilitate engraftment of the marrow. This concept has been eroded by direct experimentation. 121, 122 The second patient, now 13 months after surgery, has had a perfect clinical result since biopsy findings showed mild rejection at 2 weeks. She takes a daily dose of 6 mg of FK506 plus 5 mg of prednisone. There is evidence of chimerism in the peripheral blood lymphocytes by cytospin.

Three additional patients underwent liver transplantation and bone marrow augmentation without total lymphoid irradiation. Their courses have been uncomplicated, with the exception of one episode of rejection 2 weeks after transplantation. All are blood chimeras.

RISKS AND BENEFITS

The augmentation in liver recipients of a process of cell migration and chimerism that is already highly operational after this kind of transplantation must be closely monitored for efficacy. Whether leukocyte augmentation will do more than could be achieved naturally in tolerance induction (with earlier achievement of a drug-free state) or will only increase the risk of GVHD remains to be determined. The storage of autologous bone marrow for rescue should GVHD occur appears to be a mandatory precaution in such trials. 111

References


25. Sedmak DD, Sharma HM, Czaja CM, Ferguson RM. Recipient endotheli­


graft acceptance in rhesus monkeys given post-transplant antithymocyte globulin and donor bone marrow: I. In vivo parameters and immunohisto­


