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Acquired immunologic tolerance: with particular reference to transplantation

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Abstract The first unequivocally successful bone marrow cell transplantation in humans was recorded in 1968 by the University of Minnesota team of Robert A. Good (Gatti et al. Lancet 2: 1366–1369, 1968). This achievement was a direct extension of mouse models of acquired immunologic tolerance that were established 15 years earlier. In contrast, organ (i.e. kidney) transplantation was accomplished precociously in humans (in 1959) before demonstrating its feasibility in any experimental model and in the absence of a defensible immunologic rationale. Due to the striking differences between the outcomes with the two kinds of procedure, the mechanisms of organ engraftment were long thought to differ from the leukocyte chimerism-associated ones of bone marrow transplantation. This and other concepts of alloengraftment and acquired tolerance have changed over time. Current concepts and their clinical implications can be understood and discussed best from the perspective provided by the life and times of Bob Good.

Keywords Tolerance · Alloengraftment · Organ transplantation · Bone marrow transplantation · Clonal exhaustion-deletion · Immune ignorance · Immunosuppression · Chimerism · Microchimerism

Introduction

How do we remember the man to whom we pay homage this week? My assessment more than 10 years ago was as follows: "Bob Good, more than any other individual, is

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acknowledged to be the Father of Clinical Immunology. The pattern of essentially all of his achievements explains why. He invariably started by seeing disease in its human victims. Haunted by the appalling sight, he sought to understand the true biologic meaning of these disorders. Frequently, he started with a clean slate in that the diseases were 'mystery afflictions' of unknown cause. Finally, he evolved cures or palliation, not once, but many times. Other great men have done one or two such things, but rarely, if ever, on such a grand scale''.

After describing Good's accomplishments, this letter of nomination for an important honor concluded: ''It may be that the very diversity of Dr. Good's contributions diminished recognition of their individual importance. That is like saying it is easier to watch the stars than to gaze at the sun. Others understood the galaxies, but Good understood the universe. What he did for 50 years was to freely distribute intellectual gems, with no thought of credit or return. While being one of the most cited scientists in the world, he radiated nobility and humanity''.

What better reasons could there be to have a Robert A. Good Immunology Society? Most, if not all, of its founding members were under Bob Good's sphere of influence at sometime in their professional lives. They, and members elected from succeeding generations, will make certain that he lives on.

Proposed axiom: organ engraftment is a form of tolerance

Acquired immunologic tolerance was one of Bob Good's most enduring preoccupations. It also has been the ''holy grail'' dreamed of by organ transplant surgeons ever since the classical experiments of Billingham, Brent, and Medawar [1, 2] and Main and Prehn [3] showed that tolerance to allografts was strongly associated with donor leukocyte chimerism. Here, I will defend the concept that the successful engraftment of an organ means that the recipient has developed some degree of leukocyte chimerism-dependent donor-specific tolerance, and that the completeness of this tolerance can be inferred from the amount of immunosuppression necessary to maintain stable function and structure of the graft.

The bellwether kidney transplant cases in Denver

The evidence for this axiom has historical roots that can be traced back more than 40 years to two observations summarized in the title of a 1963 report from the University of Colorado [4]. The first finding was that kidney allograft rejection was highly reversible, rather than being inexorable as had been previously assumed. The second observation was that the reversal of rejection frequently was succeeded by what was construed to be variable acquired tolerance. The patients described in this report had been treated with azathioprine (Imuran^{R}) for one to 4 weeks before as well as after transplantation of kidney allografts from familial donors (not identical twins) (Fig. 1A). The rejections that occurred in almost all recipients were diagnosed by increases in serum creatinine, following good early post-transplant function. About 15% of the grafts were promptly lost to acute rejection despite the addition of steroids. But in the other cases, the rejections were reversed with large doses of prednisone (Fig. 1A). The ability after successful treatment of a rejection to wean immunosuppression to very low levels in many recipients (Fig. 1A) was interpreted as evidence of donor-specific tolerance. Although controversial, the descriptive use of the word ''tolerance'' proved to be appropriate. Nine (19%) of the 46 familial donor

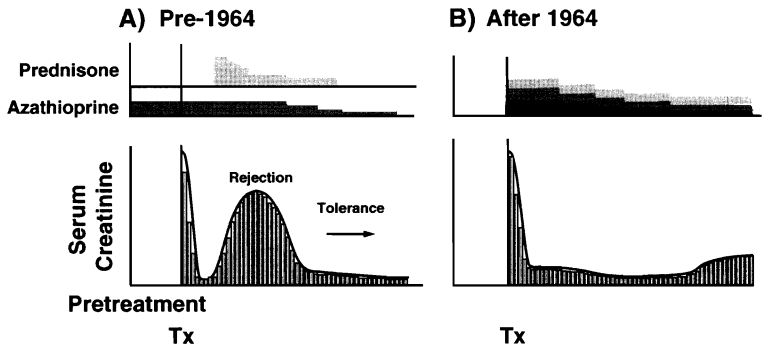

Fig. 1 An historical shift in policy of immunosuppression for organ transplant recipients. (**A**) During 1962–1964: azathioprine monotherapy was given before and after kidney transplantation at the University of Colorado, adding prednisone postoperatively only to control and reverse breakthrough rejections. Surrogate immune monitoring was done with serum creatinine tests. (**B**) Mid 1964-onward: In most centers, pretreatment was omitted and heavy prophylactic therapy with prednisone (plus additional drugs as these became available) was started at the time of transplantation. More than a third of a century later, it was recognized that these management changes essentially eliminated the possibility of achieving drug-free tolerance. Tx = organ transplantation

allografts transplanted during 1962–63 at the Denver center functioned for the next 4 decades, each depicted in Fig. 2 as a horizontal bar [5]. In seven of the nine recipients, all immunosuppression eventually was stopped without rejection for periods ranging from 7 to more than 40 years (Fig. 2). The eight patients who are still alive after 42–44 years bear the longest continually functioning organ allografts in the world [5]. Inexplicably, no comparable cohort of drug-free kidney recipients was produced again, anywhere in the world, in the next 4 decades.

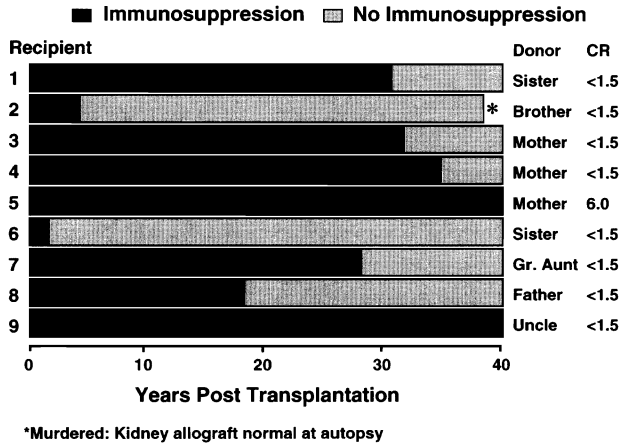

Fig. 2 Drug-free tolerance in long-surviving kidney allograft recipients of 1962–1963 whose immunosuppression was administered as shown in Fig. 1A. *Light portions of transverse bars:* off immunosuppression. By permission of Starzl et al [5]

Hepatic ''tolerogenicity''

However, drug-free human recipients of cadaveric liver grafts continued to be observed, some of whom have been off all immunosuppression for more than 30 years [5, 6]. Importantly, such liver recipients were produced only in three historical periods during which light immunosuppression was used [7]. More recently, a drug-free state has been frequently reached after parent to offspring live donor liver transplantation under minimal immunosuppression in Kyoto, Japan [8].

The higher frequency of drug-freedom in human liver compared to other kinds of organ recipients was not surprising. In canine experiments performed in the early 1960's with unrelated outbred donors and recipients, long or lifetime liver engraftment was observed much more frequently than kidney engraftment when post-transplant azathioprine therapy was limited to 120 days [9]. Prolonged ''acceptance'' of liver grafts subsequently was reported after only one or 2 perioperative doses of antilymphocyte globulin (ALG) [7, 10].

Moreover, lifetime survival after liver replacement was demonstrated in the mid-1960's without any treatment at all in about 20% of experiments with outbred unrelated pigs carried out in France [11], England [12–14], and the United States [15]. Such spontaneous tolerance later was demonstrated in all liver transplant experiments done with selected strain combinations of inbred rats [16] and mice [17]. Importantly, heart and kidney allografts also can self-induce spontaneous engraftment, although in much fewer rodent strain combinations [17, 18].

The contrasting roles of immunosuppression for organ and bone marrow transplantation

Since the foregoing clinical and experimental examples of ultimately drug-free, or entirely spontaneous, engraftment were exceptions to the usual rule of rejection, they had essentially no influence on the remarkable progress in human organ transplantation that took place worldwide after 1964. Instead, this progress was almost entirely dependent on the development of more potent immunosuppressive drugs. Both the old and new drugs were applied in remarkably divergent ways for organ and bone marrow cell transplantation.

Organ transplantation

Kidney transplantation already was a well established clinical service, and the first successful human liver [19] and heart transplantatons [20] had been accomplished by the time of the first unequivocally successful clinical bone marrow cell engraftment by Gatti et al. [21]. Progress with kidney transplantation had been slow at first. Between January, 1959, and the spring of 1963, there were only seven examples in the world of $\geq$1 year survival of kidney allografts (summarized in Ref. 22) (Table 1) [23–26]. Patients 1 and 7 were treated in Boston. Recipients 2–6 were in 2 competing Paris centers (Table 1). Since these were rare exceptions to the usual outcome of patient death, renal transplantation reached a temporary impasse until the 1-year milestone was reached by most live donor kidney allograft recipients treated in Denver during 1962–63 with azathioprine and prednisone [4, 27]. Dozens of new renal centers opened during 1963–64 in the United States and Europe, all using the double drug combination.

Table 1 Kidney transplantation with ≥12 months survival as of April 1963

Case	City [Ref]	Date	Donor	Survival (months)[a]
1.	Boston [23]	1-24-59	Frat twin	>50
2.	Paris [24]	6-29-59	Frat twin	>45
3.	Paris [25]	6-22-60	Unrelated[b]	18 (died)
4.	Paris [24]	12-19-60	Mother[b]	12 (died)
5.	Paris [25]	3-12-61	Unrelated[b]	18 (died)
6.	Paris [24]	2-12-62	Cousin[b]	>13
7.	Boston [26]	4-5-62	Unrelated	>12 (failing)

All patients received cytoablation with total body irradiation except number 7 who was given azathioprine

[a] The kidneys in patients 1, 2, and 6 functioned for 20.5, 25, and 15 years, respectively. Patient 7 rejected his graft after 17 months and died after return to dialysis

[b] Adjunct steroid therapy

Boston: J.E. Murray (cases 1 and 7)

Paris: J. Hamburger (cases 2, 4 and 6), R. Kuss (cases 3 and 5)

Beginning in 1964, however, changes were made in the way the two drugs were used. First, large doses of prednisone were added to azathioprine in most centers from the time of transplantation (Fig. 1B). The shift to heavy prophylactic treatment was caused by the 15% rate of kidney losses to non-reversible rejection when steroids were added only as needed. In a second modification, the prolonged pretreatment with azathioprine was omitted or deemphasized, in part because there was no time for this in cadaveric organ recipients (Fig. 1B).

