Seminars in Immunology 23 (2011) 304–313

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Moving to tolerance: Clinical application of T regulatory cells

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ARTICLE INFO

Keywords:
Transplantation
T regulatory cells
Cellular therapy
Drug minimization
Translational medicine

ABSTRACT

Decreasing the incidence of chronic rejection and reducing the need for life-long immunosuppression remain important goals in clinical transplantation. In this article, we will review how regulatory T cells (Treg) came to be recognized as an attractive way to prevent or treat allograft rejection, the ways in which Treg can be manipulated or expanded in vivo, and the potential of *in vitro* expanded/generated Treg for cellular therapy. We will describe the first regulatory T cell therapies that have been or are in the process of being conducted in the clinic as well as the safety concerns of such therapies and how outcomes may be measured.

1. Introduction

One of the characteristics that defines the mammalian adaptive immune system is the rapid proliferation and expansion of T and B cells following antigen exposure, but in the past two decades it has also become clear that the immune system has evolved multiple peripheral mechanisms for controlling these responses. Growing evidence indicates that it should be possible to engage these inherent regulatory pathways to suppress immune responses to alloantigens following transplantation. Ways to specifically prevent immunity to foreign cells and tissues would offer a new way to minimize reliance on non-specific immunosuppression and could ultimately allow patients to be completely withdrawn from drug-based immunosuppression.

Many different types of T cells with regulatory activity have been described including: $CD8^+$ T cells [1–3]; $CD4^-CD8^-$ double negative T cells [4,5], NK T cells [6], and γδ T cells [7], but these are all less well studied than their $CD4^+$ T cell counterparts. In this review we will focus on the potential for clinical application of $CD4^+$ T regulatory cells characterized by high and stable expression of CD25 and FOXP3 in the context of organ transplantation. $CD25^+FOXP3^+$ T regulatory cells (hereafter Treg) can arise via two distinct developmental pathways. First, so-called "naturally-occurring" or nTreg, arise directly in the thymus, and are thought to primarily function to regulate autoimmunity. Second, when conventional $CD4^+$ T cells encounter their antigen in a tolerogenic environment, e.g. when presented by immature dendritic cells (DCs), or with immunosuppressive cytokines, they differentiate into "adaptive" or aTreg. Establishment of long-term tolerance by nTregs is thought to depend on their ability to stimulate de novo differentiation of aTreg [8]. Despite distinct developmental origins, both nTregs and aTregs rely on continuous expression of FOXP3 for their suppressive function. It is difficult to distinguish nTreg from aTreg, because both are defined as $FOXP3^+$ cells, but recent data suggest that nTregs may be identified by high expression of another transcription factor, Helios [9].

The importance of Treg to the normal immune system came from two recent studies where a transgenic approach examined whether selective depletion of nTreg in otherwise normal mice might replicate some of the characteristics of profound autoimmunity seen in IPEX (*immune dysregulation*, *polyendocrinopathy*, *enteropathy*, *X-linked syndrome*) patients. Mapping studies had shown that such patients have a point mutation in the gene encoding the transcription factor FOXP3 [10,11] and a functional Treg deficit *in vitro* [12]. DREG mice were constructed in which the diptheria toxin receptor gene was inserted into the Foxp3 locus such that administration of the toxin leads to a conditional depletion of $Foxp3^+$ nTreg. Selective Treg depletion led to profound autoimmunity in neonates [13] and lethal autoimmune disease in adults [14] demonstrating that active regulation mediated by nTreg

Abbreviations: APC, antigen presenting cell; ATG, anti thymocyte globulin; aTreg, adaptive T regulatory cell; AZT, 3′-azido-3′-deoxythymidine; CNI, calcineurin inhibitor; CyA, cyclosporin A; DC, dendiric cell; DST, donor specific transfusion; GVHD, graft versus host disease; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; MMF, mycophenolate mofetil; nTreg, natural T regulatory cell; mTOR, mammalian target of rapamycin; PI3K, phosphatidyl inositol 3′ kinase; Treg, T regulatory cell; TCR, T cell receptor; Tconv, T conventional; TK, thymidiylate kinase; TSDR, Treg specific demethylation region.

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doi:10.1016/j.smim.2011.04.001

plays an indispensable role in normal immune homeostasis. The implication that nTreg play such an important role in controlling immune responses in mice and in humans gives grounds for cautious optimism that it should be possible to harness the potential of Treg to control rejection in clinical transplantation.

