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REVIEW

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Transplantation tolerance and mixed chimerism: at the frontier of clinical application

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Abstract Although the persistence of donor-type hematopoietic cells in low numbers (microchimerism) is well established in some transplant recipients, its relevance for graft acceptance is still a matter of debate. On the other hand, clonal deletion of donor-specific alloreactive cells associated with mixed chimerism (macrochimerism) has reliably produced long-term graft tolerance in pre-clinical models. So far, the cytoablative conditioning regimens required to achieve mixed chimerism have hampered the clinical development of such protocols. Here, we discuss recent observations suggesting that the deliberate induction of hematopoietic cell chimerism might become a feasible strategy to achieve transplantation tolerance in clinics.

Keywords Transplantation · Tolerance · Deletional · Non deletional · Immune-reconstitution

Abbreviations *BMT* Bone marrow transplantation · *WBI* Whole body irradiation · *GVH* Graft-versus-host disease · *NIH* National Institute of Health

Introduction

Organ transplantation is a routine clinical procedure for patients with end-stage organ failure. However, despite standardization of surgical techniques and continuous refinements in anti-rejection therapies, long-term results of transplantation have not significantly improved during the last two decades [12]. While successfully preventing acute rejection episodes, current immunosuppressive treatments are unable to control chronic rejection, which is the primary cause of long-term graft loss [27]. In addition, they cause a global immunodeficiency predisposing to severe infections and malignancies [7]. Therefore, transplantation tolerance defined as survival of the allograft in the absence of any immunosuppression remains a major goal to achieve for transplant physicians and immunologists.

Since the pioneer demonstration by Medawar and coworkers of neonatal tolerance induced in mice by injection of allogenic spleen cells [4], a number of experimental studies have confirmed that the establishment of hematopoietic chimerism, defined as the coexistence of host and donor cells, may contribute to the induction of transplantation tolerance. Isolated clinical observations of tolerance in patients treated for malignant disease with myeloablation and bone marrow infusion, and subsequently grafted with an organ from the same donor, is a principle proof supporting this approach [14, 17, 35]. Until now however, the toxicity of the host conditioning regimen required for bone marrow engraftment as well as the risk of inducing graft-versus-host disease, prohibited the application of this strategy in routine clinical protocols for patients with non-malignant diseases. Recent developments in the field of hematology, namely the definition of less toxic ablative treatments and the better understanding of the hematopoietic reconstitution taking place after bone marrow transplantation, open the possibility to introduce this concept to the clinics. Indeed, an expert panel gathered by the National Institutes of Health recently made recommendations for the design, conduct, and monitoring of clinical protocols to achieve transplantation tolerance [33].

It is therefore timely to review possible strategies for the induction of transplantation tolerance in the clinics.

Multiple pathways to transplantation tolerance

The induction and maintenance of transplantation tolerance may depend on two non-mutually exclusive pathways. The first is the clonal deletion of alloreactive T cells in the graft's recipient. In this form of deletional tolerance, also defined as central tolerance, stable engraftment of donor cells in the host hematopoietic sites (macrochimerism) induces the elimination of donor-specific T cells during their differentiation [31]. This process of negative selection is identical to the intra-thymic clonal deletion of self-reactive T cells during ontogeny. The second pathway of tolerance, peripheral tolerance, consists in the inactivation in the periphery of the alloreactive T cells causing damages to the graft. The mechanisms involved in the latter pathway include immunological ignorance, unresponsiveness (anergy), or immunodeviation with preferential production of suppressive cytokines such as interleukin-10.

The relationship between the persistence of low numbers of donor cells at sites distant from the graft (microchimerism) and graft acceptance has been extensively studied following the initial observations of Starzl's group in liver and kidney recipients [39, 40]. After the demonstration that microchimerism might persist in patients with long-term graft acceptance, it was suggested that donor leukocytes in the recipient might induce peripheral tolerance as non- professional antigen-presenting cells, such as T cells, B cells or immature dendritic cells could anergize T cells recognizing them [44]. In addition, certain donor cells might exert a veto activity resulting in inactivation of anti-donor cytotoxic T cells [37, 43]. In many models, this form of tolerance has been shown to depend on an unstable balance between microchimerism and anti-donor immunity. As a matter of fact, the elimination of donor leukocytes may correlate with graft rejection [11, 26]. However, the clinical relevance of microchimerism is still a matter of debate, and from the data published until now it appears to represent rather a consequence than a cause of long-term graft survival. Indeed, acute graft rejection may occur in patients with stable microchimerism, [15, 36] and in this setting the disappearance of microchimerism after graft removal suggests that it merely reflects a constant release of donor cells from the graft [37].