When ALG [10, 28], cyclosporine [29, 30], and tacrolimus [31, 32] were added to the therapeutic armamentarium, they were introduced clinically by reverting back to the strategy depicted in Fig. 1A. Cyclosporine and tacrolimus were begun as monotherapy, adding steroids only to treat breakthrough rejections [30–33]. When anti-human ALG was first used in 1966, it was administered in a short perioperative course that included pretreatment, or was given postoperatively as a substitute for prednisone to treat steroid-resistant rejection [10, 28] The use of all these drugs promptly drifted to heavy prophylactic immunosuppression in the same way as had occurred with the original combination of azathioprine and prednisone, and for the same reason: i.e. not all rejections that developed under baseline post-transplant monotherapy could be reversed and controlled. By the 1990's, a bewildering array of stacked drugs, begun at the time of transplantation, was in use worldwide (Fig. 1B), with the stipulated objective of reducing the incidence of acute rejection to zero.

The preemptive use of multiple drug immunosuppression was spectacularly successful insofar as acute rejection became almost a non-problem. Better short and medium term patient and graft survival was accomplished with all transplanted organs, epitomized by stepwise improvements in our own liver transplantation experience [34]. There was, however, a dark side. Chronic rejection and the devastating morbidity and mortality of long-term immunosuppression had now become unresolvable problems. Moreover, the drug-free tolerance that had not been rare in the earliest organ recipients (and just after the clinical introduction of ALG, cyclosporine, and tacrolimus) was almost never seen again.

Thus, 4 eras of transplantation were delineated by the introduction of a drug: azathioprine (1962), ALG (1966), cyclosporine (1979), and tacrolimus (1989). In each era, the new drug was soon incorporated into increasingly complex multiple drug regimens. Inexplicably, the best long-term results were obtained at the beginning, when the new baseline drug was started alone as in Fig. 1.

Bone marrow cell transplantation

In contrast to the lifetime commitment to daily immunosuppression for organ recipients, the stipulated goal after human bone marrow cell transplantation always has been drug-free tolerance. Other differences between the two kinds of transplantation were equally striking. The best known features of bone marrow (or other hematolymphopoietic) cell transplantation were a high risk of GVHD, and dependence for a good outcome on HLA matching [21, 35–39]. These and other characteristics of bone marrow transplantation did not remotely resemble those of organ transplantation: e.g. organ transplantation was almost free of GVHD risk and was routinely feasible without HLA matching (Table 2).

Potential mechanisms of organ engraftment

Almost from the beginning, the differences between organ and bone marrow transplantation prompted two questions. First, why had organ transplantation been feasible at all, seemingly in violation of all immunologic rules? Second, what was the relation (if any) of organ engraftment to tolerance? These eventually became 2 of biology's most enduring mysteries. The root cause of the intellectual cul-de-sac was disconnection of organ transplantation from the scientific base of donor leukocyte chimerism shared by the mouse tolerance models [1–3] and their clinical analogue of bone marrow transplantation [21, 35–39]. Since successful organ transplantation had been accomplished in the presumed absence of donor leukocyte chimerism, organ engraftment ostensibly required explanation by separate and distinct mechanisms. What were these mechanisms?

Table 2 Historical view of differences between clinical organ transplantation and bone marrow transplantation

Feature	Organ transplantation	Bone marrow transplantation
Host cytoablation	No	Yes[a]
HLA matching	Not essential	Critical
Principal complication	Rejection	Graft-versus-host disease (GVHD)
Immunosuppression-free	Rare	Common
Term for engraftment	Acceptance	Tolerance
Leukocyte chimerism	No[b]	Yes

[a] It was not fully recognized until the 1990's that this therapeutic step accounts for all of the other differences (see text)

[b] The discovery in 1992 of microchimerism in organ recipients meant that this notation should be "yes"

Immune ignorance

One of the scientists who tackled the question was the young surgeon, Clyde Barker at the University of Pennsylvania, who joined forces in 1967 with Rupert Billingham. In 1968, Barker and Billingham reported that skin grafts were not rejected when the grafts were placed on an island of recipient skin that had been detached from lymphatic drainage but was nourished by a vascular pedicle (Fig. 3) [40]. The simple experiment exposed at least one way in which an allograft may escape rejection: i.e. failure of the immune system to recognize the presence of antigen that fails to reach host lymphoid organs (immune ignorance).

The concept that immune activation cannot occur unless antigen reaches lymphoid organs is a crucial element of the hypothesis that clonal exhaustion-deletion is the seminal mechanism of acquired tolerance (next section). However, more than a quarter century passed before the conclusions about immune ignorance by Barker and Billingham were formally validated. The reason was the difficultly of preventing mobile alloantigen (i.e. leukocytes) from reaching host lymphoid organs in a clinically relevant transplant model. The definitive experiments were done at Yale University by Lakkis [41] in mutant aly-aly mice that possess fully competent cytotoxic T lymphocytes (CTL), but no secondary lymphoid organs except the spleen [42]. Although these animals are immunodeficient, they can reject heart allografts. But after the spleen is removed, heart transplants are not rejected because their presence is no longer detected in the absence of all organized host lymphoid collections [41].

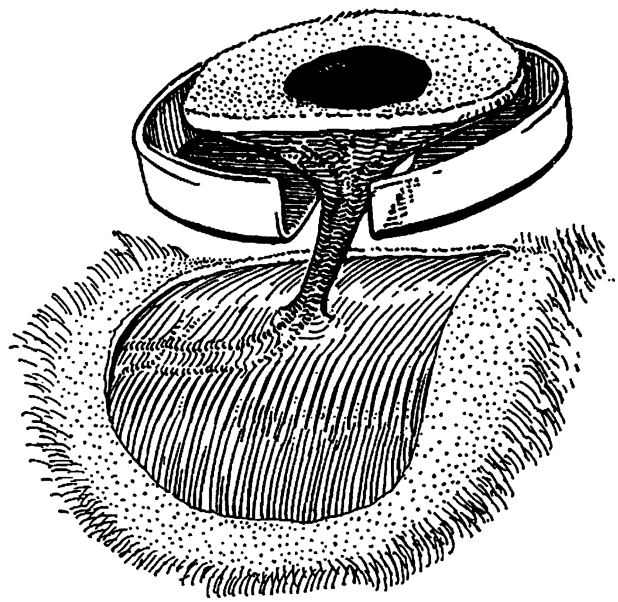

Fig. 3 Failure of the immune system to recognize and reject skin allografts whose mobile antigen (i.e. passenger leukocytes) is prevented from reaching host lymphoid organs. By permission of Barker and Billingham [40]

Clonal exhaustion-deletion

In 1969, we postulated that the seminal mechanism of successful transplantation of the liver or any other organ was clonal exhaustion-deletion. We also proposed that prolonged allo-engraftment, with or without the need for immunosuppression, was a form of variable tolerance (Fig. 4) [43, 44]. Although the overall hypothesis was correct, there were too many missing links at the time for it to be credible. First, elucidation of the mechanisms of antigen recognition by Zinkernagel and Doherty lay 5 years ahead [45, 46]. Moreover, the transport of donor antigen to host lymphoid organs was ascribed to macrophages (Fig. 4) rather than to the dendritic cells described by Steinman and Cohn [47]. Finally, clonal exhaustion-deletion, like immune ignorance, was only a theory. And like immune ignorance, it vanished from the literature until its existence and importance were formally proved in the early 1990s [48, 49].

Abandonment of immune ignorance and clonal exhaustion-deletion left a vacuum that was promptly filled with numerous alternative engraftment mechanisms (See later in Table 3).

The relation of leukocyte migration to alloengraftment and tolerance

Misinterpreted early clues

Discussions of organ engraftment mechanisms in the 1960's took place in a restrictive anatomic context. The parenchymal cells, vascular components, and passenger leukocytes of an organ were all viewed as stationary targets of the host immune response. With this assumption, a crucial observation in the Colorado kidney transplant experience of 1962–1963 could not be correctly interpreted. In essence, positive tuberculin and other skin tests of delayed hypersensitivity in the kidney donors were found to have been transferred to

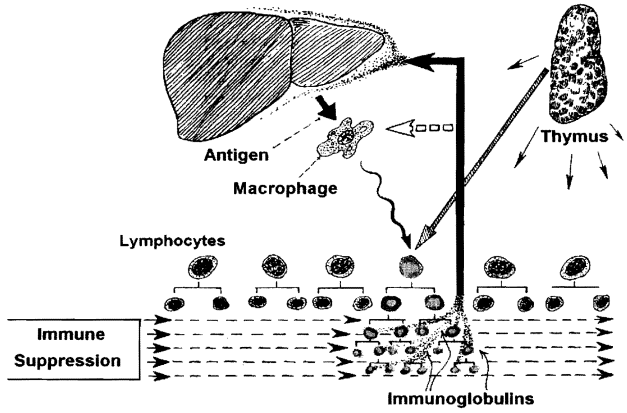

Fig. 4 This illustration and caption (in quotes) were published in 1969 to explain organ engraftment. "Hypothetical mechanisms by which non-specific immunosuppression may lead to selective abrogation of the host immune response. Special susceptibility to these agents of a fraction of the lymphoid population could lead to exhaustion of a clone and, hence, tolerance. Since maintenance of such cell lines [clones] 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". The concept proposed in 1969 was for the most part correct, but was not considered credible because of lack of scientific support. By permission of Starzl [44]

previously negative recipients [50, 51] (Fig. 5). Although this prima facie evidence of adoptive transfer indicated that donor immune cells had survived, it was thought that the donor leukocytes were still in the graft. Therefore, the findings were ascribed to a hypothetical humoral substance ("transfer factor") rather than to donor cells that had migrated into the recipient.

