

The Mystique of Hepatic Tolerogenicity

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ABSTRACT: *The evolution of clinical transplantation has hinged on 2 seminal turning points. The first was the demonstration in 1953 by Billingham, Brent, and Medawar that chimerism-associated tolerance could be induced deliberately in neonatal mice by infusing adult donor hematolymphopoietic cells. This discovery escalated in a straight line over the next 15 years to successful bone marrow transplantation in humans. The second turning point was the demonstration that organ allografts could self-induce tolerance under an umbrella of immunosuppression, or in some species without immunosuppression. Unfortunately, it was incorrectly concluded by most immunologists and surgeons that bone marrow and organ engraftment involved different immune mechanisms. In a derivative error, it became widely believed that the tolerogenicity of the liver differed fundamentally not only from that of bone marrow but also from that of other whole organs.*

These errors became dogma and were not corrected until low level donor leukocyte chimerism was found in humans and animals bearing long surviving liver, kidney, heart, and other kinds of allografts. With successful bone marrow transplantation, the trace population consisted of recipient rather than donor leukocytes. Thus, the consequences of organ and bone marrow engraftment were mirror images. From these observations, it was proposed that the engraftment of all kinds of organs as well as bone marrow cells (BMC) involved host versus graft (HVG) and graft versus host (GVH) reactions with reciprocal induction of variable degrees of specific non-reactivity (tolerance). The maintenance of the tolerance was an active and ongoing process requiring the persistence of the transplanted fragment of the donor immune system. The immune responsiveness and unresponsiveness to both organ and bone marrow allografts are thought to be governed by the migration and localization of leukocytes. The clarifying principles of transplantation immunology that have emerged from the chimerism studies are relevant to the adaptive immune response to microbial, tumor, allogeneic, and self antigens. These principles should be used to guide efforts to systematically induce tolerance to human tissues and organs, and perhaps ultimately to xenografts.

KEY WORDS: immunologic tolerance, organ allograft, liver allograft, liver tolerogenicity, liver transplantation, clonal exhaustion-deletion, immune indifference

Objectives

Upon completion of this article, the reader should be able to understand 1) the meaning and mechanisms of acquired tolerance; 2) the adaptive immune response to microorganisms *vis a vis* the response to organ and bone marrow allografts; and 3) the reasons for the liver's unusual tolerogenicity relative to that of other organs.

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Disclosure

Statements have been obtained regarding the authors' relationships with financial supporters of this activity. There is no apparent conflict of interest related to the context of participation of the authors of this article.

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The emergence of transplantation has seen the development of increasingly potent immunosuppressive agents, better methods of tissue and organ preservation, refinements in histocompatibility matching, and numerous innovations in surgical technique.^{1,2} Although these advances helped to make organ transplantation a practical and valuable clinical service, the feasibility of such procedures ultimately depended upon a natural quality of all organs, namely their ability to induce variable degrees of donor specific nonreactivity (i.e., tolerance).³

In humans, allogeneic tolerance is a normal immunologic option that generally requires a protective umbrella of immunosuppression. However, spontaneous tolerance to organs, most commonly the liver, has been recorded in pigs, rats, and mice. In addition to its greater tolerogenicity relative to other organs, the liver is more resistant to antibody mediated hyperacute rejection.⁴ Such observations, suggesting that the liver is an "immune privileged" organ led to a controversy of more than 25 years duration. The issue was whether tolerogenicity is a unique property of the liver, or a quality possessed by all organs, varying only in degree.

ORGAN TOLEROGENICITY: THE ORIGIN OF THE CONCEPT

A Pathfinder Kidney Experience

In retrospect, the inherent tolerogenicity of organs was evident from the beginning, although passionately denied. Between January 1959, and January 1963, there were 6 examples of survival of greater than one year after kidney transplantation to human recipients preconditioned with total body irradiation (TBI): one in Boston^{5,6} and 5 divided between two Paris centers.^{7,8} Under today's regulatory restrictions, these trials probably would have been blocked at an institutional review board (IRB) level. The longest survival with TBI preconditioning in an animal model had been only for 73 days, following kidney transplantation between beagle dogs.⁹ Furthermore, the first successful clinical case in Boston, in which the donor was a fraternal twin, provided the only long survival in a dozen attempts at that institution. In France the 5 long survivals also were exceptions to the usual outcome of recipient death.

In addition to these 6 cases, a non-irradiated human kidney recipient in Boston had been treated with azathioprine from the time of transplantation on April 5, 1962.¹⁰ Although this seventh long-surviving renal allograft (from an unrelated donor) was failing by January, 1963, it provided enough function to keep the patient dialysis-free for another 8 months.¹¹ Unlike the TBI trials, a track record had been established in animals. Survival longer than 100 days had been documented by

Calne¹² (with Murray¹³) in about 5% of mongrel canine kidney recipients treated with 6-mercaptopurine or its imidazole derivative, azathioprine.

The limited efficacy of azathioprine, alone or in combination with other cytotoxic drugs, was found to be the same in humans¹⁰ as in the preceding dog studies. In contrast, 8 of the first 10 human kidney recipients treated with the combination of azathioprine and prednisone at the University of Colorado had prolonged survival. These cases, compiled between October 1962, and March 1963,³ constituted the first successful series of human organ transplantation and included 2 patients who still bear the longest continuously functioning kidney allografts in the world (> 37 years).

Renal Tolerogenicity

From a practical point of view, the significance of the Colorado series was the high rate of patient and graft survival. Its far more important conceptual implications were capsulized in the title of the report published in October 1963: "The reversal of rejection in human renal homografts with subsequent development of homograft tolerance."³ Although the findings could not be explained, they provided the bedrock upon which the organ-defined specialties of clinical transplantation could be developed, using immunosuppressive drugs in combination that were ineffective when administered alone.

