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### PARADIGM CHANGES IN ORGAN TRANSPLANTATION

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## TRANSPLANTATION TOLERANCE, MICROCHIMERISM, AND THE TWO-WAY PARADIGM

### 1. INTRODUCTION

Melvin Cohn has extolled the virtues of immunologic hypotheses which accommodate all relevant observations as opposed to concise “small theories” that explain only a few facts.<sup>1</sup> Exemplifying Cohn’s argument, the inability to evolve an encompassing explanation for observations made in human and animal allograft recipients, or in surrogate *in vitro* models, has driven much of transplantation research down a reductionist pathway.

Two premises that were introduced into transplantation immunology between 1944 and 1959 explain why. One was that the events following transplantation could be defined in terms of one-way immune reactions: host versus graft (HVG) and graft versus host (GVH). When this assumption was combined with the second premise that whole organ allograft “acceptance” was fundamentally different from the chimerism-dependent acquired tolerance of Billingham, Brent, and Medawar<sup>2,3</sup>, a derivative dogma emerged that we have called “the one-way paradigm”.<sup>4</sup>

### 2. THE Y IN THE ROAD: 1962

Until 1959, the production of chimerism by donor leukocyte infusion in preparation for organ allotransplantation was a much anticipated natural extension<sup>5,6</sup> of the neonatal tolerance models of Billingham, Brent, and Medawar<sup>2,3</sup> and of the adult rodent analogues that required recipient cytoablation.<sup>7,8</sup> In both the neonatal and cytoablation models, the transplanted leukocytes caused graft-versus-host disease (GVHD) unless there was close donor/recipient histocompatibility.<sup>9–11</sup>

When long survival of functioning human kidney allografts was accomplished in sublethally irradiated recipients (1959–1962) *without donor leukocyte infusion*<sup>12–14</sup> and then regularly (1962 onward) using continuous pharmacologic immunosuppression and *no cytoreduction*,<sup>15–18</sup> the



need in organ transplantation for both chimerism and recipient preconditioning seemingly had been eliminated. These conclusions also defocused an understanding of bone marrow transplantation which was widely construed to be an example of total donor leukocyte chimerism: i.e. complete replacement of the immune system.

Because there was little room for immune interactions (i.e. combined HVG and GVH responses) in the evolving framework of transplantation immunology, objections to the simplistic concept of a one-way reaction were not taken seriously. In their alternative hypothesis, Simonsen,<sup>19</sup> supported by Michie, Woodruff, and Zeiss,<sup>20</sup> postulated in 1960–1961 that coexisting donor and recipient immune cells in neonatally tolerant mice had achieved a mutually nonreactive state while retaining the ability to function collaboratively (e.g. in a joint immune response to infection). Interest in this hypothesis evaporated when Simonsen recanted it in 1962,<sup>21</sup> largely because no experimental support could be found.

In addition, however, nothing less than host clonal deletion to explain acquired transplantation tolerance appeared to be compatible with the consensus of that time. The disputes about clonal selection as the basis for self/non-self delineation seemingly had been brought to closure by the ultimate *imprimatur* of the Nobel Prize, which was awarded to MacFarland Burnet in 1960.<sup>22,23</sup> It was no coincidence that the 1960 co-laureate with Burnet was Peter Brian Medawar, whose observations in the neonatal tolerance model<sup>2,3</sup> were widely considered to be a validation of Burnet's prediction that developing lymphocytes with an open repertoire of receptors could be purged of self-reactive cells before they achieved functional maturity. The tolerance produced in radiation chimeras<sup>7,8</sup> was viewed as an iatrogenically engineered simulation of these events of ontogeny.