Further clues about leukocyte migration surfaced in 1969 when karyotyping studies were done in human female recipients of livers that had been obtained from male donors. After 100 days, the hepatocytes, duct cells, and vascular endothelium of the hepatic grafts retained their donor (male) sex whereas most of the bone marrow-derived passenger leukocytes of the liver were replaced with female (recipient) cells [53]. Although this was an important observation, the resulting composite cellular structure of the graft (part donor, part recipient) was considered to be a unique feature of liver transplantation until it was demonstrated in the early 1990s that all other established organ allografts underwent the same transformation [52, 54, 55]. In the meanwhile (between 1982 and 1992), it had been demonstrated that large numbers of graft passenger leukocytes migrated into the recipient [56–60]. Even then, however, it was generally assumed that the donor cells missing from the graft had undergone immune destruction with selective sparing of the specialized parenchymal cells.

An epiphany

A pivotal step toward connecting the dots was taken in 1992 when multilineage donor leukocyte microchimerism was demonstrated in 30 of 30 liver, kidney, and other kinds of human organ recipients whose grafts had been functioning for up to 3 decades. The study was an extremely simple one. Using sensitive immunocytochemical and molecular methods, sparse numbers of the donor leukocytes were found in the blood or one or more

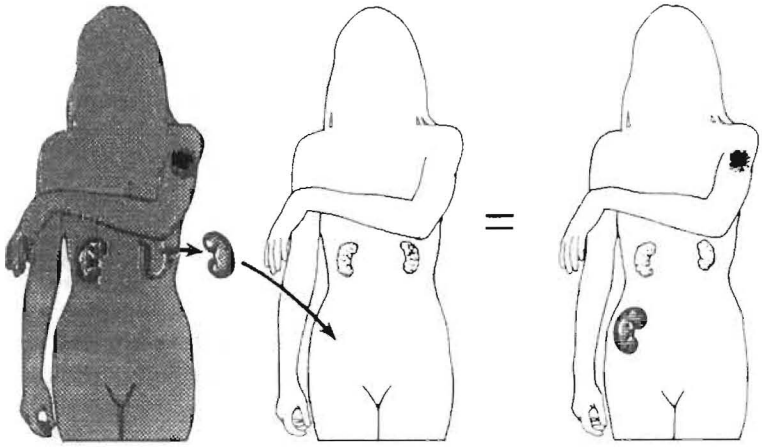

Fig. 5 Prolonged transfer from donors to recipients of positive tuberculin, coccidioidin, or other delayed sensitivity skin tests in 1962–63 cases of kidney transplantation at the University of Colorado. Although inexplicable at the time, these observations of adoptive transfer were consistent with donor leukocyte migration and relocation in the recipient. By permission of Starzl et al. [52]

of the tissue samples taken from multiple recipient sites (skin, lymph nodes, bone marrow, heart, bowel) [52, 61–64]. Biopsies of the grafts also were examined. The findings mandated a change in the perceived landscape of transplantation immunology.

The paradigm shift

An engrafted organ previously had been viewed as an island in a hostile sea in which the leukocytes were solely those of the recipient (Fig. 6, Panel A). The revised view depicting microchimerism in various non-lymphoid and lymphoid recipient sites is shown in Panel C. In the reverse image of bone marrow transplantation, the ideal result had been considered to be complete replacement of all hematolymphopoietic cells (Panel B). However, in 1991, Przepiorka and Thomas in Seattle had detected a trace population of recipient leukocytes in essentially all such "perfect" bone marrow recipients (Panel D) [65]. Now, it was evident that organ engraftment (Panel C) and bone marrow cell engraftment (Panel D) differed fundamentally only in the proportions of donor and recipient cells [66].

The double immune response

In both kinds of transplant recipient, the surviving cells of the minority populations obviously were progeny of precursor or pluripotent stem cells that had survived a double immune reaction years or decades earlier, during the first few days after transplantation. We deduced that alloengraftment occurred when "...responses of co-existing donor and recipient cells, each to the other, resulted in reciprocal clonal exhaustion, followed by peripheral clonal deletion" [61] (Fig. 7). Exhaustion-deletion of the host versus graft response during the first

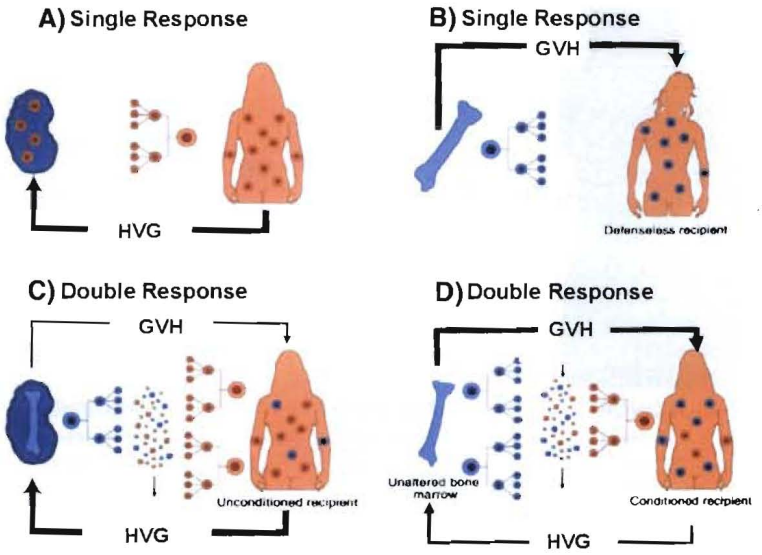

Fig. 6 Basis of a paradigm shift (see text). (**A**, **B**) Historical perception of organ and bone marrow cell recipients. (**C**, D) revised view of transplantation recipients

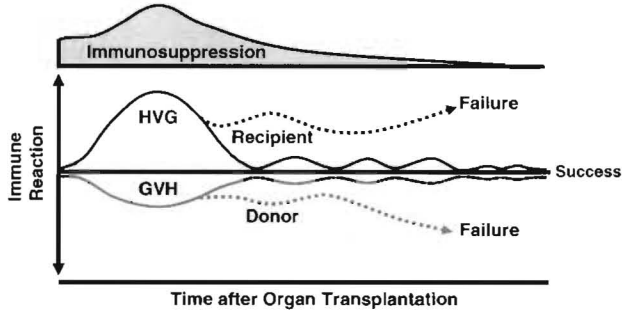

Fig. 7 Contemporaneous immune responses following allotransplantation: host versus graft (HVG, upright curves) and graft versus host (GVH, inverted curves). In contrast to the usually dominant HVG reaction of organ transplantation shown here, the GVH reaction usually is dominant after bone marrow cell transplantation to the irradiated or otherwise immune-compromised recipient. Therapeutic failure with either type of transplantation implies the inability of immunosuppression to control one, the other, or both of the responses. By permission of Starzl and Zinkernagel [67]

few post-transplant days or weeks of maximum migration of the organ's passenger leukocytes explained both the reversal of rejection, and the development of variable tolerance that had been first observed in kidney recipients 30 years earlier (Fig. 1A).

The host response (the upright curve in Fig. 7) was the dominant one in most cases of organ transplantation, but there also was a usually invisible graft versus host (GVH) reaction (the inverted curve). If the GVH response was not also exhausted and deleted, it could be expressed as clinical GVHD. GVHD was unusual, and when it occurred, it usually was in recipients of a leukocyte-rich organ (a liver or intestine) [52].

Host irradiation or other methods of cytoablation/cytoreduction in bone marrow recipients [35, 37–39], or the preexistence of immune deficiency disease as in Bob Good's first cases [21], simply transferred immune dominance from the host to the graft. The high risk of GVHD, the prerequisite of HLA matching to avoid this complication, and all of the other major features that distinguished bone marrow from organ transplantation (Table 2) were readily explained.

A unified view of tolerance models

With this paradigm, organ engraftment in the spontaneous tolerance models of organ transplantation discussed earlier could be readily related to the freemartin cattle observations of natural tolerance by Owen [68], the mouse acquired tolerance models that began in 1953 with Billingham, Brent, and Medawar [1], and the parabiosis models of Bob Good [69] (Fig. 8). Experimental evidence supporting various elements of the new paradigm was systematically compiled in Pittsburgh and elsewhere throughout the 1990s, using experimental rat and mouse models (summarized in refs. 6, 67, 70–72).

The King-Pin leukocyte

The reason why the leukocyte always seemed to be the indispensable tolerogenic cell was explicitly stated in the final sentence of our original 1992 Lancet article: "The key event

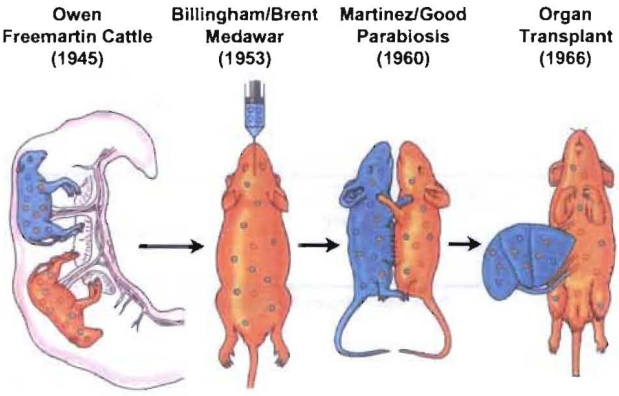

Fig. 8 The relation of organ engraftment to classical models of spontaneous donor leukocyte chimerism-associated tolerance. By permission of Starzl et al [6]

[in allograft acceptance] is cell migration and relocation'' [61]. As already emphasized, antigen that does not reach host lymphoid organs is not recognized to be present (immune ignorance). The only mobile antigen in organs consists of passenger leukocytes. Migration of these passenger leukocytes to organized lymphoid collections presumably was a prerequisite for the seminal tolerance mechanism of clonal exhaustion-deletion.

The stages of leukocyte migration

By 1992, the kinetics of the migration had been delineated in mouse, rat and human studies. The cell movement occurred in two stages [55, 58, 60, 73–77]. In stage 1, the donor leukocytes migrated selectively to host lymphoid destinations where immune activation occurs. The second stage began after 1–3 weeks when cells that had escaped initial immune destruction moved on to heterogenous sites that included skin and other non-lymphoid locations. This second phase, which could culminate in various levels of leukocyte chimerism, was essentially complete after 30–50 days [55, 73–77]. All of these migratory events were essentially the same as those of the infused leukocytes of bone marrow cell infusion [78].

The pace of the two stages was frozen in time: i.e. it remained constant throughout mammalian evolution without regard for species size, gestational duration, or interval between generations. As a consequence, one alloimmune response curve fit all extant species (Fig. 9, bottom). The first few weeks after organ allotransplantation corresponded with the maximal acute dissemination of the donor leukocytes to the host lymphoid organs, and coincided with the greatest risk from rejection. This period also provided the window of greatest opportunity for our postulated seminal mechanism of acquired allotolerance (i.e. clonal exhaustion-deletion).

