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The Unfinished Legacy of Liver Transplantation: Emphasis on Immunology

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Abstract

Liver transplantation radically changed the philosophy of hepatology practice, enriched multiple areas of basic science, and had pervasive ripple effects in law, public policy, ethics, and theology. Why organ engraftment was feasible remained enigmatic, however, until the discovery in 1992 of donor leukocyte microchimerism in long-surviving liver, and other kinds of organ recipients. Following this discovery, the leukocyte chimerism-associated mechanisms were elucidated that directly linked organ and bone marrow transplantation and eventually clarified the relationship of transplantation immunology to the immunology of infections, neoplasms, and autoimmune disorders. We describe here how the initially controversial paradigm shift mandated revisions of cherished dogmas. With the fresh insight, the reasons for numerous inexplicable phenomena of transplantation either became obvious or have become susceptible to discriminate experimental testing. The therapeutic implications of the "new immunology" in hepatology and in other medical disciplines, have only begun to be explored. Apart from immunology, physiologic investigations of liver transplantation have resulted in the discovery of growth factors (beginning with insulin) that are involved in the regulation of liver size, ultrastructure, function, and the capacity for regeneration. Such studies have partially explained functional and hormonal relationships of different abdominal organs, and ultimately they led to the cure or palliation by liver transplantation of more than 2 dozen hepatic-based inborn errors of metabolism. Liver transplantation should not be viewed as a purely technologic achievement, but rather as a searchlight whose beams have penetrated the murky mist of the past, and continue to potentially illuminate the future.

Legacy: Something immaterial, as a style or philosophy, that is passed from one generation to another. Anything handed down from, or as from, an ancestor.

During the quarter century that coincided with the birth and lifetime of the journal Hepatology (1981–2005), the philosophy and practice of hepatology were dramatically transformed by the wide acceptance of orthotopic liver transplantation. Numerous milestones in the development and use of this procedure had been reached between 1955 and 1980 (Table 1).[1–36] The prodigious task that lay ahead in 1981 was diffusion of the complex new multidisciplinary enterprise into the national and international healthcare systems (Table 1). [37–73] The extent to which this was accomplished is evident in the 2004 Action Plan for Liver Disease Research designed to "…coordinate research efforts [to treat hepatic and biliary disease] across the NIH".[74]

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Research and Development Opportunities

The NIH plan was divided into 16 chapters, one of which was devoted exclusively to liver transplantation, with primary emphasis on clinical research. The liver transplant chapter began with the simple declarative sentence, “Liver transplantation is now the standard of care for patients with end stage liver disease or acute liver failure.”[74] It was a proud statement from the government agency whose unfailing support had made this possible. But, had liver transplantation matured so completely that there is nothing left to do but fine tuning? This view is negated by links to liver transplantation in almost all of the 15 other chapters of the NIH prospectus. Most of these links were to targets of research opportunity that already had been enriched by, or even owed their provenance to, liver transplantation.

For example, techniques of liver procurement, preservation, and replacement are currently being adapted in non-transplant circumstances (*e.g.*, for subtotal hepatic resections). The discovery that portal venous blood contains substances important for maintenance of liver size, function, and the capacity for regeneration was the beginning of the still-evolving special field of hepatotrophic physiology that is concerned with the functional and hormonal interrelationships of the different splanchnic organs.[24,29,30,75–77] The hepatotrophic studies ultimately led to the cure or palliation with liver replacement of numerous hepatic-based inborn errors of metabolism,[22,48,78] providing the first examples of what might be accomplished in the future with gene therapy and the application of stem cell biology. Finally, religious beliefs, concerns about medical ethics, and public policy or legal issues that surfaced 4 decades ago with the first attempts of liver transplantation[42,79,80] remain as unresolved agenda items in the NIH master plan of 2004.

However, the most frequently identified potential research initiatives in the NIH strategic plan of 2004 concerned the immune response or the manifold consequences of modifying it, not just for transplantation but also in the context of hepatitis, HIV, and oncology (to which separate chapters of the plan were devoted). Using today’s sophisticated tools (particularly those of molecular biology), it now may be possible to expand the sphere of immunology in new directions, fill in knowledge gaps, explain long-standing enigmas, and contribute ultimately to better patient care. With this in mind, the following discussion considers specific issues of immunology that are central to the further development of liver transplantation and to improvement of treatment under multiple nontransplant circumstances.

The Relation of Alloengraftment to Acquired Immune Tolerance: The Historical View

Bone Marrow Transplantation

Transplantation immunology was brought to its current state by a series of events that began in 1943–44 with Medawar’s demonstration that rejection is an immune response.[81] A year later, Owen discovered mixed blood cell chimerism in Free- martin cattle whose fused placentas had permitted fetal cross-circulation[82]; such animals were subsequently shown to be mutually tolerant. Then, in 1953–55, the strong association of donor leukocyte chimerism and acquired donor-specific tolerance was demonstrated in experiments in which allogeneic spleen and bone marrow cells were transplanted without immunosuppression into immunologically immature mice[83] and into irradiated adult mouse recipients.[84] After hematolymphopoietic cell engraftment, the recipients could accept all other donor tissues and organs. The mouse tolerance models escalated over the ensuing 15 years to clinical bone marrow transplantation in immunodeficient and irradiated patients. However, success depended on the use of HLA-matched donors. Otherwise, the penalty for engraftment was lethal graft-versus-host disease (GVHD): rejection of the host by the graft.

Organ Transplantation

In contrast to the "bench to bedside" chronology of bone marrow transplantation, organ transplantation (initially of the kidney) was accomplished in humans[85,86] before proof of feasibility was demonstrated in an animal model and in the apparent absence of leukocyte chimerism. The first 6 kidney recipients with prolonged graft survival (1959–1962) were preconditioned with sublethal total body irradiation but were not infused with donor bone marrow cells.[86–88] In 1960–61, daily post-transplantation azathioprine was shown to prolong kidney survival in dogs[89] and taken to clinical trials. Used alone or with other cytotoxic agents, azathioprine was only marginally effective.[90,91] However, its combination with prednisone made renal transplantation a practical service by exposing 2 features of the alloimmune response[6] that later were demonstrated with liver and all other kinds of organ transplantation and under all other regimens of immunosuppression.

