Explanation for Loss of the HLA Matching Effect

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MY MAIN purpose today is to explain why HLA matching is so *crucial* for bone marrow transplantation on one hand, and yet so relatively *unimportant* for organ transplantation on the other.

THE ONE-WAY PARADIGM OF TRANSPLANTATION IMMUNOLOGY

The one-way paradigm of transplantation immunology shown in Fig 1 was of course directly derived from our patron saints—Billingham, Brent, and Medawar. In their original model,[1] or in the defenseless parent to offspring F_1 hybrid experiments, and after recipient cytoablation, the avoidance of graft-vs-host disease (GVHD) after bone marrow or spleen cell transplantation was found to be dependent on good MHC compatibility.

In analogous experiments, the same rules of histocompatibility apply to immunologically defenseless recipients of immunologically active whole organs as was emphasized by Billingham and Brent[2] and demonstrated in detail for the intestine by Monchik and Russell.[3] However, the usual immunologic context of whole organ transplantation is actually a mirror image of the bone marrow experiments, but still involving a one-way reaction. Now, the graft is the defenseless victim instead of being the aggressor.

This false conceptualization has been the paradigm of transplantation immunology for more than 40 years. In spite of its fatal intellectual flaw, the concept of an essentially unidirectional immune reaction proved useful. It was translated into successful clinical bone marrow transplantation in 1968 by Robert Good and Fritz Bach, emphasizing above all the need for HLA compatibility if engraftment was to be accomplished without the complication of GVHD.

THE 2-WAY IMMUNOLOGIC PARADIGM

The one-way paradigm has never explained what we see daily in our whole organ clinics, exemplified by the failure of HLA matching to influence more profoundly the survival of whole organ grafts. This was recognized early by transplant surgeons who by the time of the first successful clinical bone marrow cases had already recorded thousands of successful whole organ transplantations (mostly kidneys) using continuous immunosuppression—without host preconditioning, without dependence on MHC matching, and with no problems with GVHD. The avalanche of whole organ cases began after a characteristic postoperative cycle was identified when azathioprine was systematically combined with prednisone.[4] Kidney rejection could be reversed surprisingly easily with prednisone. More importantly, the need later on for maintenance immunosuppression frequently declined. The same sequence has been seen since with all other organs transplanted and with all of the immunosuppressive regimens. Something appeared to have changed in the host, the graft, or both. But what?

Thirty years and a revolution in immunology later, this question was answered. Donor leukocytes of bone marrow origin, which are part of the structure of all complex grafts (the so-called multilineage "passenger leukocytes"), had migrated from the organs and survived ubiquitously in the recipients.[5,6] Thus, successful organ transplantation was a cryptic example of cell transplantation, whereby a small fragment of disseminated extramedullary donor bone marrow (shown in Figure 2 as a bone silhouette) was assimilated into the overwhelmingly larger immunologic network of the host. The cell movement was in both directions. In this *bidirectional* paradigm, the immunologic confrontation following whole organ transplantation involves a GVH as well as HVG reaction in which the two cell populations are mutually cancelling, providing both can survive. The umbrella of immunosuppression that covers both equally allows this.

The number of chimeric donor cells is small.[5,6] Recent research has suggested how such a small number of chimeric leukocytes can down regulate the donor-specific reactivity of the vast army of recipient cells against which they are arrayed (a veto effect; Fig 2). The dendritic cells of Steinman and Cohn[7] are thought to be key participants. With the paradigm of a two-way immune reaction, virtually every previously enigmatic problem seen clinically after experimental or clinical whole organ transplantation becomes either transparent or susceptible to experimental inquiry.