The migration of non-cytopathic pathogens

What was not known in 1992 because their work was not published until the following year, was that Zinkernagel and his associates in Zurich had come to an explanation of

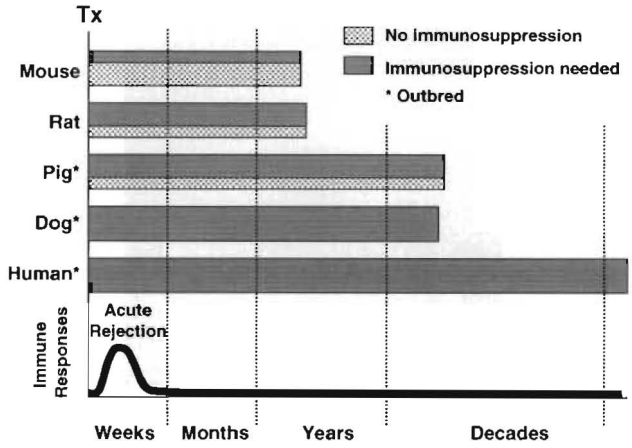

Fig. 9 The pace of development of a donor-specific response following organ transplantation in different extant species and the subsequent development of donor-specific non-reactivity. These events closely match the kinetics of donor leukocyte migration and relocation. Note that immunosuppression may not be required for liver engraftment in 3 of the 5 species shown (lightly shaded portion of transverse bars). Tx = transplantation

acquired tolerance to pathogens that was essentially the same as that of our allotolerance paradigm. In the 1970's, Zinkernagel and Doherty showed that the MHC-restricted cytolytic T cell response induced by non-cytopathic microorganisms was the same as that induced by allografts. These studies were done in highly controlled experimental models of infection with the lymphocytic choriomeningitis virus (LCMV) and other intracellular parasites [45, 46]. Their subsequent investigations of tolerance were done with the same models and described in 4 landmark articles between 1993 and 1997 [79–82].

The kinetics of destructive immunity

Figure 10A, C shows how the migration of non-cytopathic microorganisms determines the induction at host lymphoid organs of the T-cell response. The tropism of the specific pathogen then determines the targets of the response. For example, the principal spread of a hepatitis virus in a non-transplant patient is to the liver (Fig. 10A). With a cytomegalovirus (CMV) infection, the main infestation may be in the lung (Fig. 10C). Importantly, the liver (panel A) and lung (panel C) in these separate and distinct infections are not where the antiviral response is induced. Instead, the response to both pathogens is induced at host lymphoid organs to which viral antigen is carried by a relatively small number of infected antigen presenting cells. The virus-specific T cells generated at host lymphoid organs then destroy infected host cells wherever these "non-self" cells are located. The main T cell target is therefore the heavily infested liver (Fig. 10A) or lung (Fig. 10C).

The principles are the same after organ transplantation, although the details are simpler (Fig. 10B). The allograft's "non-self" passenger leukocytes migrate preferentially to host lymphoid organs and induce donor-specific T cells that target passenger leukocytes that have left the graft as well as all cells of the outlying source organ. The resulting acute liver graft rejection is therefore analogous to a bout of acute hepatitis (compare panels A and B).

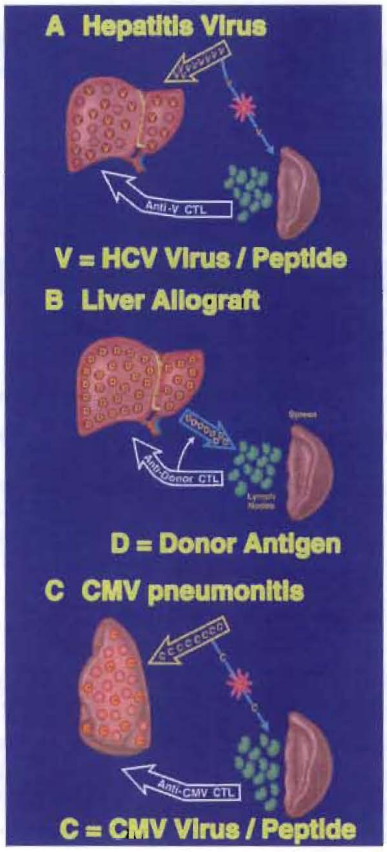

Fig. 10 How the spread and localization of antigen determines both the induction at host lymphoid organs of adaptive immunity, and then the target of this immunity. (**A**) hepatitis virus (**B**) migratory passenger leukocytes of a transplanted organ (here a liver) (**C**) cytomegalovirus (CMV) localized to the lung

In the comparable CMV analogy, the immune response that eliminates a pulmonary infection (Fig. 10C) is fundamentally the same as rejection of a lung allograft (not shown).

No matter what the infection, and no matter what kind of transplantation, the analogies between the destructive immunity against non-cytopathic microparasites and allografts were always identifiable. Moreover, it was this highly specific adaptive immune response against allografts, or against infectious agents, that was exhausted and deleted in both the transplant and infection hypotheses of tolerance.

The spectrum of transplantation/infection analogies

Recognizing that the Pittsburgh and Zurich investigations were on parallel pathways, a cross-over review was undertaken in 1997 and published in a December 1998 issue of the New England Journal of Medicine. The concept that had been independently developed in transplant and infection models was generalized in the following way: "The migration and localization of antigen govern the immunologic responsiveness or unresponsiveness

against infections, tumors, or self—and against xenografts or allografts'' [67]. The outcomes under all these circumstances were determined by the balance established between the amount of mobile antigen with access to host lymphoid organs and the number of antigen-specific cytolytic T-cells (CTL) induced at the lymphoid sites (Fig. 11) [67].

Non-response

If a virus remains localized in non-lymphoid sites or spreads by extralymphatic routes (e.g. the human papilloma [wart] virus), no immune response is induced (immune ignorance, Fig. 11A). Transplant analogues in which immune ignorance is the dominant

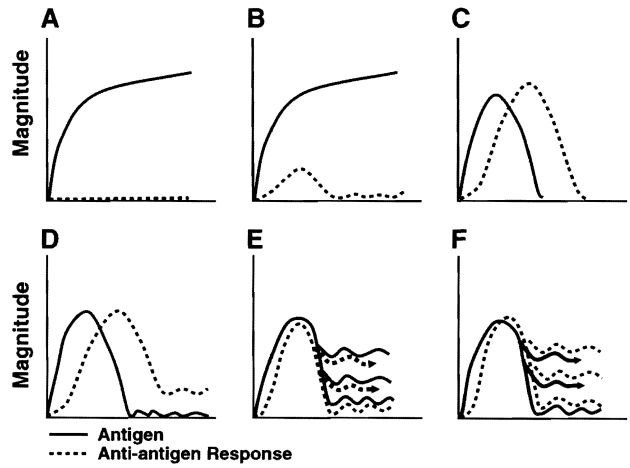

Fig. 11 Analogies between infection by non-cytopathic microparasites and the scenarios of transplantation. The outcomes are governed by the migration and localization of the respective antigens (refer also to Fig. 10). The analogies have been obscured by the presence of contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) responses after transplantation and the additional factor of therapeutic immunosuppression. (**A**) The presence of a pathogen that fails to reach organized host lymphoid tissue is not recognized (immune ignorance). Consequently, there is no immune response. The relation of immune ignorance to clonal exhaustion-deletion must be understood to comprehend alloengraftment (see text). (**B**) A highly infectious but asymptomatic and stable carrier state may be reached when a rampant non-cytopathic microorganism exhausts and deletes the antigen-specific immune response, (e.g. viral hepatitis). The transplant analogy is 'complete' repopulation of an immunodeficient or cytoablated bone marrow recipient without the penalty of GVHD. (**C**) Complete elimination of a non-cytopathic pathogen by an immune response that then subsides without memory. The transplant analogy is rejection of an organ's passenger leukocytes and the outlying source graft (refer to Fig. 10). (**D**) Instead of the outcome in Panel C, persistence of small numbers of microorganisms may maintain cellular plus antibody 'memory' (protective immunity). In the transplantation analogy, residual microchimerism may result in a ''presensitized'' state that renders a transplant candidate ''crossmatch positive'' with most donors [83, 84]. (**E**) Disease carrier states in which a balance is established that favors pathogen load over pathogen-specific T cells. The corresponding spectrum of transplantation analogues is delineated by simply substituting ''donor leukocyte'' for pathogen. For organ transplantation purposes, an umbrella of immunosuppression usually is needed to keep the various balances in stable equilibrium (see also Fig. 12). (**F**) Refractory infection syndromes in which neither adaptive immunity nor therapy with antimicrobial agents can achieve the kind of disease control shown in Panels **C** and **D** or the asymptomatic carrier state sometimes seen in Panels **B** and **E**. The treatment failure analogues of transplantation occur when the opposite objective (sustained dominance of the migratory alloantigen over an aggressive antidonor T cell response) is not achievable with immunosuppression or other means

component include the engraftment of bits of parathyroid or other endocrine tissues in privileged non-lymphoid sites [85] or encasement of these tissues in a millipore chamber that allows passage of nutrients and humoral molecules but not donor or host cells [86]. Pure or predominant immune ignorance has been an unattainable objective for purposes of whole organ or hematolymphopoietic cell transplantation.

The ''complete carrier'' state

If a rapidly expanding viral load cannot be controlled by the virus-specific T-cell response, the response may be exhausted and deleted with a resulting disease carrier state. The asymptomatic ''complete carrier'' is analogous to the idealized bone marrow recipient who has near total hematolymphopoietic chimerism (Fig. 11B) [67].

Disease control vis-à-vis transplant rejection

In most infection experiments, sufficient numbers of virus-specific CTL are induced [79–82]. If this results in complete elimination of the virus (the fall to zero of the solid line in Fig. 11C, the response ceases without T-cell memory (the fall to zero of the dashed line). This was comparable to rejection of an organ allograft including total elimination of the graft's disseminated migratory cells. However, such complete sterilization of virus was almost never seen in the infection models.

Instead, small amounts of live virus usually persist in non-lymphoid niches that are relatively inaccessible to host effector mechanisms. From these sites, residual virus may periodically migrate secondarily to host lymphoid organs and stimulate continued virus-specific immunity (Fig. 11D). The ongoing virus-specific protective immunity is analogous to the presensitization states that frequently are associated with residual microchimerism after a failed transplant procedure [83, 84]. Once microchimerism is established in an organ recipient, elimination of the donor leukocytes is extremely difficult [87].