The first unexplained observation was that rejections that developed under azathioprine were easily reversed with the addition of large doses of prednisone rather than being inexorable as previously thought. Second, a successful rejection reversal frequently was succeeded by a greatly reduced requirement for maintenance immunosuppression (Fig. 1), suggesting that the graft was inherently tolerogenic.[6] It also was learned that histocompatibility matching was not a prerequisite for success, that there was little threat of GVHD, and that perpetuation of organ graft survival almost always depended on lifetime drug treatment. In addition to these striking differences from bone marrow transplantation, none of the organ recipients were thought to have donor leukocyte chimerism (Table 2).

Because of these striking disparities, organ engraftment and successful bone marrow transplantation were considered for many years to involve fundamentally different mechanisms. Experimental therapeutic strategies were empirically developed with the objective of endowing organ recipients with the donor leukocyte chimerism-associated mechanisms of the bone marrow recipient while avoiding the penalty of GVHD.[92,93] These strategies[92,94–97] had in common the infusion of donor hematolymphopoietic cells into organ recipients that had been immunologically weakened by irradiation, antilymphoid antibody preparations, or other means (*i.e.*, non-myelotoxic cytoreduction). Although encouraging experimental results have been reported,[98,99] such protocols have not found a significant niche in clinical organ transplantation practice because of their complexity, risks, and unpredictable consequences.

Nevertheless, this body of experimental work demonstrated that the establishment of a hematolymphopoietic population composed of donor and recipient cells was possible in some models with a reasonably low risk of GVHD and could result in donor-specific tolerance, providing the donor cell contribution was at least 1% to 2% ("macrochimerism"). Levels below this ("microchimerism") were interpreted as either negative findings or artifacts. By so doing, the historical paradigm that attributed bone marrow and organ engraftment to different mechanisms required no substantive revision.

A Unification of Bone Marrow and Organ Transplantation

The historical paradigm was not challenged until 1992. When small numbers of multilineage donor hematolymphopoietic cells (microchimerism) were found in animal and human recipients of long-surviving kidney and liver allografts,[64,65,100–102] it was postulated that the mechanisms of organ engraftment differed only in degree from the leukocyte chimerism-dependent ones of successful bone marrow transplantation. Organ engraftment was now defined as a variable form of tolerance that resulted from "…responses of coexisting donor and recipient immune cells, each to the other, causing reciprocal clonal expansion followed

by peripheral clonal deletion."[64,65] The graft-versus-host arm of the double immune reaction (the inverted curve in Fig. 1) usually was clinically inapparent.

The prerequisite for the donor-specific tolerance was migration of the graft's passenger leukocytes to host lymphoid organs and induction there of the host-versus-graft response. Because the multilineage passenger leukocytes of an organ are of bone marrow origin, their hematogenous migration into the recipient was in essence the equivalent of a bone marrow cell infusion. Exhaustion and deletion of the antidonor response explained the characteristic rejection reversal and subsequent decline in need for immunosuppression in organ recipients (Fig. 1). Cytoablation of bone marrow, but not of organ recipients, was the apparent reason for essentially all of the differences between the two kinds of transplantation, including the high risk to the bone marrow recipient of GVHD, and the need to restrict marrow donors to those with a histocompatibility match[103] (Table 2). Essentially all cytoablated bone marrow recipients have a small residual population of their own hematolymphopoietic cells (*i.e.*, mirror image microchimerism) rather than complete bone marrow replacement.[104]

This unified view of transplantation was controversial,[98,105–110] in part because it was incompatible with dogmas that made up much of the foundation of transplantation immunology. One point of contention was our view that historically rooted alternative engraftment mechanisms (listed in Table 3) were either epiphenomena or "variants or stages" of the clonal exhaustion-deletion that followed the key event of leukocyte migration.[64] In addition, the role of small numbers of persistent cells (the microchimerism) in perpetuation of long-term graft survival was not yet clear. Finally, the crucial mechanism of clonal exhaustion-deletion was still generally considered to be only a theory. Although we did not know it at the time, precisely these issues were being addressed independently in Zurich but in the context of immune responsiveness and unresponsiveness to non-cytopathic microparasites (see below).

Tolerance From the Infection Perspective

The major histocompatibility complex–restricted mechanisms of T cell recognition of, and response to, noncytopathic microorganisms, and to allografts, had been elucidated by Zinkernagel and Dougherty in the 1970s,[111–113] but the mechanisms of acquired immune nonreactivity remained a puzzle. During the early 1990s, Zinkernagel and his associates in Zurich formally proved in mouse infection models that the anti-pathogen T cell response could be exhausted and deleted[114] and concluded that this was the explanation for the carrier state that may develop after infection with noncytopathic (intracellular) viruses.[115] As with transplantation, the critical step was migration of the pathogen or its peptide to host lymphoid organs. The presence of virus that failed to reach these destinations was not recognized by the host. This was termed immune indifference (now called immune ignorance). In essence, Zinkernagel et al. had clarified the interrelationship of immunity and tolerance to pathogens, and had defined tolerance in terms of two essential mechanisms: immune ignorance and clonal exhaustion deletion. Moreover, they placed these mechanisms in the same dynamic context of antigen migration as that of transplantation.[115]

Once the viral antigen reached lymphoid destinations and induced a cytolytic T lymphocyte (CTL) response, the outcomes in the highly controlled models of lymphocytic choriomeningitis virus (LCMV) infection were determined by the balance between the amount of virus antigen and the number of induced antigen-specific CTL.[115] Analogous to the maximal flood of passenger leukocytes migrating from a transplanted organ, the critical period was during the first few days or weeks of viral replication. If the CTL were induced in sufficient numbers, the result was disease control; if not, the result was clonal exhaustion-deletion and a carrier syndrome that ranged from asymptomatic to progressively more

serious disease states. No matter what balance was established acutely, perpetuation of this balance (whether immunity, deletional tolerance, or some stage in between) depended on persistence of antigen with access to host lymphoid organs. The ability of small amounts of persistent virus to survive was attributed to its relocation in non-lymphoid sites that were inaccessible to host immune effector mechanisms.[115] From these protected niches, the virus migrated secondarily to host lymphoid organs and could sustain immunity, or alternatively, maintain tolerance. Thus, persistent antigen was a two-edged sword.