THE BLINDFOLDING OF HLA

This includes the loss or blunting of an HLA matching effect. With the two-way cancellation, it is easy to understand how the expected matching influence is blind folded. With each further level of histoincompatibility, the cancelling effect is postulated to escalate both ways providing the process is chaperoned with an effective immunosuppressive

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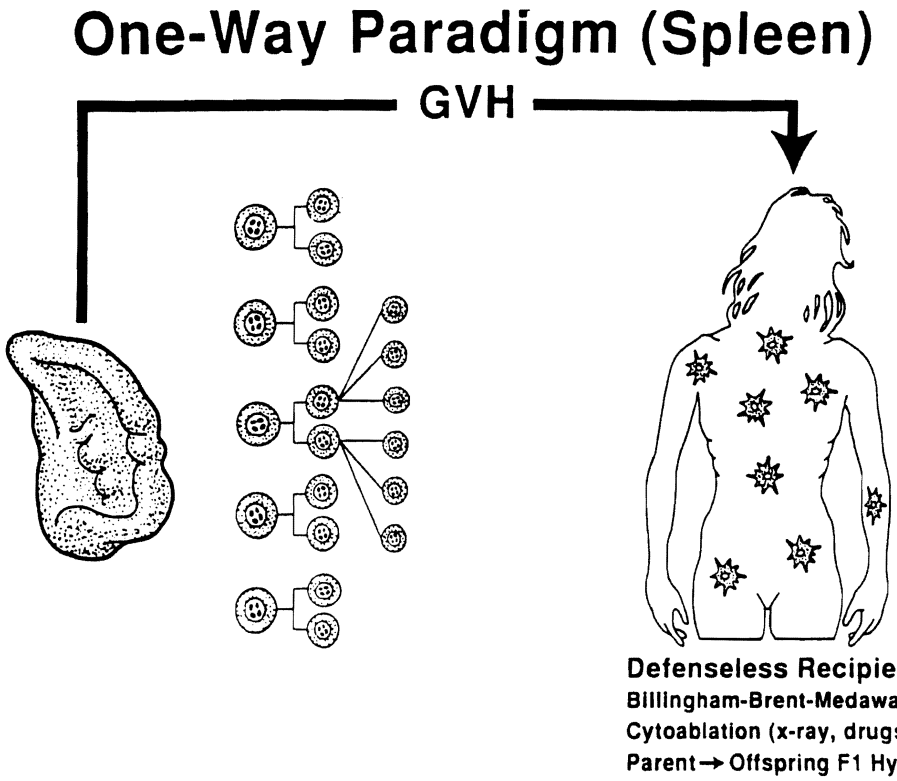

Fig 1. Transplant model used in the first demonstration by Billingham, Brent, and Medawar[1] to demonstrate the acquisition of immunologic tolerance.

umbrella, with a consequent dwindling of the matching effect as donor-specific and recipient-specific nonreactivity evolves. In this context, suppressor and veto cells, changes in cytokine profiles, and the development of enhancing antibodies are derivative from the primary event of mutual cell engagement (Fig 2).

The importance of the spontaneous chimerism and its density has been demonstrated by Robert Keenan and Adriana Zeevi (University of Pittsburgh). They stratified 15 lung transplant recipients, with follow-up of 1 to 5 years, into a clinical and histopathologically favorable group of eight with no bronchiolitis obliterans and seven who had this ominous finding of chronic rejection. The patients without bronchiolitis obliterans had dense chimerism—positive in eight of eight lymph nodes, seven of eight skin biopsies, and six of eight blood samples.[8] Chimerism also was demonstrable in several patients of the less favored group but less regularly and with a generally lower quantitative grade. Using the stored donor spleens, donor-specific nonreactivity was demonstrated in all but one of the highly chimeric recipients, but in only two of the less favored group. These results had no correlation with the preoperative HLA match.

In addition to explaining why the HLA matching effect is blind folded, this bidirectional mechanism explains why GVHD does not develop after liver, intestinal, multivisceral, and heart-lung transplantation. It also explains why all whole organs have the inherent capability of tolerogenicity. Calne's brilliant studies with Sells in pigs[9] and with Zimmerman and Kamada in rats of the leukocyte-rich liver provided the most clear examples—called hepatic tolerogenicity. However, Corry et al[10] and Russell et al[11] showed long ago the tolerogenicity of even the leukocyte-poor heart and kidney in mice but *only* with less difficult MHC barriers.

Finally, the once vast gap between the bone marrow and whole organ transplantation fields was further closed when it was shown by the teams of Donnall Thomas in Seattle[12] and others[13] using sensitive detection techniques that there is a trace population of recipient cells in patients whose bone marrow was previously thought to have been completely replaced. Thus, in these patients, as in whole organ recipients, the appearance of MHC restricted veto and suppressor cells, enhancing antibodies, and changes in cytokine profiles could be construed as by-products of and accessory to the seminal event of mixed chimerism, similar to the depiction of Fig 2. The difference in principle between the bone marrow and whole organ recipients turns on which of the coexisting cell populations is present at trace levels.