Compromise outcomes

Persistent mobile antigen was, in fact, a double-edged sword [67, 88]. In some infection models, very small quantities of virus migrating between non-lymphoid and lymphoid sites could maintain the exhaustion-deletion induced at the outset (Fig. 11E): i.e. a balance favoring viral antigen over anti-viral CTL. In Panel E, the lowest combination of solid and dashed lines also represents the analogous balance between mobile alloantigen and the CTL response reached in experimental models of organ-induced spontaneous tolerance. Here, the exhaustion-deletion achieved at the outset is sustained by microchimerism [67, 88]. Importantly, stable maintenance of any of the alloantigen dominant balances shown in Panel E may require immunosuppression, even when the donor leukocytes are at the macrochimerism level. Panel F shows a spectrum of transplant treatment failures in which no amount of clinically acceptable immunosuppression is able to maintain the desirable equilibrium.

Thus, tolerance, with or without an umbrella of immunosuppression, merely means that the mobile antigen outweighs the antigen-reactive T-cell response. Although the probability of tolerance increases with higher levels of chimerism, no arbitrary amount of

chimerism can be equated with tolerance. Thus, leukocyte chimerism is a necessary condition for, but is not synonymous with transplantation tolerance. In this view, all of the scenarios that develop after experimental or clinical transplantation are analogues of infection outcomes. Moreover, all examples of prolonged allograft survival, whether immunosuppression dependent or independent, are analogues of infectious disease carrier states [67, 88].

The microchimerism controversy

Such analogies have generated controversy, primarily because many authorities have considered the microchimerism of organ-bearing recipients to be an epiphenomenon secondary to graft acceptance by T-regulatory or other alternative mechanisms (Table 3). This ''cause or effect'' question about the role of microchimerism was answered definitively in a report by Zinkernagel's team in the January 2006 issue of the Journal of Clinical Investigation entitled: Microchimerism Maintains Deletion of the Donor Cell-Specific CD8+ T Cell Repertoire [89]. Those experiments formally proved that leukocyte chimerism, even at a micro level, is essential for perpetuation of allotolerance, and by the seminal mechanism of clonal exhaustion-deletion. The study did not exclude an accessory role for the immunoregulatory mechanisms shown in Table 3. It did indicate, however, that the alternative mechanisms are not essential.

Therapeutic implications for transplantation

The simple concept that balances between mobile antigen and antigen-specific T-cells govern immunologic responsiveness and non-responsiveness has profound therapeutic implications (Fig. 12). Tilting the balance in favor of antigen by ratcheting down the cognate T-cell response with immunosuppression has been the principal means of perpetuating organ alloengraftment. The price has been loss of immune surveillance against infections and malignant neoplasms [43, 90, 91], just as Bob Good had demonstrated earlier in patients with immune deficiency diseases.

Increasing the amount of mobile alloantigen with infusions of donor leukocytes (Fig. 12) has been shown empirically to improve allograft survival in numerous experimental transplant models. However, large-scale trials of adjunct leukocyte infusion have yielded disappointing results in human organ transplant recipients [92–94]. In these

Table 3 Mechanisms of acquired transplantation tolerance and alloengraftment

Seminal mechanisms (starzl-zinkernagel, 1998)	
Clonal exhaustion-deletion Immune Ignorance	
Alternative mechanisms	
Special cells	T-regulatory, suppressor, veto
Antibodies	Idiotypic, enhancing
Cytokine	Self-perpetuating profiles
Graft secretions	Soluble HLA antigens
Antigen presentation	Defective or deviant
Anergy	Absence of second signal

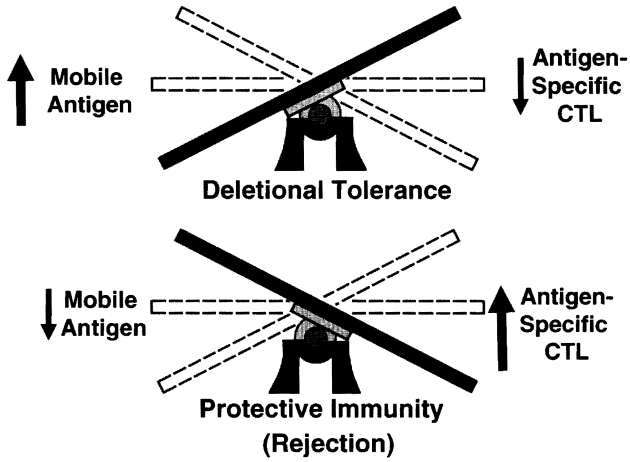

Fig. 12 Balance between antigen with access to host organized lymphoid tissue, and the antigen-specific cytolytic T cells (CTL) induced at these lymphoid sites. A balance favoring the migratory antigen over the antigen-reactive CTL (Upper teeter totter) may be achieved for transplant purposes in some models by simply adding adjunct donor leukocytes. In standard clinical practice, however, the desired balance is almost always maintained by reducing the specific CTL response with immunosuppression (discussed in text). Tilting the balance in the opposite direction results in immunity (lower teeter totter)

clinical trials, bone marrow cells, or stem cell-enriched peripheral leukocytes, usually were given on the same day as organ transplantation under conventional multiple drug immunosuppression. In a second review with Zinkernagel [88], it was suggested that the heavy post-transplant immunosuppression may have been responsible for the lack of efficacy.

It also was suggested in this review that the worldwide policy of heavy post-transplant immunosuppression for conventional organ transplantation was antitolerogenic. Our argument was that the strong immunosuppression used to drive the rate of acute rejection to near zero systematically subverted the seminal tolerance mechanism of donor leukocyte-driven clonal activation, exhaustion, and deletion. The result was the narrowing of the one time only window of opportunity for tolerogenesis in the first few posttransplant weeks (Fig. 13). We proposed avoidance of this undesirable consequence of over treatment in all conventional and leukocyte-augmented organ recipients by application of 2 therapeutic principles, singly or together: recipient pretreatment and minimal post-transplant immunosuppression.

Minimal post-transplant immunosuppression

In the experimental organ transplant models of spontaneous tolerance (Fig. 14A), no treatment is needed because the antidonor response is too weak to eliminate the donor antigen and is exhausted and deleted. The deletional tolerance is then maintained by leukocyte microchimerism. In numerous other rodent models, the normal outcome of rejection can be regularly converted to the same kind of lifetime tolerance by capping the anti-donor response with a few post-transplant doses of a single immunosuppressant [95] (Fig. 14B). However, histocompatibility and other confounding parameters in the outbred human population make it impossible to predict the effect of such treatment in any given patient.

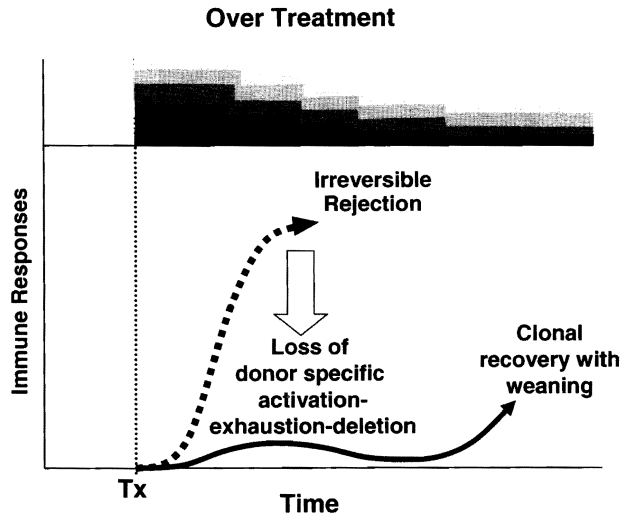

Fig. 13 Weakening or elimination of the clonal response by excessive post-transplant immunosuppression to the extent that efficient exhaustion and deletion of the clonal response is prevented. Subsequent graft survival is permanently dependent on immunosuppression

Recipient pretreatment

By reducing global immune reactivity before arrival of donor antigen, deletion of the anti-donor response is both easier and highly specific (Fig. 14C). This is what is routinely accomplished with the pretransplant cytoablation or cytoreduction of conventional bone marrow transplantation, but with the predictable penalty of GVHD. Less drastic conditioning with ALG and other lymphoid-depleting antibody preparations has been known since the 1960's to be an effective form of pretreatment with a relatively low risk of GVHD [10, 28].

Combined application of tolerogenic principles

Beginning in 2001, the therapeutic principles were combined in Pittsburgh for the treatment of conventional organ recipients: i.e. without adjunct leukocyte infusions (Fig. 15) [96]. A single large dose of antithymocyte globulin (ATG, Thymoglobulin^{R}) or alemtuzumab (Campath^{R}) was infused before graft revascularization. After transplantation, treatment was restricted to daily tacrolimus unless breakthrough rejection mandated additional agents. After about 4 months, the time between doses was increased if possible to every other day or longer intervals (spaced weaning). This strategy drastically changed the face of organ transplantation at our center.

Intestinal-multivisceral transplantation

The greatest impact was on the procedures with the most troubled histories. Intestinal transplantation alone, or as part of an abdominal multivisceral graft, had been a target for criticism because of the high short and long-term mortality. With a 25% gain in survival,

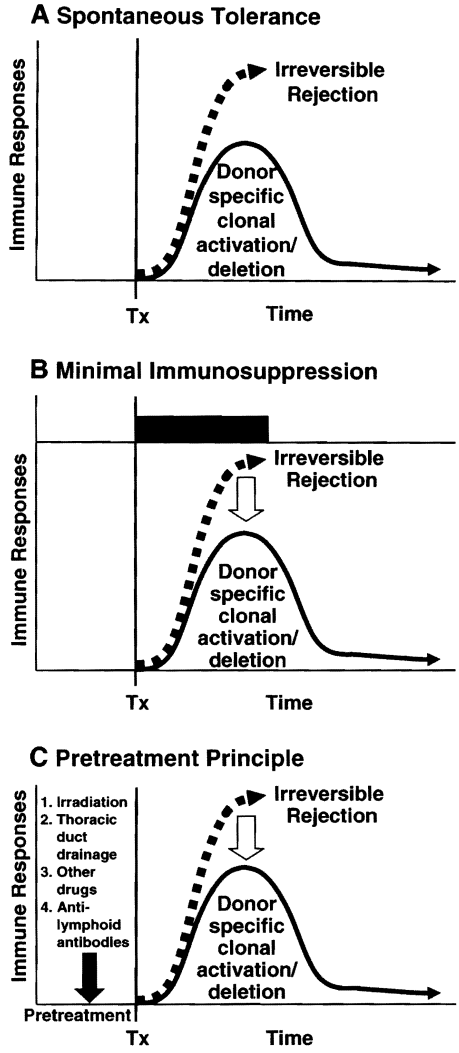

Fig. 14 Principles of tolerogenic immunosuppression. (**A**) Experimental organ transplant models of spontaneous tolerance (no immunosuppression needed). In these unusual models, the host versus graft immune response induced acutely by the migratory donor leukocytes is too weak to eliminate the donor cells and is exhausted and deleted. The deletional state induced at the outset is then maintained by microchimerism. (**B**) Organ transplant 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-transplant immunosuppression. Neither the mechanisms nor the ultimate result are different than in the spontaneous tolerance models of panel **A**. (**C**) Models in which the global recipient immune responsiveness is weakened in advance, thereby making deletion easier of the subsequently induced donor-specific T cell clone. This pretreatment (conditioning) principle is the essential basis of bone marrow transplantation. It has not been systematically exploited in organ recipients. Tx = Transplantation

intestinal transplantation promptly became a genuine clinical service [96, 97]. The improved patient and graft survival (Fig. 16) was largely due to avoidance of the infections and oncologic complications of heavy immunosuppression. After 3–5 years follow-up of the first