The Boundary Between Immunity and Tolerance

The analogies between transplantation and infection were summarized in 1998 and generalized to other branches of immunology in the following statement: "…Migration and localization are the governing factors in immunologic responsiveness or unresponsiveness against infections, tumors and self, and against xenografts and allografts."[66] All of the clinical scenarios of transplantation, and those resulting from infection by noncytopathic pathogens, could be correlated with the routes of migration and the ultimate localization of the respective antigens. The "gray area" between unequivocal immunity and durable tolerance included a diversity of transplant outcomes short of outright acute irreversible rejection, as well as a panoply of analogous virus carrier states; all represented different degrees of partial tolerance. Such immunologic "compromises" included chronic allograft rejection and its analog, chronic hepatitis.[66]

The Rejection Option

Organ Transplantation—The antigen migration is essentially the same with transplantation of the liver and any other surgically revascularized whole organ (Fig. 2A). Movement of the passenger leukocytes is selective at first to host lymphoid organs, where a clonal antidonor T cell response is induced. The CTL then target the transplanted organ as well as the peripheralized cells of the source graft (Fig. 2A).

Infection—The migratory principles after infection by noncytopathic microparasites are the same as those of passenger leukocytes, but the details differ because both the pathways of microorganism migration and the targets of the CTL response are dictated by the tropism of the various pathogens (Fig. 2B–D). For example, because of the liver tropism of the hepatitis viruses, the quantity of viral antigen that migrates to host lymphoid organs is small compared with that homing to the liver (Fig. 2B). At the lymphoid organs, hepatitis virus–specific CTL are induced by infected antigen-presenting cells (APC) displaying complexes of major histocompatibility complex molecules plus peptides derived from the pathogen. Because the induced CTL then destroy infected host cells no matter where these "non-self cells are located, the principal disease expression is hepatic (Fig. 2B). Infections with other intracellular viruses (Fig. 2C–D) are variations on the same theme (see later discussion).

The Tolerance Option Without Immunosuppression

Organ Transplantation—Exceptions to the outcome of immunity (*i.e.*, rejection) in untreated transplant recipients are rare. However, lifetime tolerance to liver allografts occurs without therapeutic manipulation in approximately 20% of outbred pig recipients[18–20,116,117] and with nearly 100% regularity using selected donor–recipient rat strain combinations,[118–120] and most mouse strain combinations.[121] Less well leukocyte–endowed mouse heart[121,122] and kidney allografts[123] also reliably self-induce tolerance, although in far fewer strain combinations. These models of spontaneous transplantation tolerance demonstrate that graft-induced immune non-reactivity is a normal potential option of the immune response (Fig. 3A). Importantly, the "spontaneous tolerance" may be abrogated in some of these models by administering immunosuppression.[124–128]

Infection—After most infections by noncytopathic parasites, the balance between antigen and antigen-specific CTL tilts within a few weeks to immunity. The exceptions in which protective immunity frequently does not develop constitute a collection of some of the world's most difficult-to-treat diseases [*e.g.*, acquired immune deficiency syndrome (AIDS), hepatitis, and malaria].

Therapeutic Implications

Infections With Noncytopathic Microorganisms

In 1994, Zinkernagel and Hengartner discussed the comparative pathophysiology of HIV (a retrovirus) and hepatitis B virus (HBV; a DNA virus) in the context largely developed with their studies of the LCMV (an RNA virus). The authors argued that the consequences of HIV were caused principally by virus-specific cytotoxic T cell–mediated immunopathology rather than by direct cytolytic effects or any other mechanism.[129] The essence of their argument is shown in Fig. 2C. After HIV antigen delivery to lymphoid organs by infected dendritic cells or other APC, a CTL response is induced that targets CD4 (T helper) and all other infected cells. With the selective destruction of infected CD4 cells and eventual decimation of the infected APC population, the inadequately renewed HIV-specific CTL clone is easily exhausted and deleted and, eventually, the entire CTL population dwindles. Thus, the immunodeficiency of AIDS may be prevented at the outset by a strong CTL response, or alternatively, the response may cause immunodeficiency. The outcome from the events shown in Fig. 2C depends on the balance established at an early time between the amount and localization of the virus versus the CTL response. A similar pathogenesis, but with variable targets and outcomes, pertains with all noncytopathic microparasites: for example, hepatitis (Fig. 2B) and cytomegalovirus (Fig. 2D).

From this point of view, Zinkernagel and Hengartner had suggested in 1994 that the foremost therapeutic objective under most circumstances is to limit the extent of the infected cell targets, or in clinical terms to reduce the viral load and restrict its spread.[129] This has since been accomplished in HIV victims with zidovudine and protease inhibitors. Once a large target is established, the deliberate resurrection of a strong CTL response could have the disastrous consequence of widespread host cell killing. A better option under these circumstances might be the administration of T cell–directed immunosuppression in just the right amount to prevent the CTL-mediated destruction of massive numbers of host cells, but not so much that runaway viremia would merely expand the target for attack from a recovering CTL clone. Thus, the therapeutic aims in an intractable noncytopathic pathogen infection would be to predict, monitor, and equilibrate beneficial balances between pathogen distribution and the absence of an immunopathologic T cell response.