The reversal of rejection in the human kidney recipients was readily monitored by renal function tests after instituting large doses of prednisone (200 mg/day) to baseline immunosuppression with azathioprine. The evidence was equally clear that the renal allografts had self-induced partial tolerance under the cover of immunosuppression. After passing through rejection crises, most of the patients had a progressively diminishing need for immunosuppression, usually to doses lower than those which initially failed to prevent rejection. The tolerance was complete enough to allow many of the kidney recipients to be released to an unrestricted environment without the lethal immunologic invalidism that had been widely predicted. More than a third of a century later, remarkably similar observations leading to what was called "prope tolerance" were reported by Calne et al.^{14,15} in patients treated with a combination of modern day immunosuppressants. In fact, 2 of the 10 recipients in the 1963 report have been free of immunosuppression for 34 and 6 years, respectively.¹⁶

Successful transplantation of the liver,^{17,18} and eventually all of the other extrarenal organs depended on the same phenomena as with kidney transplantation: the reversal of rejection and the evolution of specific non-reactivity. In 1966, adjunct antilymphocyte globulin [ALG] was added to azathioprine and prednisone¹⁹

TABLE 1. Empirical Therapeutic Dogma of Immunosuppression

<i>Ingredients of Strategy</i>	<i>Baseline Agents</i>
1. Baseline therapy	1. Azathioprine*
2. Secondary adjustments of prednisone dose, or antilymphoid agents**	2. Cyclosporine
3. Case to case trial (and potential error) of weaning.	3. Tacrolimus

*Alone or with prophylactic prednisone. Equivalent results were obtained with cyclophosphamide instead of azathioprine.

**Initially used for prophylactic "induction."

in the first "triple drug cocktail," and as more efficient baseline drugs (e.g., cyclosporine and tacrolimus) and adjunct agents (e.g., monoclonal antilymphoid antibodies) became available they were readily substituted for the previous best immunosuppressants. However, the basic strategy was always the same (Table 1).

Hepatic Tolerogenicity

Until 1965, the only published clue that the liver might be unusually tolerogenic was the observation in untreated mongrel canine recipients that the intestine and pancreas had very little histopathologic evidence of rejection if they were components of multivisceral allografts that also included the liver.²⁰ The finding was confirmed 30 years later in a rat version of the same multivisceral procedure.^{21,22} However, the first unambiguous evidence of hepatic tolerogenicity was the demonstration in 1964 that mongrel dogs bearing orthotopic liver allografts under azathioprine therapy frequently could have their immunosuppression stopped after 4 months with long subsequent survival;²³ 5 years later most of the drug free recipients remained well.²⁴

The significance of this observation in canine liver recipients was extensively discussed²³: "Although the early recovery after liver homotransplantation has many hazards . . . the frequency and rapidity with which dogs could be withdrawn from immunosuppression without an ensuing fatal rejection is remarkable. . . . The consistency of this state of host-graft nonreactivity and the rapidity with which it seemed to develop exceeds that reported after canine renal homotransplantation. The explanation for this is not apparent, but conceivably, the large antigenic mass could play a role or, alternatively, perhaps the liver with its enormous regenerative capacity is simply capable of sustained function in the face of continuing but minimal chronic rejection. [However,] findings in the serial biopsies obtained after discontinuance of therapy do not support the latter hypothesis . . ."

Soon thereafter in France²⁵ and then elsewhere,²⁶⁻²⁹ spontaneous liver engraftment in untreated

outbred pigs was reported in 1/3 to 1/4 of hepatic replacement experiments. Many of these porcine recipients first passed through one or more self-resolving rejection crises.^{28,30,31} In the 1970s, the phenomenon of tolerance induced by the liver was demonstrated in untreated rats (see later discussion).

AN EPISTEMOLOGIC COLLAPSE

The discovery that variable donor specific non-reactivity could be induced by organ allografts under an umbrella of non-specific drug immunosuppression was the second principal turning point from which clinical transplantation evolved.^{1,2} The first had been the demonstration by Billingham, Brent and Medawar in 1953 that transplantation tolerance could be induced in neonatal mice by infusing these immunologically immature (i.e., defenseless) recipients with allogeneic hematolymphopoietic cells from a histocompatible or F₁ hybrid adult donor.^{32,33} If the recipients survived to adult life, ostensibly with a "replaced" hematolymphopoietic (i.e., immune) system, skin grafts from the same donor strain (but not from third party donors) were permanently accepted.

The next step was to simulate in adult mice the immunologically defenseless state of the neonatal animals by TBI, followed by the infusion of donor bone marrow cells (BMC). When these experiments were successful,³⁴ the stage was set for clinical bone marrow transplantation. However, this application was delayed for a decade until human leukocyte antigen (HLA) matching became available.³⁵⁻³⁸ As in the experimental models, graft versus host disease (GVHD) was a lethal consequence of the procedure in humans unless there was a good tissue match. Efforts to precondition canine kidney recipients with TBI and donor bone marrow infusion yielded only a single 73 day survivor.⁹ After this strategy failed in clinical trials, the use of TBI alone was rewarded by the long survival of a handful of human kidney recipients (see earlier section, "A Pathfinder Kidney Experience"). Not unreasonably, it was concluded that successful organ transplantation was based on fundamentally different principles than bone marrow transplantation.

The two turning points had now diverged in opposite directions, one leading to clinical bone marrow transplantation, and the other to organ transplantation. Moreover, a conceptual error had been introduced that distorted the orderly development of transplantation immunology, and of all other branches of immunology. The error was the conclusion "by consensus" that organ engraftment occurred by different mechanisms than the chimerism-dependent ones associated with neonatal tolerance and its clinical analogue of bone marrow transplantation.

The Liver-Specific Dogma

The mistake subsequently was compounded by the argument that the mechanisms of hepatic tolerogenicity differed fundamentally, not only from those of bone marrow, but also from those leading to engraftment of other organs. This contention was based on the demonstration, first in spontaneously tolerant pigs³¹ and then in untreated rodent recipients of liver allografts⁴⁰⁻⁴² that the tolerization self-induced by the liver extended to donor skin transplanted at the same time or later, and to other donor organs. Except for the presumed absence of donor leukocyte chimerism in the recipients, however, the acquired donor-specific non-reactivity was indistinguishable from that in the chimerism-associated neonatal tolerance of Billingham, Brent and Medawar,^{32,33} and the experimental models of bone marrow transplantation³⁴ that by this time had been brought to the clinic.

The spontaneous tolerance in pig liver recipients was unpredictable. However, it could be reliably reproduced in about 15% of congenic rat strain combinations. Consequently, rat models came to dominate research efforts to explain the phenomenon of spontaneous liver tolerance.⁴³⁻⁴⁷ The more recent demonstration that livers self-induce such tolerance in more than 80% of mouse strain combinations⁴⁸ has generated a second windfall of mechanistic investigations.⁴⁹⁻⁵⁴ The mouse studies have been of particular interest because the principles of mammalian immunology have been derived so much from studies of this species.