Based on experimental observations by Wood and Monaco [51], several groups evaluated clinical protocols of combined donor bone marrow cell infusion and organ transplantation in conjunction with classical immunosuppression, e.g. without preconditioning the recipient with myelotoxic agents. In liver transplant recipients, such a protocol resulted in enhanced graft survival [32]. However, true tolerance was not achieved as immunosuppressive therapy was maintained and *in vitro* studies suggested non-specific immunosuppression [25].

On the other hand, it might well be that peripheral tolerance does not require hematopoietic chimerism. The observations recently reported by Kirk et al. in nonhuman primates indeed indicate that long-term acceptance of renal allografts can be achieved by a short course of anti-CD154 (CD40 ligand) monoclonal antibody early after transplantation. However, monkeys treated with this protocol still developed anti-donor antibodies as well as lymphocyte infiltrates in the graft [18]. One can therefore assume that such strategies might ultimately result in chronic rejection as in the case of protocols promoting Th2-type responses [20].

Mixed chimerism and central tolerance

Because of the limitations of peripheral tolerization, the most reliable path to long-term transplantation tolerance appears to be the induction of mixed chimerism leading to central deletion of alloreactive T cells. This approach was investigated in animal models in which immuno/myeloablative preparation of the recipient followed by bone marrow transplantation (BMT) resulted in stable mixed chimerism in which large numbers of donor-type hematopoietic cells coexist with recipient-type cells. With this type of strategy, tolerance could be achieved in the most stringent models of tissue transplantation, i.e. fully MHC-mismatched skin graft in mice [41] or xenotransplantation in large animals (reviewed in [1]).

Myelo/immunoablative conditioning of the recipient has long been considered necessary to allow long-term engraftment of donor pluripotent hematopoietic stem cells resulting in stable macrochimerism, although Cobbold and Waldmann already established a model of mixed chimerism under a non-myeloablative regimen in 1986 [6]. The cytoreductive regimens target two compartments in the host: [1] the pool of the pre-existing alloreactive peripheral T cells, in order to prevent rejection of donor cells, and [2] the bone marrow, to create "space" in order to facilitate the implantation of donor stem cells. To reach these objectives, the conditioning regimen before bone marrow cell infusion usually associates depleting or non-depleting anti-T cell antibodies, whole body irradiation (WBI) and/or thymic irradiation. The absolute necessity of thymic irradiation remains questionable in the perspective of clinical application, especially for adult recipients [22]. It is usually argued that alloantigens directly presented within the thymus are most effective in inducing negative selection of developing T cells [5]. Indeed, the absence of thymic chimerism in mice allow donor-reactive T cells to be exported in the periphery and eventually to induce graft rejection [45]. In some experimental conditions howev-

er, thymic irradiation can be replaced by repeated injections of anti T cell antibodies (reviewed in [48]).

The constant effort to reduce the toxicity of preparative regimens also leads to a tendancy to minimize or avoid whole body irradiation before marrow grafting. This has recently been achieved in a pig model of transplantation by Sachs and co-workers using a combination of non-lethal WBI, thymic irradiation and anti-T cell monoclonal antibody [16]. Moreover, the need for WBI could even be overcome in this model by the injection of very high cell numbers (reviewed in [28, 30, 41]). Thymic irradiation is still required in this type of non-myelosuppressive regimen.

Complementing host conditioning, reagents blocking T cell costimulatory pathways may help to reduce the requirement for WBI, thymic irradiation or T-cell depleting antibodies. Indeed, recent observations in mice demonstrated that the adjunction of CTLA4-Ig and anti-CD40 ligand (CD154) antibody in the preparative regimen permitted avoiding thymic irradiation or T cell depletion before bone marrow grafting for the induction of long-lasting mixed chimerism and transplantation tolerance [24, 47, 49]. As a matter of fact, costimulatory blocking agents administered simultaneously with the allogenic tissue could specifically target peripheral anti-donor T cells and induce their functional deletion, leaving the opportunity for donor bone marrow cells to migrate to the thymus and establish central chimerism and subsequent deletional tolerance. Indeed, the infusion of a high dose of allogenic marrow cells followed by the administration of anti-CD154 and anti-T cell antibody was recently shown in mice to result in long lasting mixed chimerism and associated transplantation tolerance [50].