The Organ Engraftment Objective

Comparable antigen/CTL balances must be found and kept stable for successful transplantation. The best chance to establish a balance selectively favoring tolerance is during the first few post-transplantation weeks, during which the maximal donor leukocyte migration provides the optimal conditions for reciprocal exhaustion-deletion of the double immune response (Figs. 1, 3B). This one time only the window of opportunity could be narrowed or closed by so much prophylactic immunosuppression that clonal activation is subverted. To the extent this were to occur, later reduction of the primary over-treatment predictably would lead to recovery of the ineffectively deleted clone with the clinical consequence of delayed rejection (Fig. 3C).[70] However, the penalty of too little immunosuppression during the critical early period may be irreversible rejection. In 2001, this dilemma, it was thought, could be addressed by application of one or both of the therapeutic principles depicted in combination in Fig. 4.[70]

The first principle (shown alone in Fig. 3B) consists of administration of no more post-transplantation immunosuppression than the amount needed to prevent irreversible immune-mediated damage. Because of histocompatibility and other confounding parameters in the human population, such ideal immunosuppression in individual patients cannot be accurately predicted. With the second principle (recipient pretreatment), host global immune responsiveness can be reduced by nonmyeloablative conditioning before arrival of donor antigen, thereby bringing the anticipated donor-specific immune response into a more easily controllable and deletable range (Fig. 4). The 2 principles have been combined in a practical regimen using a single large dose of an antilymphoid antibody before transplantation followed by minimalistic post-transplantation tacrolimus monotherapy with the intent of eventual weaning (Fig. 5). Satisfactory results in liver (Fig. 6) and other kinds of organ recipients with reduced overall exposure to immunosuppression have been obtained,[72,130–132] with the striking exception of liver recipients whose chronic end-stage hepatic failure was caused by hepatitis C virus (HCV)[73,133] (see next section).

The frequency and extent to which drug weaning can be accomplished in hepatitis-free liver recipients with such tolerance-facilitating immunosuppression has yet to be determined. Complete stoppage of treatment has been attempted in only a few of these recent cases. However, the feasibility of liberation from immunosuppressive drugs is evident from observations of our first 210 liver recipients: 184 at the University of Colorado (between 1963 and 1980) and 26 at the University of Pittsburgh (in early 1981). Thirty-five (17%) of the 210 have now reached or passed their post-transplantation silver anniversary (Fig. 7). Thirty-two (15%) are still alive from 24.5 to 35.7 post-transplantation years (mean, 27.1 ± 3.1 SD), and 3 died after 25 years of lung disease (#42), widespread metastases from colon carcinoma (#82), and *de novo* HCV infection (#93).

Importantly, 16 of the 35 quarter-century survivors had periods of 3 to 31 years off immunosuppression, as indicated by the green portion of the horizontal bars in Fig. 7. In 5 of these 16 recipients, immunosuppression was resumed, but not because of breakthrough rejection in any case. The reason for treatment reinstitution in 2 patients was liver retransplantation necessitated by intractable biliary tract complications (#202 in Fig. 7) or because of HCV hepatitis (#125). A third recipient whose cyclosporine-based immunosuppression was stopped after 11 years was returned to treatment 6 years later because of cadaver kidney transplantation (#192). The other 2 recipients who were asymptomatic and had rejection-free biopsies were restarted on treatment because of patient and physician anxiety (#64 and #105, in Fig. 7).

The Price of Chronic Immunosuppression

The consequences of a decision to resume immunosuppression without a clear justification may not be evaluable for years or decades. When a detailed account of our 210 first recipients was published in the June 1993 issue of HEPATOLOGY,[65] 50 (23%) of the original patients had survived for at least a decade (maximum, 23 years). However, 7 had died 1 to 11 years after their 10th anniversary, leaving 43 (20.5%). Rejection had not caused any of the late deaths. Instead, the most common causes of death were hepatitis, other infections, malignant neoplasms, and drug-specific side effects. The same pattern has been apparent in the further shrinkage of survivors between 1993 and 2005 (from 43 to 32) for reasons other than rejection. Chronic immunosuppression clearly has been the principal direct or indirect cause of late mortality.

Organ Engraftment in HCV-Infected Patients

Although the importance of reducing or eliminating long-term immunosuppression is clear, when the tolerogenic strategy depicted in Figs. 4 and 5 was applied in Pittsburgh in HCV-

infected liver recipients, the results fulfilled the 1994 prophecy of Zinkernagel and Hengartner[129] (see earlier discussion). First, the lymphoid depletion caused viremia. Then, when later attempts were made to wean from tacrolimus monotherapy, the heavily infected liver graft was targeted by recovering CTL, resulting in early and severe recurrent HCV disease. With recognition by Eghtesad and Fung et al.[73] of what had happened, the explanation also was apparent for the worldwide epidemic of HCV recurrence that was associated with the various regimens of viremia-inducing heavy multiple drug immunosuppression.[134–138] Because strong prophylactic immunosuppression cannot be administered indefinitely without fatal consequences, such treatment eventually must be reduced. The consequent CTL recovery was leading to widespread destruction of infected allograft cells in the same way as with the weaning of our lymphoid-depleted patients.[73]

In contrast, light but continuous double-drug immunosuppression with tacrolimus and prednisone has allowed the systematic development of a relatively asymptomatic carrier state: a stable equilibrium between HCV and HCV-specific CTL.[73] Thus, treatment protocols that minimize disease recurrence in HCV-infected liver allograft recipients must balance the desire to reduce immunosuppression or induce allotolerance with the need to prevent antiviral immunopathology. Curtailment of the epidemiologic implications of producing HCV-carrier recipients, and of the probability of insidious disease recurrence, will depend on containment of the viral load with yet to be developed HCV-specific drugs. One candidate is the protease inhibitor recently described in Hepatology by Reiser et al.[139]

Tumor Surveillance

By the late 1960s, convincing evidence existed that immunosuppression in organ recipients could result in accidental engraftment of donor malignancies, accelerated growth of tumor metastases, or the development of new malignancies (summarized in[140,141] Because the highest risk from these consequences has been in liver recipients, liver transplant centers have become hotbeds of oncology research. Two tumors have been of particular interest because of their etiologic association with intracellular microorganisms: post-transplantation lymphoproliferative disorders (PTLD) with the Epstein-Barr virus, and hepatocellular carcinoma with HBV. The PTLD, most of which are B cell lymphomas, provided the first unequivocal proof of immune surveillance of a human malignancy when they disappeared after withdrawal of T cell–directed immunosuppression.[45]