The argument that clonal deletion is the key mechanism of either neonatal, cytoablation, or drug-induced tolerance has not been considered defensible by Schaffner,<sup>22,23</sup> Cohn,<sup>1</sup> Nossal,<sup>24</sup> and Tauber.<sup>25</sup> If it depended solely on clonal deletion, organ transplantation was difficult to envision as a biologically sound undertaking. In fact, Burnet did not. In 1961, he wrote in the *New England Journal of Medicine* that "... much thought has been given to ways by which tissues or organs not genetically and antigenically identical with the patient might be made to survive and function in the alien environment. On the whole the present outlook is highly unfavorable to success ..."<sup>26</sup>

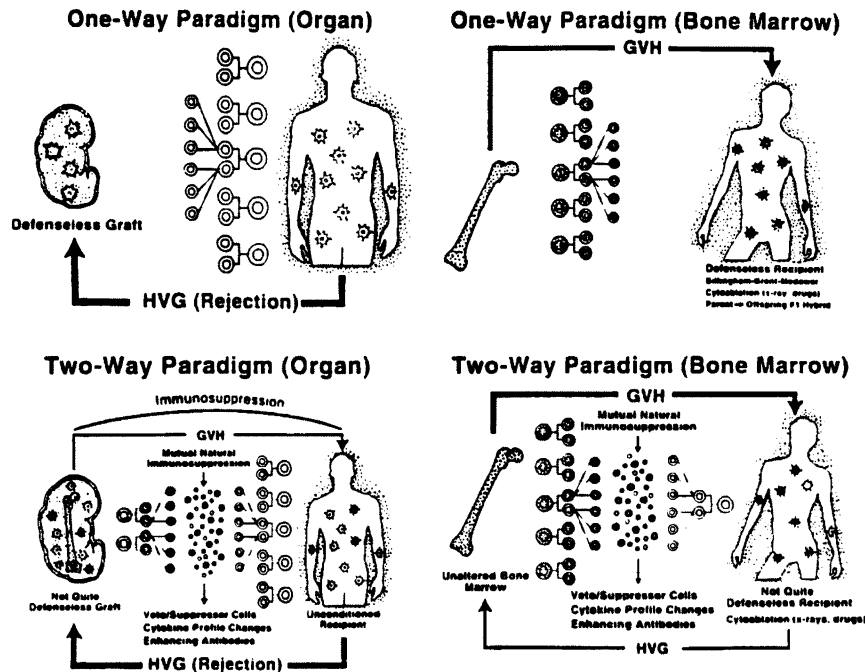


Figure 1. (Upper panels) One-way paradigm in which transplantation is conceived as involving a unidirectional immune reaction: (left) host-versus-graft (HVG) with whole organs and (right) graft-versus-host (GVH) with bone marrow or other hematopoietic transplants. (Lower panels) Two-way paradigm in which transplantation is seen as a bidirectional and mutually cancelling immune reaction that is (left) predominantly HVG with whole organ grafts, and (right) predominantly GVH with bone marrow grafts.

### 3. THE ONE-WAY PARADIGM

#### 3.1. Organ Transplantation

With the widespread acceptance of the simplistic one-way paradigm that emerged from the 1959–1963 period, the unidirectional *in vitro* tests of immune reactivity<sup>27,28</sup> were automatically accepted as “minitransplant models”, the results from which were assumed to be directly applicable to *in vivo* circumstances of transplantation. An organ allograft was envisioned as a defenseless island in a hostile sea (Figure 1 upper left). With the opposite conditions of bone marrow transplantation, histoincompatible allografts rejected the defenseless recipient (GVHD) (Figure 1 upper right).

The one-way paradigm guided the strategies of recipient cytoablation and “immune system replacement”<sup>7,8</sup> that eventually led to successful clin-

TABLE I

Differences between conventional bone marrow and organ transplantation

Bone marrow		Organ
Yes	← Recipient cytoablation <sup>a</sup> →	No
Critical	← MHC compatibility →	Not critical
GVHD	← Principal complication →	Rejection
Common	← Drug free State →	Rare
Tolerance	← Term for success →	"Acceptance" <sup>b</sup>

<sup>a</sup> Notes: All differences derive from this therapeutic step which in effect establishes an unopposed GVH reaction in the bone marrow recipient whose countervailing immune reaction is eliminated.

