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EDITORIAL

Tolerance for Organ Recipients: a Clash of Paradigms

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In this issue of *Liver Transplantation*, Donckier et al.[1] report on 3 patients with primary hepatic malignancies who underwent live donor liver transplantation, followed 1 week later by an infusion of fractionated stem cells obtained from the blood of the granulocyte colony-stimulating factor (G-CSF)-conditioned donor. The intention of the Brussels team was to limit immunosuppression to the first few postoperative weeks by promptly establishing donor leukocyte chimerism–associated tolerance. In cases 1 and 2, immunosuppression was discontinued as planned, about 3 weeks postoperatively. Approximately 3 months later, mild acute rejections occurred that were reversed with a short course of immunosuppression. Treatment was then successfully stopped, with subsequent in vitro evidence of donor specific nonreactivity. These 2 patients were immunosuppression free for >95% of the time between liver replacement and death from recurrent malignancy at 561 and 356 days. For patient 3, immunosuppression discontinuance was put off until 213 days because of a surgical complication. When a reversible rejection developed, no further effort was made to stop treatment. This patient is currently on small daily doses of tacrolimus and is tumor free at 498 days.

As the authors emphasized, these results were incompatible with the immunologic paradigm upon which the strategy was based. The paradigm evolved historically in the following way. After the demonstration in 1953 that acquired allotolerance is strongly associated with donor leukocyte chimerism,[2] attempts were made in the 1950s to produce chimerism in cytoablated organ recipients by infusing donor leukocytes before or at the time of organ transplantation.[3,4] Such efforts failed, because the donor cells either caused lethal graft versus host disease (GVHD) or were rejected. When human kidney transplantation was accomplished in the early 1960s under continuous drug immunosuppression without cytoablation/reduction or leukocyte infusion, it was widely concluded that leukocyte chimerism played no role in the induction or the perpetuation of organ alloengraftment.[5] With this assumption, experimental efforts were renewed to give organ recipients the tolerance advantages of leukocyte chimerism while avoiding GVHD. This proved to be possible in selected cytoablation and cytoreduction models,[6-8] but only when donor cells made up ≥1% of the recipient blood leukocytes (macrochimerism).[9] Lower levels (microchimerism) had no effect. When these model-specific observations were generalized, the sharp distinction between macrochimerism and microchimerism became dogma.[10-13] In addition, the dogma that organ engraftment occurs by mechanisms different from bone marrow transplantation appeared to be strengthened.

The foregoing dogmas, as well as the overarching immunologic paradigm that had been distorted by the incorporation of those dogmas, were challenged in 1991-92, when multilineage donor leukocyte microchimerism was detected in all long-surviving human organ recipients.[14,15] It was evident that migration of an organ's passenger leukocytes (including stem cells) into the recipient had been the previously unrecognized equivalent of a bone marrow cell infusion. The picture was made complete by the demonstration of "reversed proportion" mixed chimerism (i.e., small numbers of persisting host leukocytes) in essentially all bone marrow recipients previously thought to have total hematolymphopoietic cell replacement.[16] These findings unified the immunology of the 2 kinds of transplantation.[17]

Abbreviations: GVHD, graft versus host disease; CTL, cytolytic T cells.

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Moreover, only 2 mechanisms involving the mobile leukocytes were needed to explain how an allograft could avoid rejection.[18] One mechanism was immune ignorance: i.e., failure of the immune system to recognize the presence of donor leukocytes that failed to reach host lymphoid organs.[18-20] The other mechanism (and the seminal one of acquired tolerance) was exhaustion-deletion of the clonal T-cell response induced by cells that reached the lymphoid sites.[14,15,18] The paradigm shift mandated by the microchimerism and related discoveries was facilitated by formal proof of the existence and importance of both immune ignorance[20] and exhaustion-deletion,[21,22] and ultimately by formal proof that leukocyte chimerism, even at a micro level, is essential for perpetuation of clonal exhaustion-deletion and tolerance.[23]

In the new paradigm,[18] it was proposed that all outcomes after transplantation are determined by the balance established between the amount of mobile donor antigen (donor leukocytes) with access to host lymphoid organs and the number of donor-specific cytolytic T cells (CTL) induced at the lymphoid sites (Fig. 1A). In this view, it is axiomatic that the prolonged engraftment of an organ, with or without the aid of maintenance immunosuppression, connotes some degree of partial donor leukocyte chimerism-dependent tolerance. In some cases, very small numbers of donor leukocytes may be sufficient to retain ascendancy over donor-reactive CTL. Even if the CTL arm gains supremacy, immunosuppression can restore and maintain a favorable balance as depicted in Figure 1A. This is the usual outcome after clinical organ transplantation.[14,15,17,18]