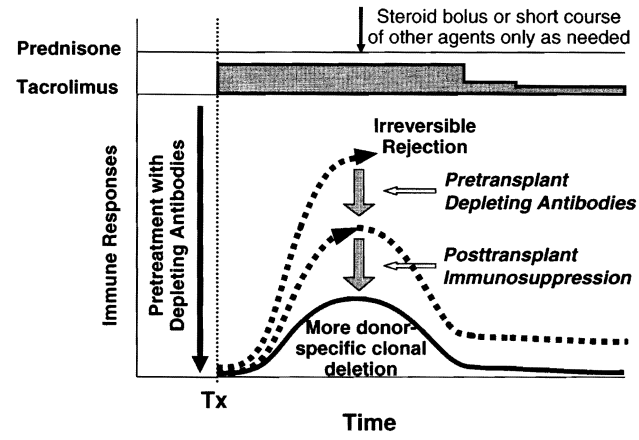

Fig. 15 Combination of the two principles shown in Fig. 14**B** and **C** in a "tolerance friendly" immunosuppression protocol routinely used since 2001 at the University of Pittsburgh Medical Center [96]. Lymphoid depletion was done before organ allograft revascularization. Weaning from post-transplant monotherapy was systematically attempted (see text). The usually silent graft versus host (GVH) reaction depicted in Fig. 7 is not shown here. Tx = Transplantation

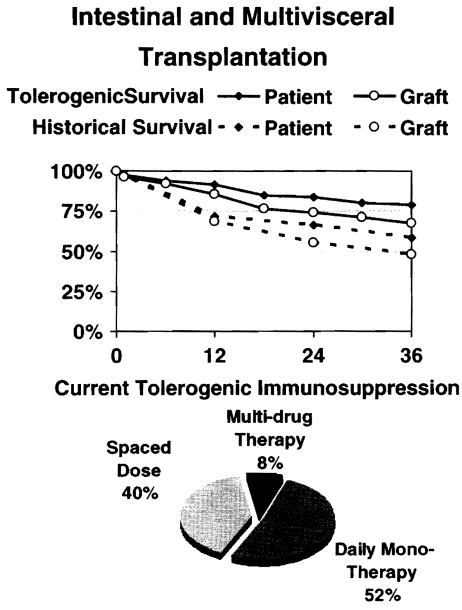

Fig. 16 The patient and graft survival of the first 89 intestinal or multivisceral allograft recipients treated with the tolerogenic immunosuppression shown schematically in Fig. 15. Note that only 8% of the surviving patients with functioning grafts are on more than a single immunosuppressant, and that 40% are on spaced weaning

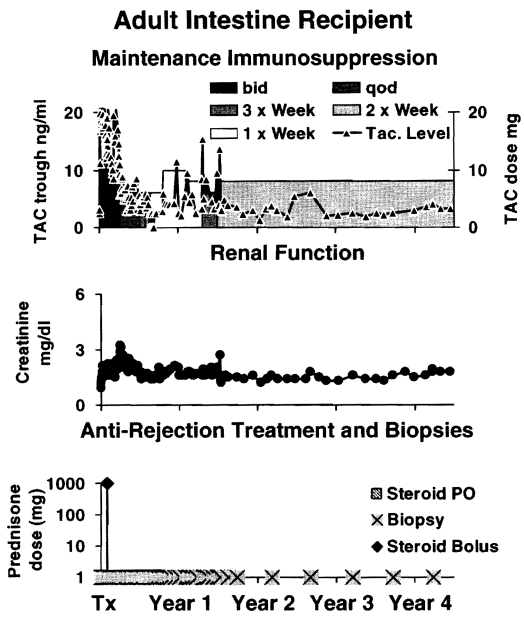

Fig. 17 Course of an intestinal recipient who was pretreated with ATG and managed postoperatively with tacrolimus monotherapy. She has been on full oral alimentation for nearly 5 years and has been treated with two doses of tacrolimus per week for the last 4 years. The xxx's at the bottom indicate intestinal biopsies. Note the stable creatinine (i.e. avoidance of tacrolimus nephrotoxicity) with this regimen

89 ATG-depleted intestinal recipients, only 8% of those with functioning grafts are on more than one drug and nearly half are on spaced doses (Fig. 16) [96, 97]. After the switch from ATG to alemtuzumab for lymphoid depletion, the results improved again (not shown).

Figure 17 summarizes the course of a space-weaned 68-year old woman who underwent full small bowel transplantation on August 5, 2001 after lymphoid depletion with ATG. After the first 4 months of daily tacrolimus dosing, spaced weaning was begun. She ultimately settled into the 2 tacrolimus doses per week schedule of tacrolimus monotherapy on which she has been maintained for the last 4 years. In a recent biopsy, the karyotyped leukocytes of her male donor accounted for about 3% of the total cells of the exquisitely preserved Peyer's patches of her engrafted intestine. Her peripheral blood at the time showed only microchimerism.

Lung transplantation

The other procedure of previous dubious value was lung transplantation in which the one year survival was barely 60% in our experience, and lower than that in multicenter registry reports. After adoption of the tolerogenic principles, and using alemtuzumab for lymphoid depletion, 1 year survival jumped to 90% [98].

Kidney transplantation

The more common procedures of kidney and liver transplantation also were upgraded [96, 99, 100], especially after substituting the more potent lymphoid-depleting agent, alemtuzumab for ATG in 2002. With both agents, patient and graft survival in adults was as good or better than in our historical controls. Approximately 80% of the patients with

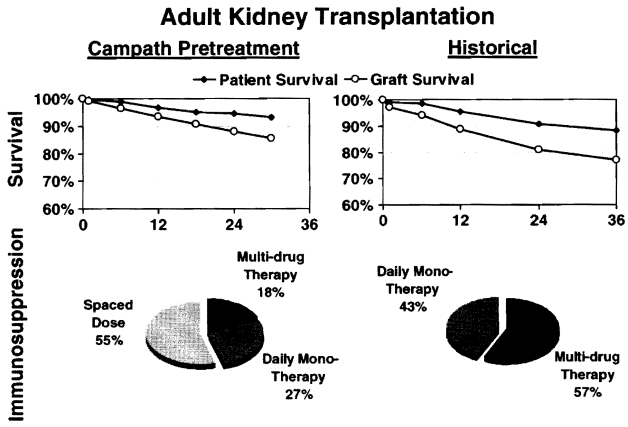

Fig. 18 Patient and kidney graft survival using alemtuzumab (Campath^{R}) lymphoid depletion and minimalistic post-transplant immunosuppression, versus historical experience. The pie shows the current immunosuppression of survivors with functioning grafts

functioning grafts require maintenance treatment with more than one drug and the majority are on spaced doses (Fig. 18).

The "quality of life" gains from minimizing immunosuppression in adults are too obvious to dwell on. In pediatric recipients, the growth retardation and other side effects of chronic immunosuppression [101] have been all but eliminated [99]. After minimum follow-up of 2 years, patient survival in our recently reported pediatric experience is 100% and graft survival is 97% [99]. All of the patients with functioning grafts are on monotherapy, and more than 80% are on every other day or longer dose spacing. Height and weight increases during the first post-transplant year of the first 16 patients are shown in Fig. 19.

Liver transplantation in hepatitis virus-free patients

Infants and children undergoing liver replacement have had the same quality of life benefits as kidney recipients. Cadaveric liver transplantation in adults also has yielded good results but only when the original hepatic disease was caused by something other than hepatitis C virus (HCV) (Fig. 20). In these hepatitis-free adults, there was a high rate of weaning including a small number of recipients who had immunosuppression discontinued (Fig. 21).

However, because the original intention in all of the adult cadaveric liver recipients was to stop immunosuppression, the stipulated objective of complete weaning was not consistently achieved. The patient whose course is summarized in Fig. 21 was one of the encouraging exceptions. Stepwise total weaning was started after 7 post-transplant months and completed after 15 months, with drug freedom for the succeeding 2-1/2 years.

The HCV-infected liver recipient

In contrast to these results, the tolerogenic protocol (Fig. 15) was unsatisfactory for adults with chronic HCV hepatitis. The rapid decline of patient and graft survival (Fig. 22) was

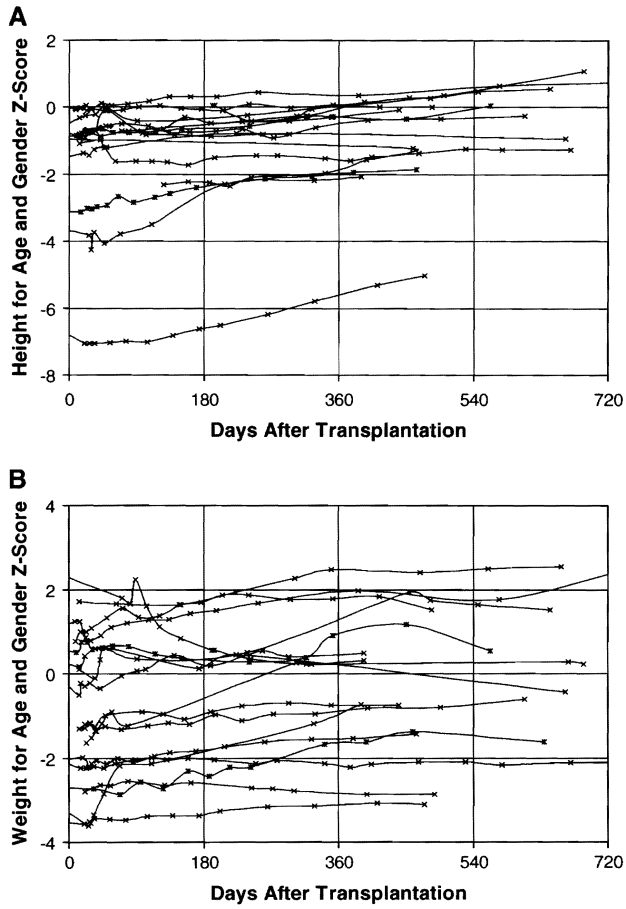

Fig. 19 Height (**A**) and weight (**B**) for age and gender Z scores for 16 kidney transplant recipients who have born functioning grafts for 2 years or longer under tolerogenic immunosuppression. Reference population data is from the 2000 Center for Disease Control, growth data available at www.cdc.gov/growthcharts. By permission of Shapiro et al. [99]

caused mainly by accelerated HCV recurrence. An explanation for the poor results is shown schematically in Fig. 23. As discussed earlier, the typical patient who comes to liver transplantation because of chronic HCV hepatitis has been a partially tolerant disease carrier for a long time (see also Fig. 11E and F), connoting a relatively stable balance between HCV antigen and HCV-specific T cells.