<sup>b</sup> Or "operational tolerance".

ical bone marrow transplantation in humans,<sup>29–33</sup> a procedure that was (and is) feasible only with a perfect or good tissue (HLA) match. The belief that cytoablation (or cytoreduction) to "make microenvironmental space" was a prerequisite for leukocyte engraftment (reviewed in <sup>34</sup>), became dogma in spite of early<sup>35,36</sup> and recent evidence<sup>34,37</sup> that it was not true.

To explain the differences between organ and bone marrow transplantation (Table I), it was necessary to ascribe "graft acceptance" following the two kinds of procedures to disparate mechanisms. The assumption that chimerism, the *sine qua non* of bone marrow transplantation, was irrelevant to an explanation of successful organ transplantation lay at the root of the dilemma.

#### 4. THE TWO-WAY PARADIGM

A connection between bone marrow and organ transplantation was made in 1992 when donor leukocytes (microchimerism) were discovered up to 30 years postoperatively in the peripheral tissues or blood of human recipients of kidneys, livers, and other organs <sup>38–43</sup> (Figure 1 lower left). The donor-derived cells were so few in number that sensitive immunocytochemical and polymerase chain reaction (PCR) techniques were required for their detection. However, we postulated that they were surviving leukocytes from one limb of originally antagonistic but ultimately reciprocally attenuated or abrogated HVG (rejection) and GVH reactions (Figure 2).

In the first few days after organ transplantation, multilineage bone marrow derived ("passenger") leukocytes constitute 1–20% of the host

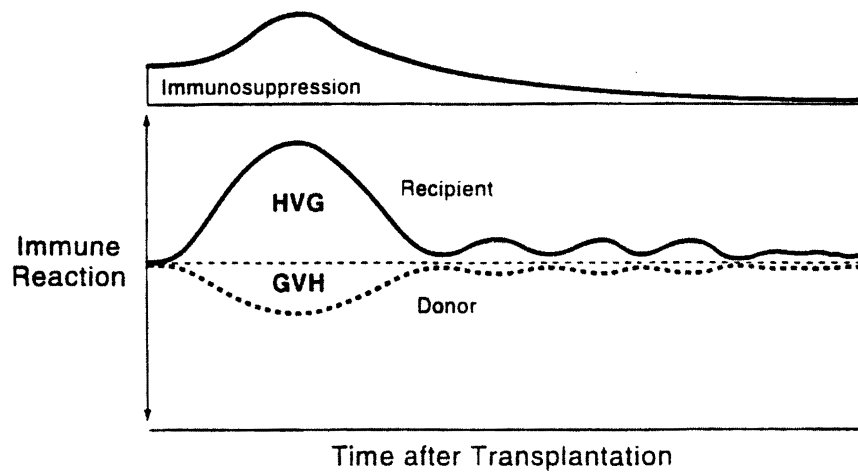


Figure 2. Contemporaneous host versus graft (HVG) and graft versus host (GVH) reactions in the two-way paradigm as applied to organ transplantation. Following the initial interaction, the evolution of non-reactivity of each leukocyte population to the other is seen as a predominantly low-grade stimulatory state that may wax and wane, rather than one of absolute or irreversible clonal deletion.

circulating mononuclear cells, depending on the kind of organ (i.e. highest with liver and intestine, lowest with heart or kidney).<sup>38,40,44</sup> These leukocytes, which include pluripotent stem cells<sup>45,46</sup> and dendritic cells<sup>38,40–43</sup> migrate to the recipient lymphoid organs and are largely replaced in the graft by similar recipient cells.<sup>40,41,44,47–50</sup> After about 2 weeks, small numbers of donor leukocytes can be found increasingly in other tissues and by 3 months they are mostly in non-lymphoid sites (e.g. skin and native heart).<sup>40,51,52</sup>

Thus, even with the limited information available in 1992, it was possible to suggest that organ allograft acceptance involved "...[acute] responses of co-existing donor and recipient immune cells, each to the other, causing reciprocal clonal expansion, followed by peripheral clonal deletion".<sup>38</sup> Much evidence has accrued subsequently in support of this bidirectional mechanism (summarized in<sup>51,52</sup>).