It follows that the completeness of tolerance short of the drug-free state can be inferred from the amount of immunosuppression needed to maintain graft stability.[18,24,25] None of the patients of Donckier et al.[1] had macrochimerism. But the first 2 recipients clearly achieved a high degree of drug-free tolerance that can be considered with certainty to be microchimerism dependent.[23,26,27] Moreover, their third patient was in no sense a tolerance-induction failure. Although the balance between mobile donor antigen (microchimerism) and antigen-reactive T cells was close to the null point shown in Figure 1A (the lower dotted lines), the small amount of daily tacrolimus needed to maintain a stable equilibrium in this patient reflected partial tolerance in spite of the in vitro evidence of ongoing antidonor reactivity.

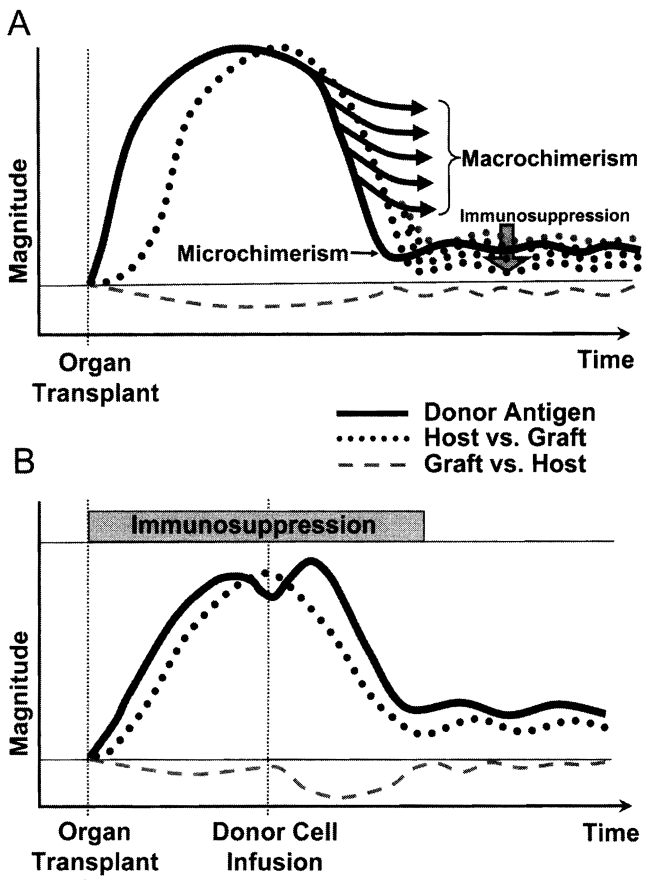

Figure 1. The balance of mobile donor antigen (passenger leukocytes) and donor-reactive T cells that determines destructive immunity (rejection) or nonreactivity (variable tolerance) after organ transplantation. (A) If the quantity of self-renewing leukocytes retain dominance over the antigen-specific cytolytic T lymphocytes, the T cell response may be exhausted and deleted. This is most likely to result in a drug-free state if there is a large quantity of persistent donor cells (macrochimerism), but variable drug-free stable balances may be reached with microchimerism. Alternatively, a balance in which the T-cell response dominates can be tilted in favor of the leukocytes with immunosuppression. This is the usual scenario of organ transplantation under conventional immunosuppression, and it is epitomized by the third patient of Donckier et al. (B) The outcome in liver recipients 1 and 2 reported by Donckier et al. The solid line denotes donor antigen (leukocytes). The upright dotted line denotes the host versus graft response. The inverted dashed line denotes a larger graft versus host reaction than that seen in (A).

The concept that balances between mobile antigen and antigen-specific T cells govern immunologic responsiveness and nonresponsiveness[18] has profound therapeutic implications.[25] Ratcheting down the response arm with immunosuppression as shown in Figure 1A has been the usual means of perpetuating organ alloengraftment, but at the costly loss of T-cell surveillance of malignant neoplasms and infections. The less damaging possibility of tilting the balance by simply giving organ recipients adjunct donor leukocyte infusions 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.[28-30] In these clinical trials, the infusion of bone marrow cells, or stem-cell-enriched peripheral leukocytes, usually was given on the same day as organ transplantation under conventional multiple-drug immunosuppression. What was the reason for the lack of efficacy?