The possibility of preventing virus-associated tumors with vaccines was demonstrated by Coggin, Larson, and Hilleman[142] with proof of principle studies in primates of the SV_{40} virus, and extended to humans 20 years later with the recombinant HBV vaccine trials. The HBV- associated hepatoma scourge was virtually eliminated in immunized Asian populations.[143] How the anti–HBV-induced antibodies produced by the vaccine interrelate with T cell immunity has been thoroughly studied with LCMV[144] and explained by Klenerman.[145] The research and therapeutic opportunities opened by these and other observations is too vast to dwell on here beyond emphasizing that the mechanisms of immune reactivity and non-reactivity to tumors are the same as those of transplantation.

Autoimmunity

The chimerism-dependent mechanisms of non-reactivity to allografts, all of which depend on mobile leukocytes, can be identified in a continuum of classical tolerance models that began with the observation by Owen of mixed blood cell chimerism in Freemartin cattle[82] and ended in 1992 with the discovery of microchimerism in liver and other kinds of organ recipients (Fig. 8). Of historical interest, Paul Ehrlich recognized more than a century ago that a patient’s tissues could be destroyed by an immune system run amuck. Ehrlich’s term of “horror autotoxicus” is today’s autoimmune disease. He postulated that there must be

mechanisms to prevent this by "…a regulatory contrivance as yet undescribed."[146] In the context of immunology described here, the contrivance that Ehrlich envisioned consisted of the mechanisms that have made transplantation feasible.

A Need for Closure

A coherent profile of immune function and governance has emerged from the studies of transplantation and infection summarized here. However, mechanisms of nonresponsiveness other than the essential ones of immune ignorance[115,147–150] and clonal deletion (listed in Table 3) have generated a large body of historical[151] and recent publications.[152–155] In turn, these model-specific and poorly understood alternative mechanisms have become elements of recurring immunological dogmas and theories. Is assigning them essential roles in transplantation tolerance justifiable? Evidence of their existence derives from phenomena observed in experimental models in which the immune system is drastically perturbed (*e.g.*, under conditions of lymphopenia) or the antigenic barriers to transplantation are significantly weakened (*e.g.*, transplantation across minor histocompatibility mismatches). To complete the picture, it will be necessary to definitively determine the conditions for the development of these phenomena and to accurately assess their functional significance.

Conclusion

The article entitled "The Evolution of Liver Transplantation" published in 1982 in Hepatology concluded with the statement that "… what was inconceivable yesterday, and barely achievable today, often becomes routine tomorrow."[38] Liver transplantation proved to be a prime example. The accomplishment was more than the addition of the crucial centerpiece for the treatment of otherwise lethal end-stage hepatic disease, however. Liver transplantation was from the beginning, and will continue to be, an instrument of scientific discovery in multiple fields, and above all in immunology.

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Abbreviations

GVHD	graft-versus-host disease
LCMV	lymphocytic choriomeningitis virus
CTL	cytolytic T lymphocyte
APC	antigen-presenting cells
HBV	hepatitis B virus
HCV	hepatitis C virus
PTLD	post-transplantation lymphoproliferative disorders

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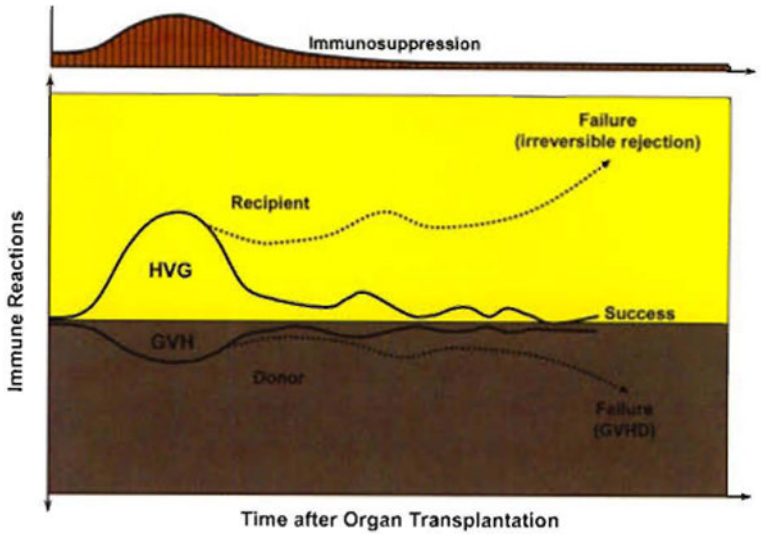

Fig. 1.

Contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) immune responses after transplantation. The presence of the weaker GVH response in organ recipients (the inverted curves on a gray background) was not recognized until the discovery of microchimerism in 1992.[64,65] However, the demonstration in 1962–63[6] that the HVG response (rejection) is reversible and may be succeeded by a state of variable tolerance (yellow panel) allowed the practical development of immunosuppression-aided clinical organ transplantation.