A second mechanism also was proposed,<sup>38,40</sup> namely that the departure from the transplanted organ of the donor leukocytes that drive the HVG response reduces the allograft's immunogenicity. This effect of leukocyte depletion has been demonstrated in many different experimental models.<sup>53–58</sup> For successful engraftment, the progressive change in organ immunogenicity and the clone-specific deletion of the recipient as well as donor leukocyte populations presumably take place in a close temporal

relationship. In time, a stable allograft emerging from this triple process may come to resemble an immunologically neglected infection.<sup>52</sup>

## 5. PREVIOUS ENIGMAS

### 5.1. *Organ Transplantation*

The characteristic cycle of immunologic crisis and resolution, first observed in drug immunosuppressed kidney recipients and most practically monitored by serial changes in allograft function,<sup>16</sup> was the product of a dual immune reaction (Figure 2). With the peripheral migration of the donor cells, and the influx of recipient cells which do not cause graft damage when adequate immunosuppression is given, both the allograft and recipient become genetic composites (Figure 1 lower left).

The mutually cancelling effect of the donor and recipient cell populations explains the rarity of GVHD following the engraftment in non-cytoablated recipients of immunologically active organs such as the intestine and liver. Disruption of the leukocyte interaction with the host cytoablation used to prepare bone marrow recipients, but not the recipients of whole organs, obviously is responsible for the differences between the two kinds of procedure (Table I) including absolute dependence on HLA matching to avoid GVHD in the first instance but not the second.

### 5.2. *Bone Marrow Transplantation*

In the context of the two-way paradigm, it can be seen that bone marrow (Figure 3) and organ transplantation (Figure 2) are, in fact, mirror images, resulting from the drastically different treatment strategies<sup>4</sup> (Table I). This conclusion has been supported by reports describing a trace residual population of recipient leukocytes in essentially all human bone marrow recipients who previously were thought to have complete donor cell chimerism (Figure 1 lower right).<sup>59,60</sup>

### 5.3. *Post-Transplant Lymphoproliferative Disorders (PTLDs)*

The two-way paradigm casts new light on the B cell lymphomas (PTLD) that usually are of host origin in organ recipients and of donor origin after bone marrow transplantation. Except for their frequent Epstein-Barr virus (EBV) association, these human malignancies are indistinguishable from those induced by Robert Schwartz in a mouse chimerism model<sup>61</sup> 3 years before the PTLD complication was first recognized clinically.<sup>62</sup>

Although these tumors correctly are explained in part by a loss of immune tumor surveillance,<sup>63</sup> Schwartz attributed the development of

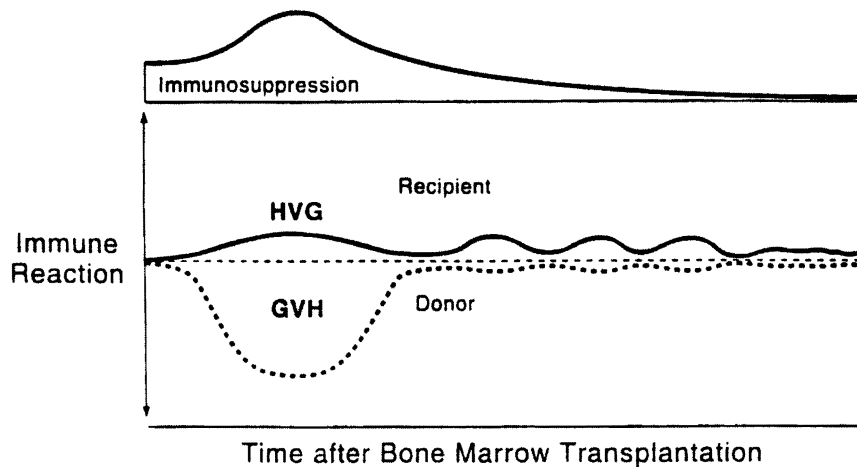


Figure 3. The mirror image of Figure 2 after bone marrow transplantation to a recipient who has undergone cytoablation.