In the ostensible absence of donor leukocyte chimerism, Davies, Kamada, and Rosen⁵⁵ demonstrated specific deletion of cytotoxic antidonor lymphocytes from the thoracic duct, lymph nodes, and hepatic tissue of spontaneously tolerant rat liver recipients. The possibility implicit in these findings that the liver is a focus of peripheral tolerance induction has resurfaced with the studies by Crispe⁵⁶ of interactions within the liver between thymus-dependent circulating T cells and thymus-independent T cells with natural killer (NK) cell markers.

In addition, it was postulated that soluble major histocompatibility complex (MHC) Class I antigens secreted by the hepatocytes were responsible for the spontaneous hepatic tolerance.^{39,40,55,57-59} A subsequent but still liver-specific modification of this soluble antigen hypothesis^{60,61} accommodated the discovery in 1992 of donor leukocyte chimerism in human liver recipients (see later discussion). In the revision, Calne suggested that the soluble Class I antigen was a critical co-factor without which engraftment and persistence of donor leukocytes could not occur, while concluding that the persistence of late stage microchimerism was epiphenomenal.⁶⁰

Studies in mice⁴⁸ including those with "knocked-out" MHC class I genes⁶² have not supported the liver-specific hypotheses. Importantly, it has been established

that spontaneous tolerance also can be induced in mice by heart^{48,63} and kidney allografts,⁶⁴ although in a much smaller number of donor-recipient strain combinations than with mouse liver allografts.

The Colorado Hypothesis

The exhaustion and deletion of an antigen specific clone had been postulated in the late 1950s to explain the acquisition in animals of tolerance to heterologous protein (with the aid of 6-mercaptopurine⁶⁵), and to allogeneic splenocytes (without the need for immunosuppression⁶⁶). The acceptance of clonal exhaustion-deletion as a "real phenomenon" by early workers was evidenced by extensive reviews in 1967⁶⁷ and 1968⁶⁸ by some of the most distinguished pioneers in immunology. Clonal exhaustion-deletion also was invoked at a very early time to explain the characteristic immunologic confrontation (rejection) and resolution (graft acceptance) that occurred with kidney engraftment.⁶⁹

Liver engraftment from the Colorado perspective was a form of donor specific tolerance that differed from kidney engraftment only quantitatively, exemplified by the ability of long-surviving canine^{23,24} and human liver recipients⁷⁰⁻⁷² to stop all immunosuppression more frequently than kidney recipients (Fig. 1). As with the kidney allografts, the seminal mechanism of hepatic tolerogenicity was postulated to be clonal exhaustion-deletion ("clone stripping"). However, the ideas depicted in Figure 2 were not taken seriously. In addition to showing clonal exhaustion-deletion as the primary explanation of allograft acceptance, other features proposed in this first book on liver transplantation (published in 1969⁷³) were ahead of their time.

For example, alloantigen presentation leading to induction of the expanded clone was depicted via host macrophages (Fig. 2) rather than by the dendritic cells (DC) that would not be described by Steinman and Cohn⁷⁴ until 1973. The discovery of apoptosis also lay ahead. Nevertheless, the immune competent cells proliferating in response to alloantigen were depicted in Figure 2 as selectively vulnerable, accounting for the emergence of donor-specific non-reactivity (tolerance) under treatment with immunologically non-specific immunosuppressants.

Despite the logic of clonal exhaustion-deletion, this mechanism of acquired tolerance abruptly disappeared from the literature. To our knowledge, clonal exhaustion-deletion was not mentioned a single time as an explanation for acquired tolerance in the voluminous immunology and transplantation literature spanning 1970-1990. The dismissive treatment could be explained in part by the theoretical status of clonal exhaustion-deletion. However, a more important factor

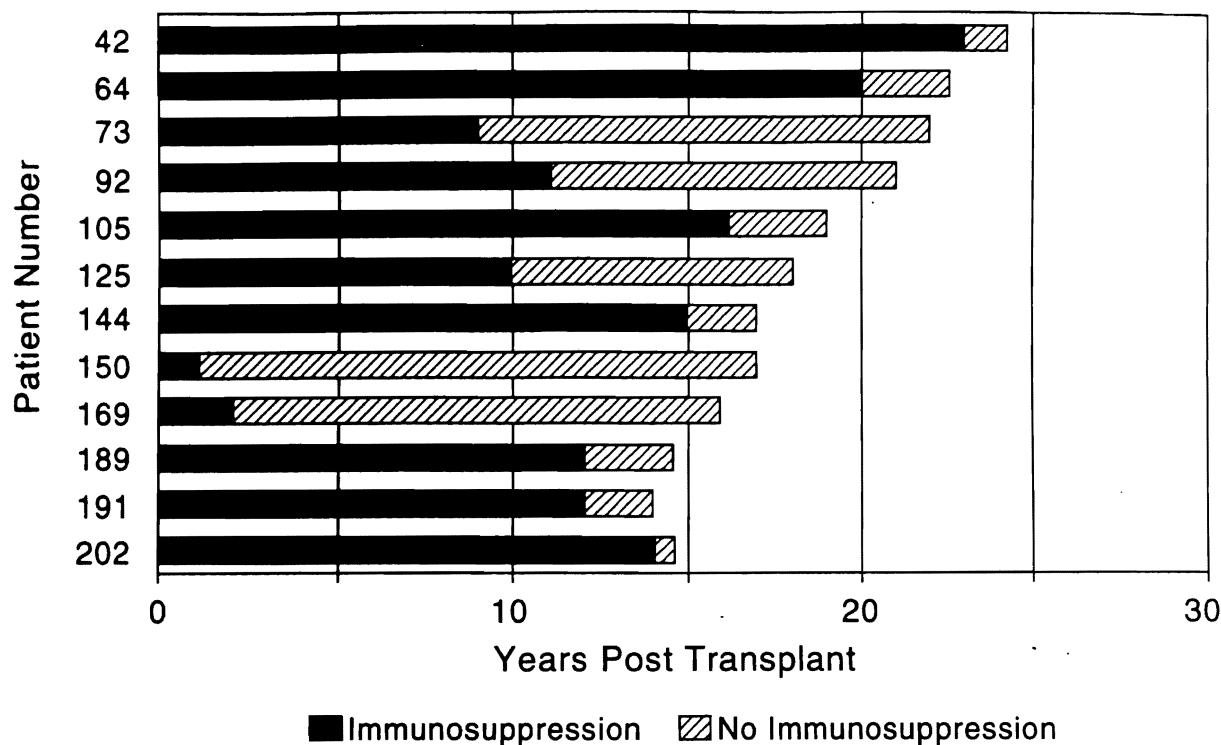


FIG. 1. Time on and off immunosuppression (as of October 1995) of 12 long surviving liver recipients. Patients 150 and 169 stopped medication less than 2 years posttransplantation because of noncompliance. The others were weaned because of complications of chronic immunosuppression. These 12 patients represented 28% of the 42 patients in our total experience who had continuously borne hepatic allografts for 15 to almost 26 years. Four years later, these patients remain drug free. Data from ref. 16.