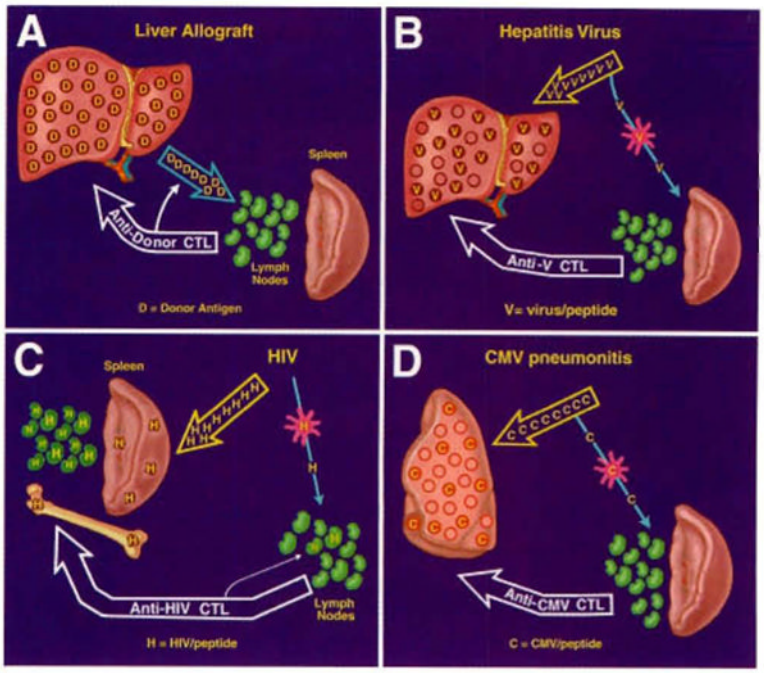

Fig. 2.

The consequences of antigen spread and localization (see text): (A) with liver or other kinds of organ transplantation; (B) with a viral hepatitis infection; (C) with a human immunodeficiency virus (HIV) infection; (D) with a pulmonary cytomegalovirus infection.

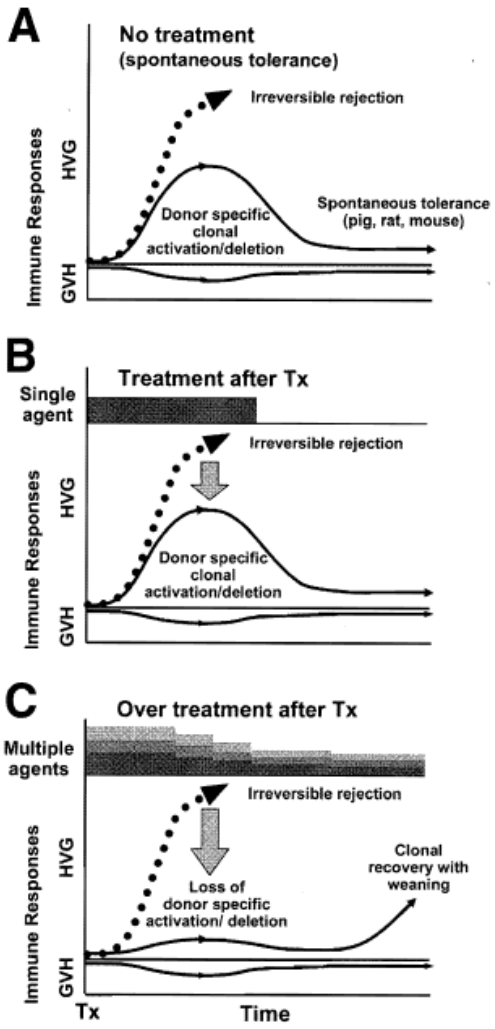

Fig. 3.

Organ-induced tolerance. (A) Spontaneous tolerance induced by the liver in experimental models[18–21,118–121] in which the host-versus-graft immune response induced by the migratory donor leukocytes is too weak to eliminate the donor antigen and is exhausted and deleted (the rise and fall of the continuous thin line). Maintenance of engraftment depends on small numbers of persistent donor leukocytes (microchimerism). The less well–leukocyte-endowed heart and kidney also can “self- induce” engraftment but in a much smaller number of models. (B) Immunosuppression-aided tolerance in organ transplantation models in which the recipient response that normally would cause rejection (dashed line) is reduced into a deletable range (continuous thin line) with a short course of early post-transplantation immunosuppression. Lifetime tolerance after stopping immunosuppression was first convincingly demonstrated in canine liver recipients.[11] (C) A self-defeating consequence of excessive prophylactic over-immunosuppression (depicted with multilayered bars) that subverts efficient clonal exhaustion- deletion. The initially over-treated recipient may be committed to unnecessarily high maintenance immunosuppression. This undesirable effect of too much immunosuppression was not recognized until 2001.[70] Tx = Transplantation

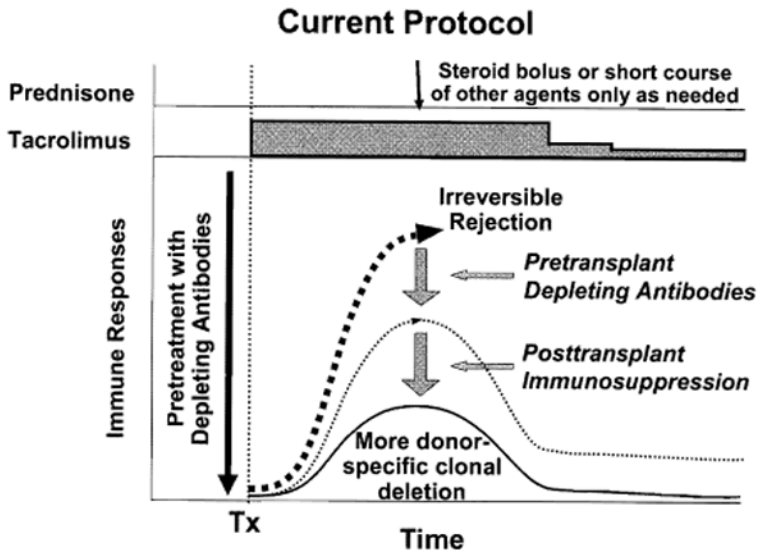

Fig. 4.