DONOR LEUKOCYTE AUGMENTATION

At a practical clinical level, the observations establishing the two-way paradigm exposed a perioperative window of opportunity, which is not HLA dependent during which unaltered HLA incompatible bone marrow can be engratted safely in organ recipients without recipient conditioning, without T-cell depletion, and with no deviation

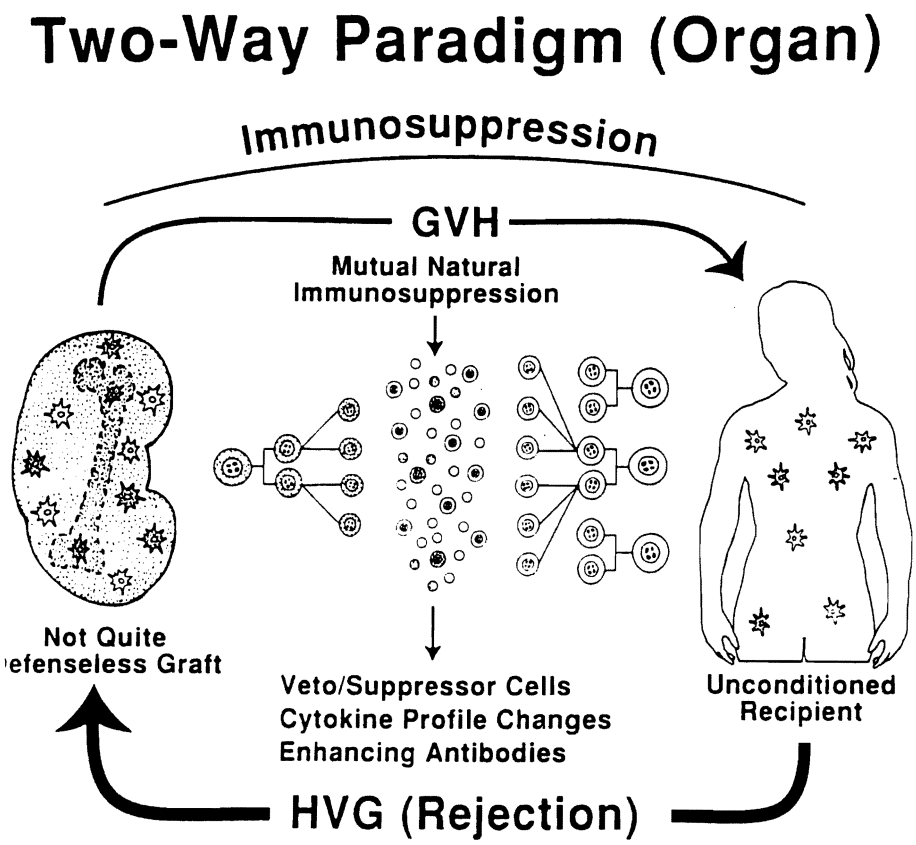

Fig 2. Bidirectional mechanism of whole organ graft acceptance involving a graft-vs-host (GVH) reaction by the bone marrow derived donor leukocytes in the graft that are pitted against the whole recipient immunologic apparatus (host-vs-graft [HVG] rejection). For conventional whole organ clinical transplantation, the recipient is not preconditioned.

from the standard practices of postoperative immunosuppression used in whole organ centers. This has been done in Pittsburgh with the infusion of 3×10^8/kg unaltered donor bone marrow cells perioperatively in 64 consecutive primary recipients of cadaveric kidneys, livers, hearts, and lungs (Fig 3). Immunosuppression was with the standard strategy developed a third of century ago with azathioprine and prednisone but now with the combination of FK 506 and prednisone instead.