appears to have been a lack of understanding about the role of the organ's "passenger leukocytes."

Passenger leukocytes are of bone marrow origin, and have been known for more than 3 decades to be the principal immunogenic component of allografts.⁷⁵⁻⁷⁹ Consequently, one explanation of organ engraftment was that it involved the destruction of the immunogenic donor leukocytes by the recipient immune system, with selective sparing of the specialized parenchymal cells. The disappearance of most of the resident donor leukocytes and their replacement by recipient cells of the same lineages had been reported in 1969 in the first long-surviving human liver allografts.^{31,80} Because this was considered for the next 2 decades to be a unique feature of the liver allograft, the passenger leukocyte replacement became a candidate co-factor for hepatic tolerogenicity.^{45,58} However, when the same cell replacement was demonstrated in intestinal allografts in 1991^{22,81} (Fig. 3, lower graph), it was obvious that it probably was a generic phenomenon, that is, it occurred in all successfully transplanted organs. This was promptly confirmed.

Exemplifying the power of ossified dogma, however, the possibility was not generally entertained, even at this late time, that the missing donor leukocytes had simply migrated and survived in the recipient. Consequently, organ engraftment continued to be explained

by hypotheses in which donor leukocyte chimerism played no role. The hypotheses included the presence or development of suppressor, veto, and other immune regulatory cells; cytokine profile changes; a role for various idiotypic and/or enhancing antibodies; and failure of delivery of a second (co-stimulatory) signal following primary antigen presentation.

AN EPIPHANY

The requirement for nonchimerism mechanisms was eliminated when a link was established in 1992 between organ transplantation and classical neonatal and/or bone marrow transplant-induced tolerance. Using sensitive immunocytochemical and polymerase chain reaction (PCR) techniques, low level (micro) chimerism was detected in the tissues or blood of all 30 human organ recipients studied 2.5 to 30 years after transplantation of continuously functioning liver ($n = 25$) or kidney allografts ($n = 5$).^{70,82-85}

From this finding, it was deduced that organ engraftment was a dynamic process that had begun at the time of transplantation with "... widespread responses of coexisting donor and recipient immune responses, each to the other, causing reciprocal clonal expansion followed by peripheral clonal deletion."^{70,82} Although

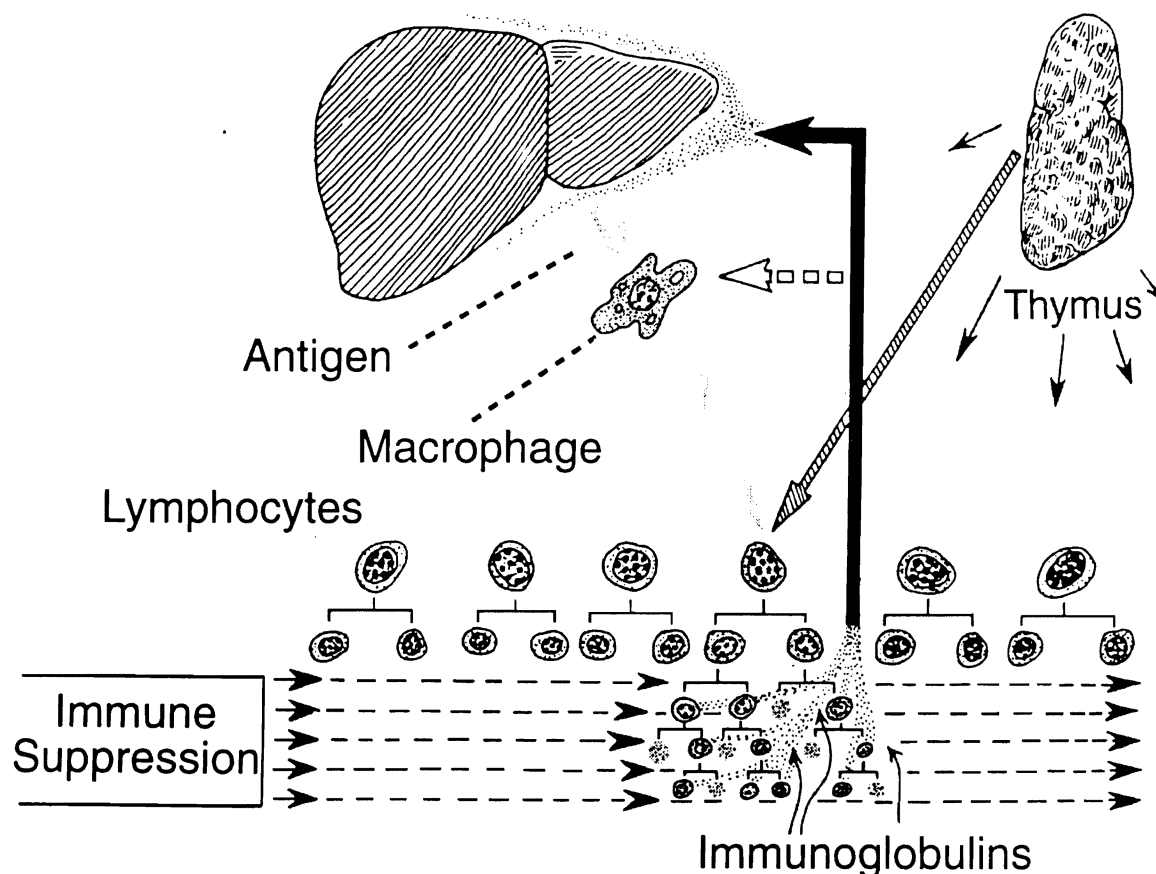


FIG. 2. Original caption published in 1969: "Hypothetical mechanisms by which nonspecific immunosuppression may lead to selective abrogation of the host immune response. Special susceptibility to these agents of a fraction of the lymphoid population could lead to exhaustion of a clone and, hence, tolerance. Since maintenance of such cell lines, even in adult life, is apparently thymic dependent in experimental animals, thymectomy would be expected to aid the process; this appears to be true in rodents, but such an effect of thymus removal has not been detected in dogs or humans." Note that antigen presentation was depicted via the macrophages rather than by the dendritic cells (which were not described until 4 years later.⁷⁴ The principal gap in the 1969 hypothesis was the failure to stipulate the location of the immune activation (by permission of WB Saunders).