Protocol of “tolerance friendly” immunosuppression introduced at the University of Pittsburgh Medical Center.[70] An antilymphoid antibody infusion before arrival of an allograft reduces the anticipated antidonor response into a more readily deletable range and allows maintenance treatment to begin with daily monotherapy to which other agents are added only for rejection. Weaning from the monotherapy may be possible later. The inverted curve at the bottom shows the usually silent graft-versus-host (GVH) reaction shown more clearly in Figure 1. Tx, transplantation

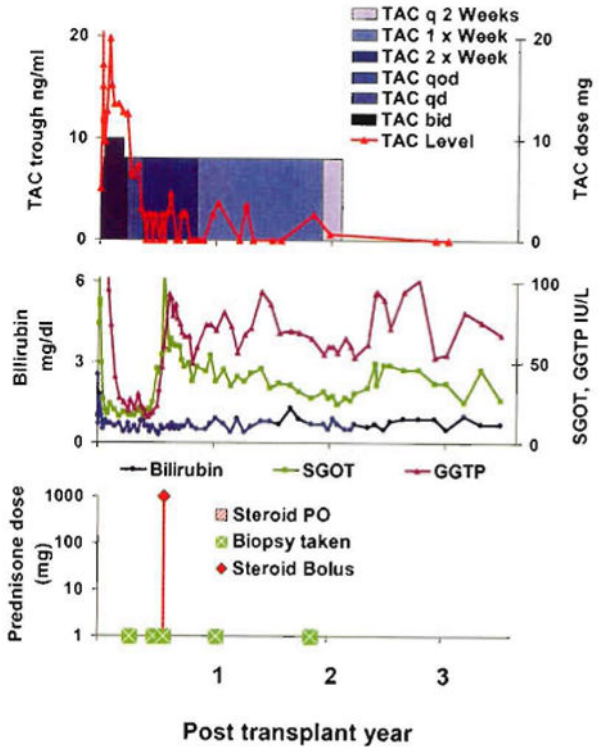

Fig. 5.

An example of the strategy shown in Fig. 4. A woman with a hepatic hemangioendothelioma who was infused with 5 mg/kg antithymocyte globulin (ATG, thymoglobulin) before liver allograft revascularization. Tacrolimus (TAC) monotherapy was reduced from daily to every other day at 100 days, and to once per week by 10 months. Treatment was stopped at 22 months. She has been immunosuppression-free for 11/2 years. Serum bilirubin and measures of hepatic parenchymal function have been normal throughout. Although enzyme levels have been stable, these have hovered at a high normal range or slightly above since a rejection at 6 months, which was treated with 1 g methylprednisolone. SGOT: serum glutamic-oxaloacetic transaminase, GGTP: gamma-glutamyl transpeptidase.

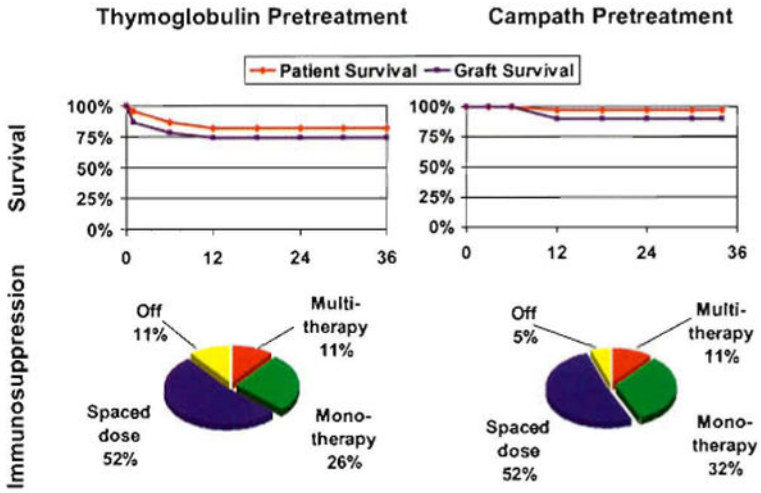

Fig. 6.

Patient and graft survival (above), and current immunosuppression (below) of hepatitis-free primary liver transplant recipients treated in Pittsburgh with the principles of immunosuppression shown in Fig. 4, and exemplified in Fig. 5. Left: pretreatment was with an infusion of 5 mg/kg rabbit ATG (thymoglobulin) (n = 23). Right: pretreatment was with 30 mg alemtuzumab (Campath®) (n = 38).

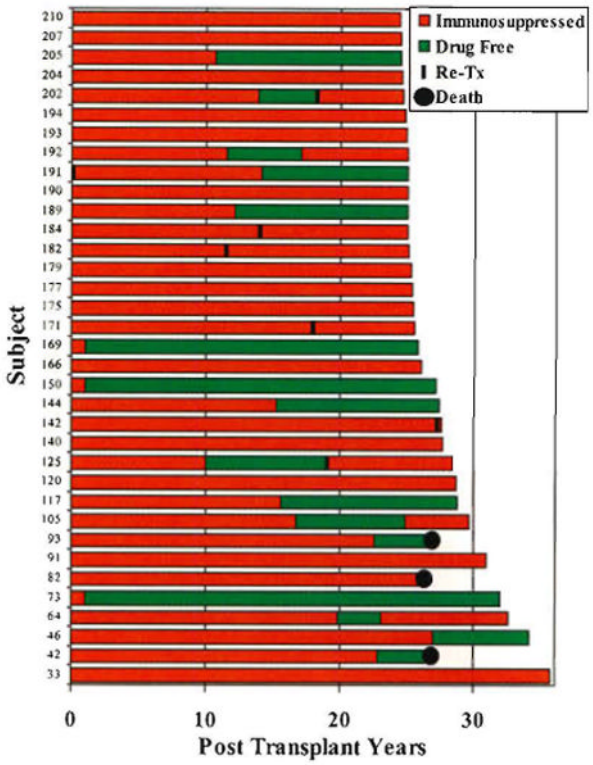

Fig. 7.

The 35 patients from the first 210 who survived 25 years or longer after liver replacement at the University of Colorado (n = 184) or University of Pittsburgh (n = 26). The times on (red) and off immunosuppression (green) in individual patients are displayed in the horizontal bars. Note that 6 of the 32 still-surviving recipients had retransplantation 11 to 28 years after the primary procedure.