All 64 of these patients are well except for a cardiac recipient who died with a normal heart of nonimmunologic complications 9 months after transplantation (Table 1). Chimerism, estimated to be more than 1000 times greater than that occurring spontaneously, could be reliably produced and sustained. The first 18 of these patients were recently reported in *The Lancet*.[14] The familiar events were observed of crisis and recovery that are also seen in nonmarrow augmented control recipients. The notable absence of serious GVHD, and the presence of well-functioning grafts in all of the patients, has marked these recipients as an advantaged cohort. Although drug weaning in these densely chimeric patients is expected to take several years, they are the first recipients to undergo HLA mismatched cadaveric whole organ transplantation with the reasonable prospect of eventually becoming drug free.

TERASAKI'S SEMINAL OBSERVATION

In closing, it is worth reflecting on the previous work of Paul Terasaki, who in 1970 announced at the Third Transplantation Congress at The Hague that tissue typing did not accurately predict outcome after kidney transplantation except when there was a perfectly matched donor (unpublished manuscript). This courageous report was treated as a scandal by the HLA community and was the beginning of a dispute that has never come to closure. Now we can explain Terasaki's results as well as those obtained by us from a 6-year prospective trial of HLA matching for kidney transplantation carried out in collaboration with Terasaki at the University of Colorado between 1964 and 1970.[15] This was the first such matching trial ever done. When matching proved to be nonpredictive of outcome except when there was a perfect match, we were stunned, because we had no explanation.

Ironically, the explanation was published as early as 1953 at least in concept, by Simonsen—one of this years' Medawar laureates—who wrote: "... our only real hope of making homotransplantation of the kidney a clinically feasible procedure ... must actually be based on the following conditions: First, that the most important individual-specific antigens are shared by the kidney and the blood cells, so that a gross biological incompatibility can be eliminated by detailed blood determination. Second, that

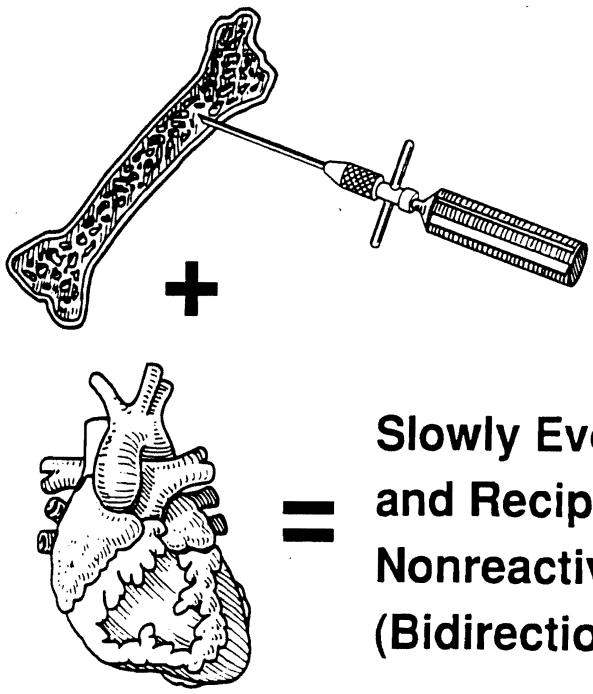

Fig 3. Strategy for donor leukocyte augmentation in recipients of whole organ allografts.[14] The bone marrow is given at the time of the surgical transplant procedure.

the kidney's defense reaction against the recipient's antibodies and/or antigens can check a less significant biological incompatibility . . . and thus provide a more or less complete adaptation to the recipient."[16] As it has turned out, the first of these conditions, histocompatibility matching, is apparently less important in the whole organ field than the second which is the primitive outline of what we now call the two-way paradigm.

CONCLUSIONS

As in Edgar Allen Poe's (1809 to 1849) famous short story, *The Purloined Letter*, the reason for the failure of an HLA matching effect was not found for such a long time because it was hidden in such obvious locations—throughout the body of the recipient in the form of donor chimeric cells (Fig 2).

Table 1. Whole Organ Transplantation With 3 × 10^8/kg Bone Marrow Cells (December 14, 1992 to August 5, 1994)

Organ	Survival	
	Patient	Graft
Kidney	17	17
Kidney + islets	6	6
Kidney + pancreas	2	2
Liver	28	28
Liver + islets	1	1
Heart	8	7*
Lung	2	2
Total	64	63

Note: The first 18 patients were reported in *The Lancet*, July 17, 1994. There was no host conditioning.
*Nonimmunologic death, 9 months—cardiac allograft normal.

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