there had been only one study with substantive evidence that clonal exhaustion-deletion actually existed,⁸⁶ numerous confirmatory reports have been published since.⁸⁷⁻⁹² Moreover, the critical importance of acute clonal exhaustion-deletion has been confirmed directly in experimental heart and liver transplantation models.^{47,53,93}

The persistence of the disseminated donor leukocytes for as long as 3 decades implied (as was subsequently proved^{94,95}) that precursor or stem cells are included in the organ's passenger leukocytes. After organ transplantation, donor leukocytes briefly constitute 1% to 20% of the recipient circulating mononuclear cells (Fig. 3, upper graph). Although the number of the donor cells is greatest with transplantation of leukocyte-rich organs (e.g., the liver), the same events on a smaller scale occur with transplantation of the kidney and heart.⁴⁶

Whatever the organ, the donor cells are multilineage, and include many of the dendritic cells

(DCs)^{46,48,70,82,96-98} that previously had been associated with antigenicity and rejection^{99,100} rather than tolerance. Individual samples from organ recipients often do not contain donor leukocytes, which wax and wane.^{101,102} However, disseminated donor cells including DCs, or other lineages, are consistently demonstrable if host non-lymphoid and lymphoid tissues and blood samples are thoroughly studied in rodents bearing long-term grafts.^{46,48,103}

Thus, it had become obvious that both the engrafted organ and the recipient become genetic composites (Fig. 4A). It also has been established that the extent of donor leukocyte chimerism in the recipient is reflected or exceeded by the quantity of donor leukocytes in the graft.^{96,104} In fact, Sakamoto et al.¹⁰⁵ have shown that the organ allograft, whose parenchymal cells are syngeneic to the donor passenger leukocytes, provides the optimal microenvironment for donor leukocytes including the precursor and stem cells that presumably are responsible for the multilineage nature of micro- as well

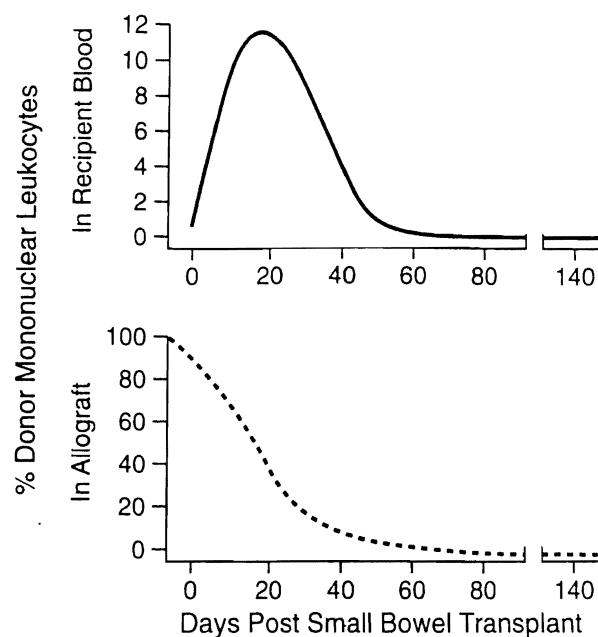


FIG. 3. The dynamic events after transplantation of all organs, exemplified here by intestinal engraftment. Upper Panel: The surge of migratory donor mononuclear leukocytes in the recipient blood during the first 2 or 3 weeks posttransplantation. Lower Panel: The contemporaneous disappearance of the passenger leukocytes from the allograft: these were replaced by recipient cells of the same lineages (schematically redrawn from data in Iwaki et al [81]). (Reprinted from Starzl et al. Transplant Proc 1999;31:1406–1411, with permission.)

as macrochimerism.^{48,96} Therefore, the organ allograft may be a critical site from which donor leukocytes are renewed and exported to destinations in the host.¹⁰⁵

A mirror image condition exists after bone marrow transplantation in which the dominant cell population is donor¹⁰⁶ (Fig. 4B). As demonstrated by Przepiorka et

al.¹⁰⁷ and by Wessman et al.,¹⁰⁸ a trace residual population of host leukocytes can be found in essentially all stable human bone marrow recipients who previously had been thought to have complete donor leukocyte chimerism.

PREVIOUS ENIGMAS

In the context developed with this new information, the donor and host leukocytes in organ recipients respond reciprocally (i.e., they mount HVG and GVH responses), and also can present antigen and induce variable degrees of specific non-reactivity (tolerance)^{16,46,70,82–84,106,109} (Fig. 5). If a significant level of mutual tolerance does not occur, one of the immune competent populations will destroy the other, this may be manifested at one extreme by rejection, at the other by graft-versus-host disease (GVHD), or by some combination of the 2 kinds of responses^{46,109} (Fig. 5). The time course of the tolerance induction is highly variable. However, the progression from the immunologic confrontation to either early or delayed graft loss, or to a variable stable state of graft acceptance (with or without immunosuppression), is monitored clinically by serial changes in organ allograft function.