By weakening the T cell restraint on the virus with lymphoid depletion and other transplant-related immunosuppression, the balance was disequilibrated [102]. The resulting astronomical increases in viral load (Fig. 23) resulted in prompt and widespread infection of the new liver. With subsequent drug weaning and recovery of global immune responsiveness, the liver was subjected to potential attack by one, the other, or both viral-specific and de novo donor-specific T-cell responses (Fig. 23) [102].

In our report of this experience, we described a compromise strategy for HCV-infected recipients that requires a lifetime commitment to a relatively fixed level of daily

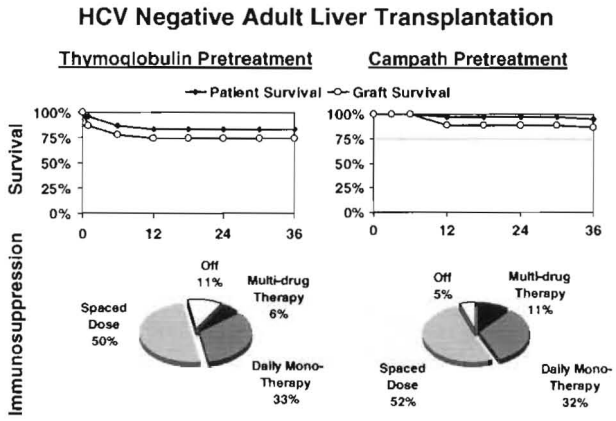

Fig. 20 Patient and cadaveric liver allograft survival of HCV-negative adults who were lymphoid depleted with ATG or alemtuzumab and treated after transplantation with minimal post-transplant immunosuppression. The pies indicate the maintenance immunosuppression at 3 years

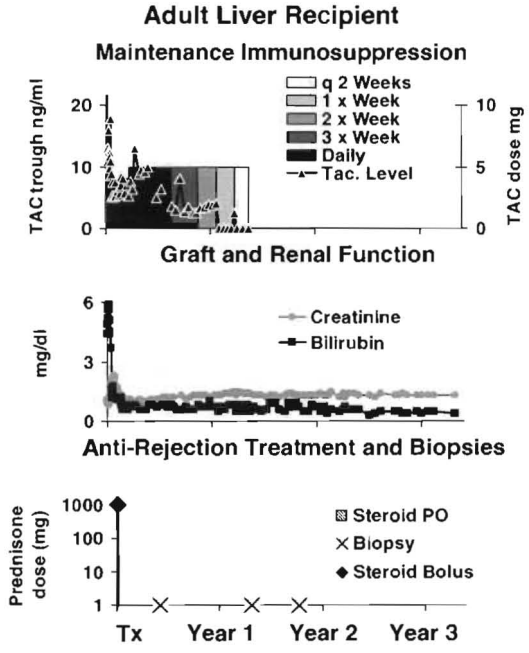

Fig. 21 Course of an alemtuzumab-conditioned cadaver liver recipient who was completely weaned from immunosuppression without incident

immunosuppression that allows prophylactic control of the separate and distinct CTL responses [102]. The compromise requires the systematic production of potentially infectious HCV carriers, an epidemiologic risk that must be made known to all concerned. This unsatisfactory solution to the problem will change only with yet-to-be developed drugs or other means to better contain the HCV load.

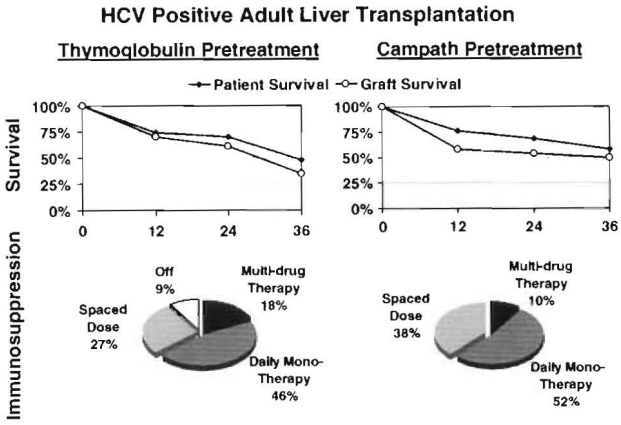

Fig. 22 Results in HCV-infected liver recipients who were lymphoid depleted with ATG or alemtuzumab and treated with tacrolimus monotherapy from which spaced weaning was attempted. The drastic decline in survival was due almost exclusively to accelerated recurrence of hepatitis [6, 102]

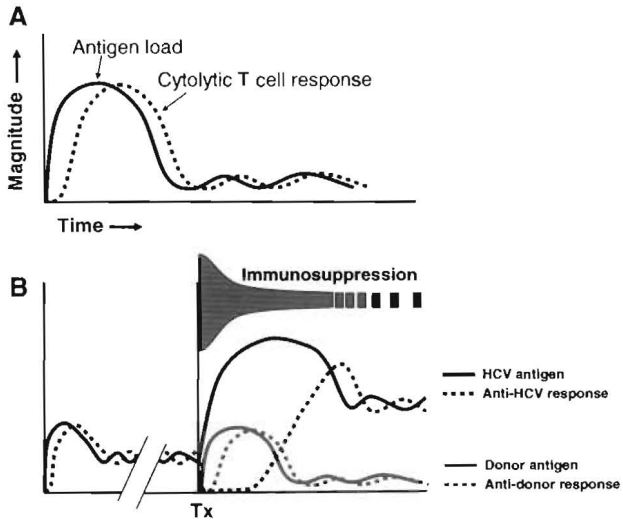

Fig. 23 Explanation for the loss of poor results shown in Fig. 22 (see text and Refs. [6, 102)

The history of clinical transplantation: in retrospect

I recently characterized organ transplantation as "a practical success and an epistemologic collapse" [103]. The epistemologic collapse (i.e. failure to understand what was being accomplished) was caused by the incorrect conclusion in the 1960s that organ engraftment involved mechanisms other than the donor leukocyte chimerism-associated ones of bone marrow cell transplantation. This error had pervasive consequences. First, it distorted the interpretation of experimental and clinical observations. Second, it spawned numerous derivative dogmas and theories. Exposure of the primal error by the microchimerism

discoveries of 1991–92 served notice that the entire superstructure of transplantation immunology had to be reassessed.

At a clinical level, the therapeutic advances that had been made empirically in organ transplantation could now be viewed as the addition of floors to an increasingly habitable house that had been constructed piecemeal without a global architectural blueprint (Fig. 24). The house foundation was laid with the demonstration 44 years ago that rejection is a highly reversible event that frequently can be succeeded by variable tolerance. Better immunosuppressive drugs were developed during the succeeding 4 decades, but the new tools were used like sledge hammers for organ transplantation and like scalpels for bone marrow transplantation. The detection in 1991 and 1992 of leukocyte chimerism in organ recipients, and of the mirror image chimerism in bone marrow recipients, intellectually unified these two kinds of transplantation.

However, efforts to endow organ recipients with the tolerance advantages of bone marrow recipients by infusing adjunct leukocytes yielded disappointing results because of the self-defeating effects of heavy immunosuppression (Fig. 24). Once it was understood how immunosuppression could subvert the essential mechanisms of leukocyte chimerism-driven tolerance, the timing and dosage of the drug treatment (and of adjunct leukocytes) could be adjusted in ways that protected these mechanisms. Now that the pieces of the transplantation puzzle were reassembled in their proper places, the fresh insight could be put to practical use.

Clinical tolerance for organ recipients revisited

The resulting protocol (Fig. 25) was best implemented with live donor transplantation because tolerogenic mechanisms could be set in motion well in advance of organ transplantation. Three weeks before the organ transplantation, recipients were lymphoid-depleted with a single 30 mg dose of alemtuzumab, followed 12 h later by an infusion of unfractionated fresh leukocytes obtained by leukopheresis from the G-CSF-conditioned organ donor. Daily tacrolimus was administered during the 3 weeks between the cell

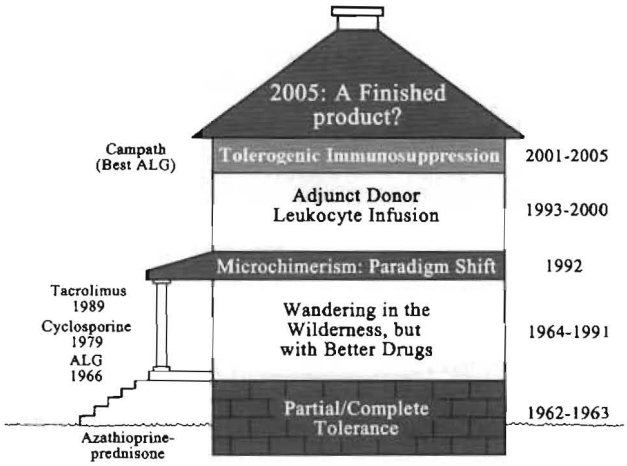

Fig. 24 The "house of transplantation", viewed in hindsight (see text)

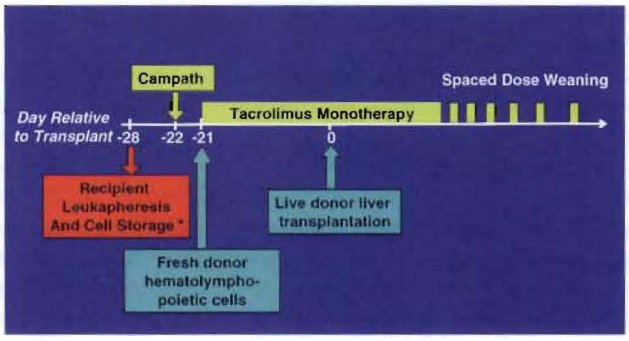

Fig. 25 Tolerance protocol applied in 2005–2006 based on the concepts summarized from the historical perspective of Fig. 24

infusion and the organ transplantation, and for a limited period thereafter. The interval between tacrolimus doses was then increased to every other day or longer.