On the basis of these recent experimental developments, clinical protocols with an acceptable level of toxicity can now be considered. Indeed, Sykes et al. reported in patients with refractory lymphoma the successful induction of mixed chimerism after infusion of HLA-mismatched bone marrow cells using a conditioning regimen based on cyclophosphamide, anti-lymphocyte globulins and thymic irradiation [42]. In the context of solid organ transplantation, the same group reports a case of successful induction of tolerance to a kidney graft in a patient with a multiple myeloma, using a protocol based on cyclophosphamide, anti-thymocyte globulins and thymic irradiation before combined bone marrow and renal transplantation [38].

One critical issue for the design of clinical protocols to achieve mixed chimerism is the nature of the donor cells to infuse. Ideally, the donor inoculum should establish stable macrochimerism and provide precursors for immune reconstitution without mediating graft-versus-host (GVH) disease. These imperatives could be contradictory, as the cells responsible for the GVH overlap with those favoring cell engraftment and restoring immunocompetence. Indeed, T cells favor engraftment but some of them endowed with alloreactive potential are the GVH effectors. Studies investigating the infusion of purified allogeneic donor $CD34^+$ pluripotent stem cells suggest that they may present some selective advantage for long-term engraftment [3, 21]. Moreover, they appear to be endowed with veto activities [30]. We verified that the infusion of $CD34^+$ cells purified from the bone marrow of cadaveric donors is feasible and safe in kidney transplant recipients, although long-term chimerism was not achieved in the absence of a conditioning regimen [8]. One can therefore anticipate that the successful induction of mixed chimerism will critically depend both on the conditioning of the recipient and on the balance between the numbers of donor $CD34^+$ stem cells and donor T cells infused.

Immune reconstitution after stem cell transplantation

Since transplant tolerance should be specific for donor alloantigens, it is essential to consider the reconstitution of the immune system after the induction of mixed chimerism. As a matter of fact, studies in bone marrow transplant recipients indicate that the conditioning regimen may induce a state of immunodeficiency, which might be prolonged even after withdrawal of immunosuppressive drugs [2]. In the first months after immuno/myeloablation, T cell reconstitution preferentially concerns the $CD8^+$ T cell subpopulation, leading to an inversion of the $CD4^+/CD8^+$ ratio [29, 46]. Within the $CD4^+$ T cell compartment, regenerating cells first show a predominance of memory-type ($CD45RO^+$) over naive ($CD45RA^+$) cells [23]. The origin of these memory cells depends on the type of bone marrow transplant. In the absence of T cell depletion, they are almost exclusively of donor origin while, after T cell-depleted bone marrow transplantation, they expand from recipient peripheral cells that survive the conditioning regimen [34]. In addition to their limited capacity to recognize foreign antigens, repopulating T cells may present intrinsic functional defects including low proliferative responses, reduced cytotoxic potential, and abnormal cytokine profile [2]. However, in the setting of autologous stem cell transplantation for severe autoimmune disease, we observed a recovery of T cell repertoire and function within one year, without disease recurrence [10].

The factors involved in T cell reconstitution after immunoablation were thoroughly reviewed in a recent article [13]. One critical factor is the age of the patient, as the thymic function declines with time [23]. The identification of a specific molecular marker of early thymic migrants (TREC) allowed confirmation of this fact but also established that a substantial thymic output persists in adults [9]. Experimental studies suggest that cytokines and/or hormones could stimulate the regeneration of thymic tissue in adults (reviewed in [13]). Although

the efficiency and safety of such treatments in humans remain to be established, such methods of treatment offer interesting perspectives to accelerate immune reconstitution after induction of mixed chimerism.

Conclusion

The advances made in experimental models have brought the induction of mixed chimerism very close to the frontier of clinical application, and further refinements in preparative regimens will probably make an acceptable level of toxicity possible. At this point, a critical question should be considered: Which patients should be selected for this type of protocol, in view of the current results achieved with classical immunosuppression? Although the NIH expert panel suggests initiating these procedures in kidney or pancreatic islet transplantation [33], we feel, for several reasons that liver recipients may also be appropriate candidates. First, the inherent capacity of the liver to produce stem cells may facilitate the establishment of mixed chimerism. Moreover dendritic cell progenitors originating from the liver may promote the deletion/inactivation of donor-specific T cells [44]. Finally, in the absence of any reliable *in vitro* test for the induction of tolerance, the ultimate demonstration of operational tolerance will depend on immunosuppression withdrawal. The relative resistance of the liver graft to acute rejection and its ability to recover rapidly from anti-rejection treatment should allow this type of challenge more easily.

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