The dynamic modulation of the two immunocompetent leukocyte populations, each by the other, readily explains the poor prognostic value of human leukocyte antigen (HLA) matching for any kind of organ transplantation^{70,82,110} and especially transplantation of the leukocyte-rich liver.^{111,112} The nullification effect also explains why GVHD rarely occurs after transplantation of immunologically active organs such as the intestine and liver, providing the recipients have not been conditioned with cytoablation and do not have underlying im-

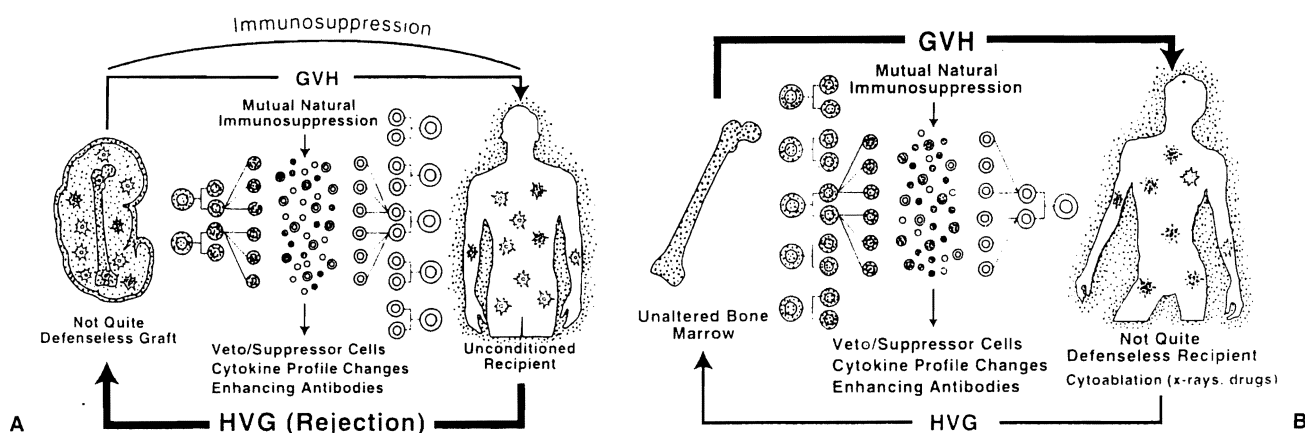


FIG. 4. Paradigm in which transplantation is seen as a bidirectional and mutually cancelling immune reaction that is (A) predominantly host-versus-graft (HVG) with whole organ grafts, and (B) predominantly graft-versus-host (GVH) with bone marrow grafts. The persistence of the minority leukocyte population after both kinds of transplantation was not recognized until the 1990s (see text). Importantly, both the recipient and the allograft after organ transplantation contain a donor and host mixture of immune cells (i.e., leukocytes) and are therefore genetic composites (i.e., chimeric).

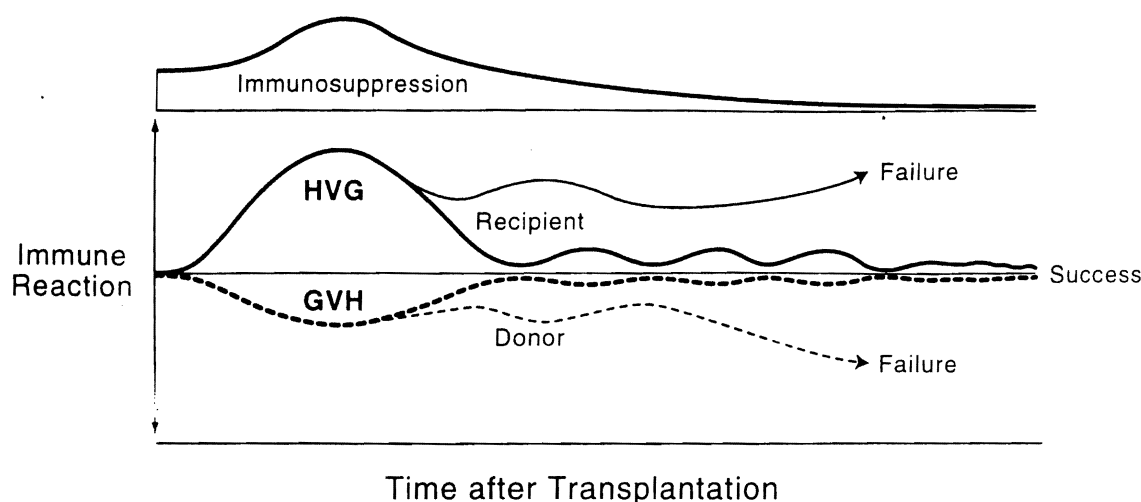


FIG. 5. Contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) reactions after transplantation. Failure is defined as the inability to control rejection, GVHD, or sometimes both. Acute reciprocal clonal exhaustion after successful transplantation is maintained subsequently by chimerism-dependent low-grade stimulation of both leukocyte populations. The responses may wax and wane.

mune deficiency disorders. Deletion of the host arm by cytoablation, as is routinely done before clinical bone marrow transplantation, shifts immune dominance to the donor hematolymphopoietic cells, predisposes the recipient to GVHD, and is responsible for essentially all of the disparities between bone marrow transplantation and organ transplantation (Table 2).

It also was apparent why the liver is more tolerogenic than other organs. The leukocyte load contained in the normal liver is many times greater than that carried by any other organ, and it contains a larger proportion of immature precursor cells and cells of myeloid lineage.⁴⁶ As a corollary, it was predicted that recipients of organs less well-endowed with leukocytes could be safely infused with adjunct donor bone marrow cells (BMC).^{113–115} In practice, BMC infusion has been shown to be safe in such patients and even in recipients of liver and intestinal allografts. However, the need to provide the same immunosuppression as for conventional transplantation of the various organs has imposed

a fundamental limitation on the efficacy of the adjunct BMC strategy (see discussion below).

Finally, the ostensible differences between the adaptive immune response to allografts and the analogous response to microorganisms were explained by the double immune reaction after transplantation versus the single host versus pathogen response of infections. Microorganisms that induce an MHC-restricted adaptive immune response are generally intracellular, and have no or low cytopathic qualities.^{116–118} Host cytolytic T lymphocytes recognize only microorganism-derived peptides that are presented in the context of host MHC molecules. Because elimination of all the infected cells could disable or even kill the host, the mechanism of clonal exhaustion-deletion has evolved with which the immune response can be tempered or terminated, allowing both host and pathogen to survive.^{109,116–120}

The highly variable clinical manifestations of clonal exhaustion-deletion in a patient infected with disseminated non-cytopathic microorganisms (e.g., the common hepatitis viruses) versus the clinical findings in the organ recipient are shown in Figure 6A-C. In one scenario, the pathogen (antigen) load may rapidly increase during the so-called latent period, but then be dramatically and efficiently reduced by antigen-specific effector T-cells. Following control of the infection, the cytotoxic T lymphocytes (CTL) subside (Fig. 6B). The events are similar to those of irreversible organ rejection (or rarely GVHD) in the unmodified or ineffectively treated recipient (Fig. 6, subtext of panel B).