The first patient to be treated with this regimen was a 19-year old woman with sclerosing cholangitis who received the right liver lobe of her HLA mismatched sister in October 2005 (Fig. 26). Low grade increases in transaminases and canulicular enzymes developed when tacrolimus doses were reduced from 3 to 2 doses per week after about 6 months. The differential diagnosis was rejection versus recurrence of sclerosing cholangitis. After a short course of prednisone, a maintenance schedule of 3 doses/week tacrolimus was settled upon. According to the axiom proposed at the beginning of this lecture, the "need for maintenance immunosuppression" defined the extent of her acquired tolerance. Determination of this endpoint will take one or 2 years since the effects of conditioning with a single infusion of a potent lymphoid-depleting agent, alemtuzumab, are long lasting [98, 104, 105].

Further experience suggests that space-weaning and maintenance dose finding can be done earlier in some cases. In the second patient, a 65-year old liver recipient, tacrolimus was stopped 4 weeks after right hepatic lobe transplantation from her HLA-mismatched

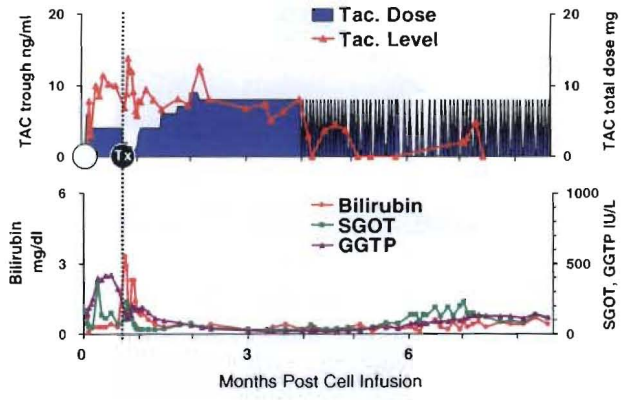

Fig. 26 Course of the first patient treated by the protocol shown in Fig. 25. Complete drug discontinuance has not been possible (see text). TAC = tacrolimus

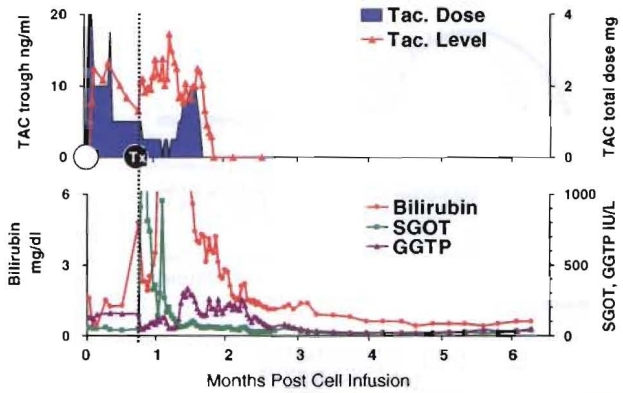

Fig. 27 Second patient treated by the protocol in Fig. 25 (see text). The patient has been drug-free for a half year

son. Multiple organ failure had developed secondary to thrombosis of the graft's hepatic artery. The ischemic liver graft was successfully rearterialized with recovery of completely normal function. She has been off all immunosuppression for a half year (Fig. 27). The fourth patient received a liver allograft from her son and was jaundiced for the first several weeks because of a biliary complication that was corrected with reoperation. She has been on homeopathic or no maintenance immunosuppression for all of the 7 months post-transplant course except the first 2 weeks (Fig. 28).

Three more live donor liver recipients (all HLA mismatched) and 3 live donor kidney recipients (2 HLA mismatched, one identical) have had transplantation with this strategy. All are at different stages of spaced weaning. None of the nine patients has had sustained macrochimerism. This has been a welcome finding. By the time the passenger leukocytes of an organ graft arrive, the recipient has been partially tolerized (Fig. 29). Since the graft passenger leukocytes are naïve, the circumstances at the time of organ transplantation mimic those of a parent to defenseless offspring F1 hybrid model [73, 106] in which there

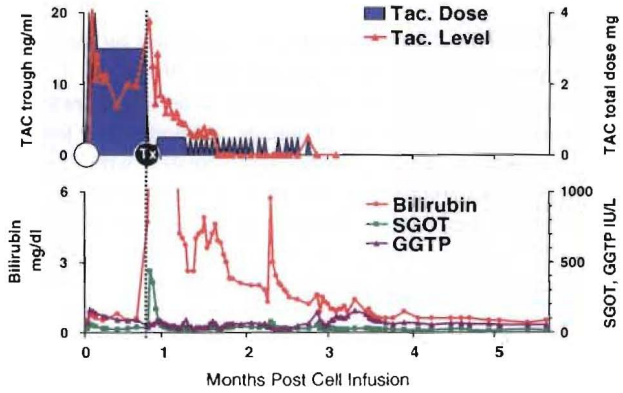

Fig. 28 Course of a patient whose post-transplant course was complicated by a major biliary fistula that was corrected at reoperation. Note that little or no immunosuppression was given from the third postoperative week onward

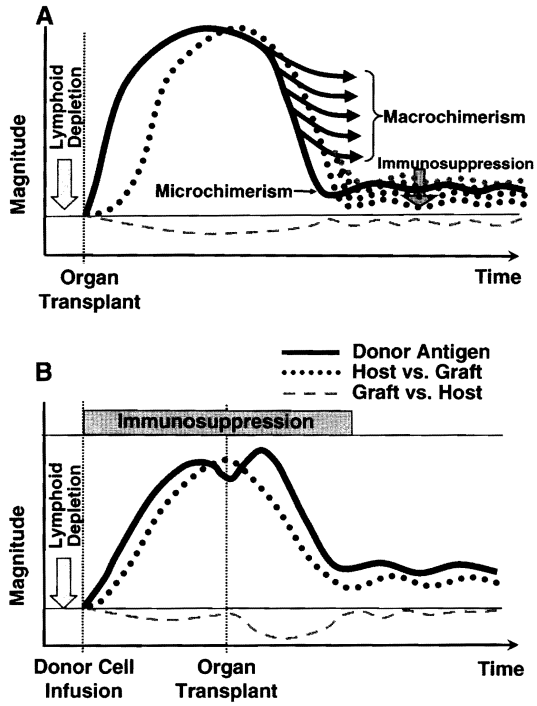

Fig. 29 Balances of mobile donor antigen (donor leukocytes) and donor-reactive T cells after organ transplantation. (**A**) With organ transplantation alone under the immunosuppression shown in Fig. 15, a balance favoring drug-free antigen supremacy over the T cell response induced by passenger leukocytes is theoretically most likely if there is a large quantity of persisting donor cells (macrochimerism). A positive antigen balance also is possible with the microchimerism but this usually requires continuous immunosuppression to weaken the CTL arm. (**B**) With leukocyte infusion in advance of organ transplantation (see Fig. 25), the patient is partially tolerized by the time the second load of donor cells (the passenger leukocytes) arrive and boost the tolerogenic process while theoretically increasing the risk of GVHD (see text). Preliminary experience with this protocol in liver and kidney recipients has been encouraging

is an increased risk of GVHD. This potential risk is depicted by the second expansion of the inverted GVH response curve at the bottom of Fig. 29.

From our perspective, sustained macrochimerism in an organ recipient is more apt to connote a serious complication than an advantage. Although we have not encountered GVHD in any of our nine patients, all recipients treated with this protocol undergo reliminary leukopheresis with cryopreservation of the collected cells (Fig. 25). These naïve recipient cells constitute a safety net, for thawing and re-infusion in the event of GVHD. In 1993 [52], and in more detail 1 year later [107], we described how infusion of such stored cells could quickly switch off an otherwise lethal GVHD.

What lies ahead

Collectively, the nine patients described here make up a promising consecutive series of organ recipients. As has been emphasized, however, the follow-ups are too short to know

Table 4 Transplantation dogmas that require reassessment

1	Macrochimerism is the "holy grail" of organ transplantation
2	Tolerogenesis is more protracted and more difficult to accomplish in humans than in lower species
3	Organ engraftment occurs by leukocyte chimerism-independent mechanisms
4	Immune activation does not require the presence of lymphoid organs
5	Immune responses are generated "directly" in transplanted organs
6	Passenger leukocytes of organs are uniquely tolerogenic because of their cell surface expression of MHC class II or other (e.g. co-stimulatory) molecules
7	Antigen-specific "memory cells" do not require persistence of the antigen

how close this protocol is to optimum. In addition, the extent to which the principles of such treatment can be applied to cadaveric organ transplantation remains to be determined.

It also should be emphasized that the paradigm upon which the protocol is based is incompatible with numerous dogmas that have made up much of the foundation of transplantation immunology. The first 3 dogmas of the incomplete list in Table 4 have major clinical implications. The foremost example is the historical conviction that acquired organ tolerance requires sustained macrochimerism [108–110]. In the context developed here, macrochimerism is equivalent to the microbial infestation of an infectious disease carrier, and is fraught with a similar range of risks to the host, with particular reference to GVHD.

With today's sophisticated research tools, it should be possible to examine the validity, or lack thereof, of the listed as well as other suspect dogmas. Alternatively, the vast amount of meticulously acquired existing data could be reexamined with a different mind set about what these data really mean. Putting the big picture together in this way was, in fact, Bob Good's unique talent as a scientist.

What conclusions and theories ultimately will be validated? Good surely would be content with the philosophy of natural science capsulized 275 years ago in six short lines of poetry:

"All Nature is but Art unknown to thee;
All Chance direction which thou cans't not see;
All Discord Harmony not understood;
All partial Evil universal Good.
And spite of Pride, in erring Reason's spite,
One truth is clear, "whatever is, is RIGHT."
Alexander Pope ("Essay on Man"): 1730A.D.

Good himself loved poetry. One of my most prized possessions is a gift from him of the collected work of Robert Frost. Good's prediction about immunology is contained in the last 4 words of his lengthy inscription: "...the best lies ahead!!". Inside the Frost book (page 105), a poem is found entitled "The Road Not Taken". The last 5 lines read:

"I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I -
I took the one less traveled by,
And that has made all the difference."

Through his life, Bob Good chose the tough less-traveled road into the scientific unknown. And the road has led today to the Society that now bears his name.

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