similar neoplasms in his mouse model to the additional factor of a lymphoproliferative response by the dominant immune apparatus against the persistent subclinical GVH and immunogenicity of the minority leukocyte population. The clinical relevance of Schwartz's observations, and of his "rules" of pathogenesis could not be appreciated until three decades later in the context of the two-way paradigm.<sup>64</sup>

## 6. THE "DEBATE"

Non-vital antigen can also induce donor-specific non-reactivity, albeit inefficiently and of limited duration, compared to live leukocytes.<sup>3</sup> In the chimerism-exclusionary context of the one-way paradigm, it became axiomatic that antigens of the parenchymal (or vascular endothelial) cells of transplanted organs induced allograft acceptance by ill-defined alternative mechanisms.<sup>65</sup> In an extension of such reasoning, it has been argued that the microchimerism associated with successful transplantation, and conversely its disappearance with or just after irreversible rejection in experimental models,<sup>66–70</sup> is epiphenomenal.<sup>71</sup>

Skepticism about the significance of microchimerism (summarized in<sup>71,72</sup>) has been based on: (1) the inconsistency with which donor leukocytes can be found in blood or tissue samples from organ recipients, (2) the development of acute or chronic rejection despite chimerism, and (3) the inability to use microchimerism to guide post-transplant drug weaning. All



of these ostensibly contradictory observations can be readily fitted into the concept of the various chimeric states, providing these states are considered along with the leukocyte depletion and altered immunogenicity of the allograft. Both of these factors vary with time, and according to antigen migration and localization.<sup>52</sup>

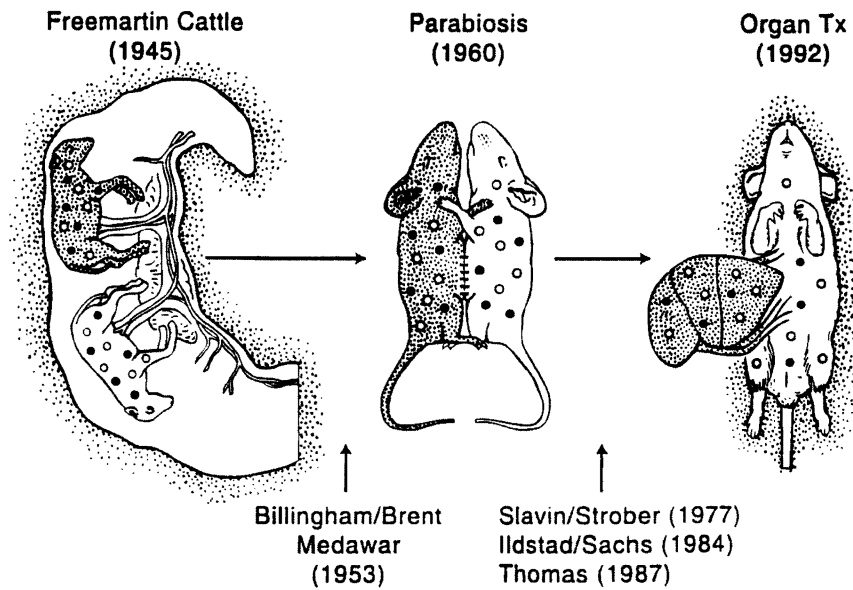
Starzl and Zinkernagel<sup>52</sup> have proposed that the immunologic response or non-response against infections or tumors, and under the conditions of clinical transplantation, are governed primarily by the migration and localization of antigen. In their view, immune reactivity depends on migration of antigen to organized lymphoid tissue and can be viewed as "... a balance between potentially reactive lymphocytes versus the qualities, quantity, kinetics, and distribution of the antigen (foreign or self) within the host".<sup>52</sup>