Alternatively, however, such infections may lead to a continuously high antigen load and an antigen specific immunologic collapse (Fig. 6A). The resulting asymptomatic carrier state is analogous to unqualified accep-

TABLE 2. Differences between Clinical Organ Transplantation and Bone Marrow Transplantation

Feature	Organ Transplantation	Bone Marrow Transplantation
Host cytoablation	No	Yes*
HLA matching	Not essential	Critical
Principal complication	Rejection	Graft-versus-host disease
Immunosuppression-free Term for success	Uncommon Acceptance	Common Tolerance

*This therapeutic step allows a relatively unopposed graft-versus-host reaction and accounts for the other differences.

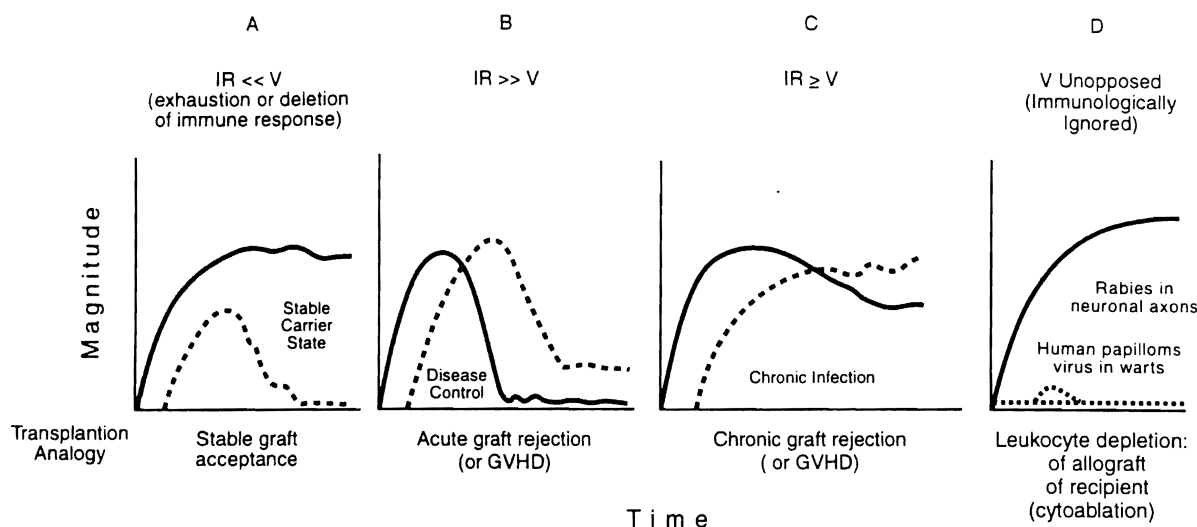


FIG. 6. Potential outcomes after infection with non-cytopathic microorganisms and analogies (expressed as Rejection or graft-versus-host disease) to organ and bone marrow transplantation. The horizontal axis denotes time. The vertical axis shows the magnitude of the viral load (V, solid line), and the host immune response (IR, dashed line).

tance of an allograft. This kind of result after transplantation, which may include independence from immunosuppression, is not often achieved by organ recipients and usually is associated with donor-recipient compatibility of HLA alleles (a good tissue match). Complete tolerance induced by an organ is a mirror image version of successful bone marrow transplantation (Figs. 4 and 5; also compare panel A [organ-induced tolerance] with panel D [BMC-induced tolerance in a cytoablated recipient]).

Between the 2 extremes of destructive (protective) immunity and a carrier (tolerant) state, a persistent infectious agent may induce an unrelenting immune response that results in serious immunopathology such as the chronic active hepatitis following a B or C virus infection. This is equivalent to chronic rejection (or uncommonly GVHD) after liver transplantation (Fig. 6C). Chronic rejection may range from aggressive to indolent, despite the best immunosuppression available today.

IMMUNE REGULATION

We have proposed that the HVG and GVH responses of transplantation as well as the responsiveness or unresponsiveness to the antigens of pathogens, tumors, and self are governed by the migration and localization of the respective antigens.¹⁰⁹ The donor passenger leukocytes represent the only mobile donor antigen in an organ allograft that is capable of reaching host lymphoid organs in large quantity. After organ transplantation, the donor leukocytes migrate hematogenously to the recipient lymphoid organs¹²¹⁻¹²³ (see Fig. 3) where they may induce and exhaust allograft T cells

while activated donor antihost T cells are deleted.¹⁰⁹ If the respective donor and recipient alloantigens do not have access to organized lymphoid collections (epitomized by but not limited to the lymphoid organs) HVG and GVH cytotoxic T cell responses either are not induced (*immune indifference*) or cannot be sustained.^{109,116-118,124} Thus, the only 2 mechanisms needed to explain all permutations of immune responsiveness or non-responsiveness are clonal exhaustion-deletion and immune indifference.

Once initiated, termination of the immune response occurs by 2 kinds of apoptosis, each with distinct molecular pathways (reviewed by Lenardo¹²⁵). If one leukocyte population eliminates the other, its antigen-specific clonal expansion ceases, stopping the secretion of IL2 and other pro inflammatory molecules (corresponding to Fig. 6B). The ensuing "passive" apoptosis of the cytokine-deficient clone requires new protein synthesis, is strongly inhibited by Bcl-2 and related anti-apoptotic molecules, and is thought to involve mitochondrial apoptosis mechanisms rather than the death cytokines such as Fas ligand (FasL) and tumor necrosis factor (TNF).¹²⁵

In contrast, organ engraftment occurs when the alloantigen is not eliminated and when the continuing response is terminated by apoptosis involving FasL and TNF.¹²⁵ If the clonal exhaustion-deletion is complete enough, the recipient may become immunosuppression-independent (Fig. 6A). With the partial tolerance that usually characterizes immunosuppression-dependent organ "acceptance" both kinds of apoptosis may be involved,^{126,127} corresponding to some version of Figure 6C.