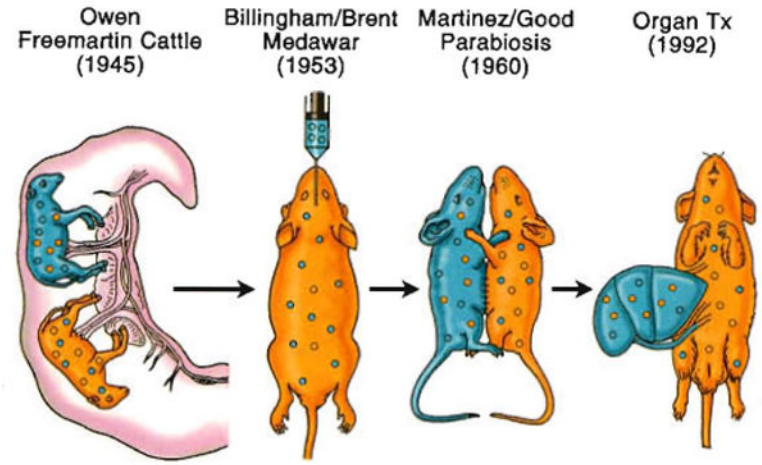

Fig. 8.

The relation of organ-induced engraftment (right) to classical models of spontaneous donor leukocyte chimerism-associated tolerance.

Table 1

Milestones of Liver Transplantation

Year	Description	Reference
1955	First article in the literature on auxiliary liver transplantation	1
1956	Concept of liver replacement first mentioned	2
1958–1960	Results reported of canine total hepatectomy and liver replacement without immunosuppression	3,4
1960	Abdominal multivisceral transplantation described in non-immunosuppressed dogs	5
1963	Azathioprine-prednisone cocktail introduced (kidneys first, then livers) and recognition of organ-induced tolerance	6
1963	Description of *in situ* preservation and procurement of cadaveric organs	3,7
1963	First attempts to transplant the human liver	8
1964–1965	Evidence reported of hepatotrophic (liver-supporting) factor(s) in portal venous blood	9,10
1965	Liver-induced tolerance under a short course of azathioprine reported in dogs	11
1966	First liver xenotransplantation on July 15, 1966 (chimpanzee donor)	12
1966	Clinical introduction of ALG for kidney, then liver recipients	13
1966–1970	Evidence that HLA matching would not be a major factor in cadaveric organ transplantation	14,15
1967	First 1-year survivals after human liver replacements	16
1967–1968	Acceptance of brain death concept	17
1967–1969	Liver-induced tolerance in pigs without immunosuppression	18–20
1969	First textbook of liver transplantation based on 25 Denver cases	21
1969	First palliation (or cure) of inborn error of metabolism with liver transplantation	22
1973–1983	Evidence accrued that the liver controls cholesterol homeostasis	23–27
1973	Description of the liver's resistance to antibody-mediated rejection	28
1973–1975	Principal portal blood hepatotrophic factor identified as insulin	29,30
1976	Causes of failure analyzed in first 93 Colorado cases of liver transplantation	31
1976	Improved slush liver preservation permitted long-distance procurement	32,33
1979	Systematic use of arterial and venous grafts for cadaver liver revascularization	34
1979	Cyclosporine introduced for organ transplantation including 2 liver recipients	35
1980	Cyclosporine-steroid cocktail introduced clinically	36
1981	80% 1-year liver recipient survival reported using cyclosporine-prednisone	37
1982	Review of progress in liver transplantation generates widespread interest of hepatologists	38
1983	Introduction of pump-driven venovenous bypass without anticoagulation	39–41
1983–1995	USA consensus development conference conclusion that liver transplantation is a service (1983) is followed by rapid proliferation of transplant centers worldwide	42
1984	Standardization of *in situ* preservation-procurement techniques for cadaver multiple organ donors	43,44
1984	Reversibility reported of B cell malignancies (PTLD) in liver and other organ recipients	45
1984	Reports of reduced-size liver grafts for pediatric recipients	46,47
1984	Liver transplantation of patient with hypercholesterolemia verified hypothesis that the liver is the site of cholesterol homeostasis	48,49
1987–1989	First successful transplantation of liver-containing multivisceral grafts	50,51
1987	University of Wisconsin (UW) solution improves preservation of liver and other organs	52–54
1987	Successful extensive use of livers from "marginal" donors reported	55
1988	National adoption of Pittsburgh point system for cadaver kidney and liver distribution complies with Organ Transplant Act of 1984	56,57
1989	Popularization of the "piggyback" variation of liver transplantation	58

Year	Description	Reference
1989	Clinical introduction of FK 506-(tacrolimus)-based immunosuppression	59,60
1989	First report of split cadaver liver for transplantation into 2 recipients	61
1990	First use of live liver donors (left side fragments)	62,63
1992–1993	Discovery of donor leukocyte microchimerism in liver (and other organ) recipients, placing organ and bone marrow cell transplantation on common ground.	64,65
1998	Delineation of analogies between transplantation and infection immunology	66
1994–1999	Live donor transplantation of right side liver fragments	67–69
2001	Mechanism-based tolerogenic immunosuppression proposed	70
2003	Double knockout of porcine α1,3GT gene, revitalizing hopes of clinical xenotransplantation	71
2003	Clinical use of tolerogenic immunosuppression	72
2005	Mechanisms of recurrent hepatitis under transplant immunosuppression elucidated.	73

Abbreviation: ALG, antilymphocyte globulin.

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	Uncommon	Common
Term for success	Acceptance	Tolerance
Leukocyte chimerism	No	Yes

Abreviation: HLA, human leukocyte antigen.

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

Table 3

Mechanisms of Immune Nonreactivity*

Essential	
1	Clonal exhaustion-deletion
2	Immune ignorance
Alternative	
1	Special recipient cells: T-regulatory, suppressor, veto
2	Antibodies: Idiotypic, “enhancing”
3	Cytokines: Self-perpetuating combinations
4	Inhibitory molecules: Down-regulation
5	Graft secretions: Soluble HLA antigens
6	Antigen presentation: Defective or deviant
7	Anergy: Absence of second signal; or cytokine exhaustion

* Modified from Starzl TE. J Am Coll Surg 2005;201:160–170. Reprinted with permission.