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

Vincent Donckier
Michel Toungouz
Michel Goldman

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 cytotoxic ablative 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 (microchimerism) 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 immunodepletion 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 have 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 immunomyoeloblativ 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/myeloblativ 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-myeloblativ regime 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 allows donor-reactive T cells to be exported in the periphery and eventually to induce graft rejection [45]. In some experimental conditions howev-