In 2001, it was suggested that heavy posttransplant immunosuppression systematically narrowed the window of opportunity for initial clonal exhaustion-deletion that coincides with the sudden passenger leukocyte migration.[25] Such heavy treatment also would be expected to abrogate the potential benefit of additional

infused cells. When over-immunosuppression is later reduced, recovery of the inefficiently deleted clone would leave the patient prone to chronic rejection and dependent on lifetime drug treatment. On the other hand, failure to give enough early immunosuppression would lead to irreversible damage to the transplanted organ. It was proposed that the dilemma of over- vs. under-immunosuppression in the organ recipient (without or with adjunct leukocytes) could be addressed by applying 2 therapeutic principles, singly or together: recipient pretreatment and minimal posttransplant immunosuppression.[25] These principles have been applied in Pittsburgh since 2001 with encouraging results in liver and other organ recipients.[31-34]

The same principles can be readily identified in the strategy reported by Donckier et al. Immunosuppression was begun on the day of liver replacement. This consisted of 5 daily doses of antithymocyte globulin (ATG, thymoglobulin®), a 1-week course of medium-dose prednisone that was tapered at the time of stem cell infusion, and conventional doses of sirolimus until liver function normalized. The strategy provided pretreatment for the second load of donor leukocytes on postoperative day 7. Neither the cells infused at day 7 nor the recipient were subjected to much immunosuppression since the already minimal treatment was stopped a short time later. In their discussion, the authors raised the possibility that the inability to eventually wean their third patient may have been due to their decision against early immunosuppression stoppage. Although they also drew attention to theoretical antitolerogenic qualities of the tacrolimus that replaced sirolimus after 30 days, the best explanation may be that the unnecessary continuation of any potent T-cell-directed immunosuppressant can variably thwart the objective of eventual drug freedom.

Figure 1B shows what we believe to have been the role of the stem cell infusion in the Belgium cases. In essence, the recipients already were partially tolerized by the graft's passenger leukocytes at the time the cell infusion was given. Because the infused cells were a naïve population, they could have posed an increased risk of GVHD because circumstances had been created that potentially mimicked those in a parent to defenseless offspring F1 hybrid rodent model.[35,36] The added theoretical risk is depicted by the bulging inverted GVH response curve shown at the bottom of Figure 1B. This threat was effectively defused in the Belgium cases by the nearly complete removal of mature T cells, as originally described in 1981 by Reisner et al.[37] In an important thoughtful comment, the authors correctly pointed out that the second cell delivery might not have been essential, since the passenger leukocytes of the graft could have been all that was necessary to achieve tolerance (Fig. 1A).

What, then, was the single-most important element of their protocol? With or without a cell infusion, the critical step in the induction of tolerance probably was the minimum use of posttransplant immunosuppression. If just enough treatment is given at precisely the right time and then stopped, tolerogenesis is "permitted." This was described in 1992 as ". . .responses of coexisting donor and recipient cells, each to the other, resulting in reciprocal clonal exhaustion, followed by peripheral clonal deletion."[14,15] Assessment of the completeness and durability of the tolerance may require follow-ups of 2 or more years since the effects of conditioning with a potent lymphoid depleting antibody are long-lasting. Nevertheless, the observations in the 3 cases described by Donckier et al. add to the considerable historical evidence[15,34,38-42] that drug-free tolerance in a significant number of HLA-mismatched liver transplant recipients is a realizable objective.

There are several summary caveats: (1) Although hematolymphopoietic chimerism will be a prerequisite, sustained macrochimerism may be disadvantageous to the host, and it is more apt to be a complication (e.g., GVHD) than the desired objective of tolerance; (2) the ability of microchimerism to perpetuate alloengraftment under no (or low) immunosuppression will depend on the efficiency of the exhaustion-deletion achieved at the outset; (3) heavy immunosuppression with agents that block T-cell activation must be used sparingly after arrival of donor antigen, because T-cell activation is a prerequisite for donor-specific T-cell deletion; (4) permanent deletion or suppression of all donor specific T cells is simply unrealistic; (5) rather than being a state of absolute immune quiescence, tolerance is a low-grade active process (with or without the aid of maintenance immunosuppression) that depends on finding a stable dynamic balance favoring mobile donor antigen over donor-specific CTL; (6) stability of this balance in organ recipients with microchimerism requires avoidance of the destruction of the minority population by mechanisms of immune ignorance that are not yet fully understood; and (7) chimerism-dependent or chimerism-independent adjunctive tolerance mechanisms other than immune ignorance may be contributory but they are not essential.

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