In this context, donor leukocyte chimerism is a prerequisite for, but neither synonymous with nor a consequence of, the evolution of allograft tolerance.<sup>38,40,51,52</sup> Although the association of chimerism with organ allograft acceptance was discounted for a third of a century,<sup>51</sup> the principle of chimerism-linked organ allograft acceptance is no different than in the rodent neonatal,<sup>2,3</sup> cytoablation-dependent,<sup>7,8</sup> parabiosis-induced<sup>73</sup> and more complicated "mixed chimerism" tolerance models<sup>74-76</sup> (Figure 4). The theme came full-circle back to the observations by Owen<sup>77</sup> 53 years ago of natural tolerance in freemartin cattle.

## 7. MECHANISMS IN THE TWO-WAY PARADIGM

The two-way paradigm defines success and failure after transplantation in a different way than before. Success implies that chimerism has been introduced which may or may not be immunosuppression-dependent. Treatment failure connotes the therapeutically uncontrollable ascendancy of HVG or GVH.<sup>4,38,40,78</sup> Pathologic evidence of both processes is found frequently in failed cases. Although the ultimate result almost always is predominantly one or the other, the two cell populations cross-modulate and are actively self-protective as a general rule. The reciprocal "defensive" mechanism is particularly important if one cell population is outnumbered and if there is severe major histocompatibility complex (MHC) disparity.

"T-cell tolerance" is an apposite term for donor-specific non-reactivity, but it is unlikely that allograft acceptance can be fully comprehended from the results of studies of individual leukocyte phenotypes.<sup>43</sup> The totality of events leading to tolerance suggests learning (cognitive) functions that have been explained by "networks".<sup>79-82</sup> Network activity under



*Figure 4.* The continuum of chimerism from observations of Ray Owen in Freemartin cattle (77) to the present. Chimerism was incorrectly discounted as a factor in organ allograft acceptance from about 1960 until the discovery in 1992 of microchimerism in organ recipients.<sup>38,40</sup>

the circumstances of transplantation presumably would be multimechanistically influenced by cytokines, immunoregulatory cells, antibodies, and other factors.

Considering the shifting details of signalling and effector response involved in the merger of two interactive and genetically controlled immune systems, computer modelling based on known and experimentally verifiable events may be required to fully comprehend the process. However, this level of information is not required to understand the seminal governance role of antigen migration and localization.<sup>52</sup>

## 8. NON-TRANSPLANT ISSUES

Transplantation does not involve unique immunologic responses.<sup>52</sup> For example, although the relation between infectious and transplantation immunity is complicated by the presence of a double immune reaction and the additional factor of therapeutic immunosuppression after transplantation, the mechanisms and rules are basically the same and governed by antigen migration and localization. The two essential mechanisms

are antigen-driven clonal exhaustion/deletion, and “indifference” of the immune system to antigen that either does not reach lymphoid organs or survives passage through them.<sup>52</sup>

Because the fetus possesses T cell immune function in the every early stages of its development,<sup>51,82,83</sup> it can be suggested that antigen migration and localization is the basis for the ontogeny of self/non-self discrimination in the same way as it is for acquired tolerance. Autoimmune diseases would then reflect unacceptable post-natal perturbations of the prenatally established lymphoid/non-lymphoid balance (self tolerance). Thus, we have suggested an extension and modification of the meaning of “immunologic self”<sup>52</sup> which has been enigmatic since it was described a half century ago by FM Burnet.<sup>26</sup>

## 9. CONCLUSION

The assumption that hematolymphopoietic chimerism was irrelevant to successful whole organ transplantation as currently practiced led to alternative inadequate explanations of organ allograft acceptance, clouded the meaning of successful bone marrow transplantation, and precluded for more than three decades the development of a cardinal principle of transplantation. Recognition of this error and the incorporation of the chimerism factor into a two-way paradigm has allowed previous enigmas of organ as well as bone marrow engraftment to be explained and may allow a better understanding of immune function generally.

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