The significance of the patchy donor leukocyte (micro) chimerism remaining after the acute post-transplant period has been questioned because of the inconsistency

with which donor leukocytes can be found in blood or tissue samples from organ recipients; the development of acute or chronic rejection despite chimerism; and the inability to use microchimerism to guide post-transplantation drug weaning. Far from eroding the significance of microchimerism, these observations (summarized in¹²⁸) are readily accommodated by the migration/localization concept.^{46,70,82,109} Moreover, most of the ostensibly negative studies have consisted essentially of looking for chimerism in single blood samples.

With serial sampling, Terakura et al.¹²⁹ have shown in a rodent allograft model that a large proportion of the donor leukocytes surviving the early post-transplant period leave the blood and lymphoid tissues during the first 60 transplant days and find niches in non-lymphoid tissues and organs of the host such as the skin, and particularly in the allograft itself,^{96,104} which may provide an optimal syngeneic environment for donor precursor and stem cells.¹⁰⁵ Periodic leakage of the chimeric cells from such non-lymphoid areas to the host lymphoid compartment after transplantation has been suggested as an explanation for the maintenance of clonal exhaustion,^{109,124} analogous to the stable equilibrium between destructive and non-destructive immunity described by Ohashi et al.^{130, 131} in a model of autoimmune diabetes mellitus.

In experimental animals, in which a complete search for donor cells is possible, chimerism always can be found in stable organ recipients.^{103,132} In contrast, the disappearance of microchimerism in an organ recipient presages loss of the outlying allograft from chronic or acute rejection.^{46,98,133} Allograft rejection was associated with thymus-dependent recovery of precursor CTL in the mouse model used by Ehl et al.¹³³ Thus, persistence of donor leukocyte chimerism is a prerequisite for, but not synonymous with and not a consequence of, the evolution of organ-allograft acceptance under clinically relevant circumstances.^{70,82,109}

A THERAPEUTIC IMPASSE?

It is self-evident why it has been so difficult in human organ recipients to achieve the closely related objectives of drug-free tolerance and freedom from chronic rejection, and why these objectives are achieved more frequently with the leukocyte-rich liver than with other less well endowed organ allografts. Tolerance induction, no matter what the organ, depends on the acute clonal activation induced by the migratory donor leukocytes. However, the prevention with immunosuppression of destructive immunity (i.e., rejection) for long enough to allow the variable induction of tolerance has been the sine qua non of organ transplantation. In turn, the penalty for inhibiting either the critical step of cell migration to the host lymphoid organs, or the subse-

quent immune activation (immunosuppressants do both) may be the inability to ever stop drug therapy. In such cases, the reduction of maintenance immunosuppression below the threshold necessary to complement the variably incomplete tolerance, is followed by the disappearance of the donor leukocytes from the host tissues and allograft, and the development of chronic rejection (Fig. 6C).

This chain of events is seen in the majority of human recipients of long surviving organ allografts, but with the lowest incidence of chronic rejection in liver recipients. Clinical efforts to facilitate tolerance in organ recipients with adjunct donor BMC have been hampered by the fact that the same potentially antitolerogenic immunosuppression is required as that used for conventional organ transplantation.¹¹³ The low level chimerism normally found in organ recipients has been increased many fold by the additional load of donor leukocytes and has been reported in some studies to result in a higher incidence of donor specific non-reactivity.^{113-115,134} However, discontinuance of immunosuppression has not been achieved.

DERIVATIVE FALSE DOGMAS

The paradigm that emerged from the chimerism discoveries has necessitated reevaluation of other aspects of transplantation immunology. Studies of allogeneic tolerance mechanisms commonly has been done with "parking" techniques.^{75,76,79,135-137} At Stage 1, tissue or an organ is engrafted with the aid of recipient irradiation, a few post-transplant doses of an immunosuppressant or in spontaneous tolerance liver transplant models^{136,137} without treatment. At Stage 2 of a prototype experiment, the retransplanted allograft is not rejected by naïve animals of the original recipient strain.

It is self evident that parking experiments are inappropriate for most studies of tolerance mechanisms. Stage 1, if it is successful, is an example of tolerance induction per se, although the term tolerance has been studiously avoided by most investigators using these models. By replacing most of the passenger leukocytes with those of the strain to which the altered graft is secondarily transplanted, the essential tolerogenic step of immune activation is eliminated from the retransplantation stage. In addition, the donor strain leukocytes in the parked allografts are not uniformly replaced¹⁰⁴ as has been commonly assumed. Furthermore, neither the residual donor cells nor the replacement cells from the recipient can be viewed as either naïve or purely in terms of antigen. Finally, completeness of graft "acceptance" during the parking or after retransplantation cannot be assumed without examining the allograft for histopathological signs of chronic rejection and without analyzing its leukocyte composition.

The most important dogma to be questioned is the hitherto unchallenged assumption that passenger leukocytes are highly immunogenic while parenchymal cells (especially hepatocytes) are not. The immunogenicity of nonparenchymal cells has been widely attributed to the expression by these bone marrow derived leukocytes of MHC class II and/or co-stimulatory (i.e., B7) molecules. In the antigen migration/localization paradigm,¹⁰⁹ passenger leukocytes appear to be uniquely immunogenic primarily because of their ability to migrate to lymphoid organs whereas organ parenchymal cells are non-immunogenic largely, if not entirely, because they are immobilized within the organ architecture. This would readily explain the otherwise enigmatic finding by Bumgardner and Orosz et al.,¹³⁸⁻¹⁴⁰ that when hepatocytes are isolated and given by infusion, they are as immunogenic, if not more so, than hepatic passenger leukocytes.

Acknowledgment: This work was supported by the National Institutes of Health grants DK 29961, R01 AI/DK 38899, AI 40329, DK 49615, and R01 DK 54232 and the Juvenile Diabetes Foundation 4-1999-807.

ABBREVIATIONS

ALG	antilymphocyte globulin
BMC	bone marrow cells
CTL	cytotoxic T lymphocytes
DC	dendritic cells
FASL	fas ligand
GVH	graft versus host
GVHD	graft versus host disease
HLA	human leukocyte antigen
HVG	host versus graft
IRB	institutional review board
MHC	major histocompatibility complex
PCR	polymerase chain reaction
TBI	total body irradiation
TNF	tumor necrosis factor

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