2. Regulatory T cells in vivo: an historical perspective

Although much of our current understanding of immune regulation has come from autoimmunity models, it is important to recognize that transplantation provided some of the earliest evidence for Treg function in vivo. Almost 30 years ago in a rat heart transplant model, Hall et al. showed in the MHC mismatched PVG to DA strain combination that a two-week course of cyclosporine (CyA) led to indefinite allograft survival without further therapy. Importantly, when harvested 100 days post transplant and tested in adoptive transfer models, T cells from these animals had the capacity to prevent rejection mediated by normal effector cells [15]. These data provided a clear indication that long-term allograft survival independent of long-term immunosuppression (*operational tolerance*) involved T cells with the ability to regulate naïve alloreactive T cells. Subsequently, Hall and colleagues demonstrated that regulation was associated with $CD4^+$ T cells [16,17] and were the first to suggest that CD25 is a useful Treg marker [18]. Similar data were obtained in a rat renal allograft model where operational tolerance was induced by donor-specific blood transfusion [19,20].

To determine whether cells isolated on the basis of CD25 expression could be used therapeutically in the transplant setting, Hara et al. reconstituted immunodeficient CBA mice with naïve CBA effector T cells with or without $CD4^+CD25^+$ T cells isolated from CBA mice bearing fully allogeneic B10 heart allografts. The reconstituted mice were then transplanted with test B10 skin grafts. Mice reconstituted with effector T cells alone rejected their skin grafts acutely but, in stark contrast, co-transfer of $CD4^+CD25^+$ T cells from tolerant animals led to indefinite skin graft survival in 80% of recipients [21]. Strikingly, when used at equivalent cell doses, naïve $CD4^+CD25^+$ T cells were unable to control rejection suggesting that exposure to alloantigen in a tolerogenic environment either enhances nTreg function and/or generates a population of induced Treg.

Whilst the observation that long-term tolerant mice contain populations of alloantigen reactive $CD25^+$ Treg was important, these experiments were unable to distinguish between Treg that were generated by the induction strategy itself and those that arose simply by the presence of the accepted allograft. In terms of developing potential clinical approaches, a much more important question is whether induction strategies that ultimately lead to long-term operational tolerance can drive Treg development independently of the graft itself. The presence of sufficient numbers of donor-reactive Treg pre-transplant might offer immediate active regulation perhaps allowing early drug-minimization. In a fully mismatched mouse transplant model, pre-treatment of $H2^k$ CBA mice with $H2^b$ donor alloantigen (donor specific transfusion, DST) under the cover of a non-depleting anti-CD4 antibody 28 days before transplant leads to the indefinite survival of donor-specific $H2^b$ hearts without further therapy [22]. Importantly, when $CD4^+CD25^+$ T cells were isolated from mice 28 days after pre-treatment but *without* transplant, these cells prevented test skin graft rejection in a sensitive adoptive transfer model [23]. Critically, protection was not seen with similar populations isolated from naïve, anti-CD4-only or DST-only mice demonstrating that tolerance mediated by $CD25^+$ Treg can be indeed induced in vivo *prior to* transplant.

Although a significant body of work has demonstrated that Tregs can control alloreactive responses, most experiments involved adoptive transfer of cells into immunodeficient recipients where allograft rejection is driven by relatively small numbers of effector T cells—typically of the order of 10^5 per mouse. In terms of translational medicine, a much more relevant question is what role do Tregs play in an intact immune system? In transplantation, Treg-specific inactivation was used to show that in the anti-CD4/DST tolerance induction model described above, the survival of primary heart allografts in normal, lymphoreplete recipients is also unequivocally dependent on aTreg driven by the tolerance induction protocol [24]. These data suggest that it should indeed be possible to boost the function of Tregs in non-lymphopenic transplant patients.

3. How might Treg be exploited for therapeutic benefit?

3.1. In vivo induction of Treg

The current success of clinical transplantation depends on immunosuppression and, as in rodent models [15,25,26], it may be possible to tailor immunosuppression to promote the generation and/or expansion of donor-reactive Treg. Attempts to identify the emergence of Treg in such circumstances are essential but a number of factors make such identification far from trivial. Firstly, although FOXP3 and CD25 have been and continue to be useful for the identification of Tregs, in humans neither marker is unique to Tregs, and both can be up-regulated on activated non-regulatory populations [27–31]. Thus, accurate identification of Treg is problematic and historical data that did not take this possibility into account must be viewed with caution. Secondly, it is quite likely that different immunosuppressive drugs will be more or less permissive for Treg development/function. For example, some studies have found circumstantial evidence that calcineurin inhibitors (CNI) have a negative impact on regulation whilst rapamycin may preserve or enhance Treg development and or function. Thirdly, the heterogeneity of donor-recipient populations and the use of many different induction and maintenance immunosuppressive regimens will make it challenging to identify protocols that promote Treg function. Fourthly, even if a given transplant protocol were to induce functional human Treg, identifying the in vivo contribution of such cells against a background of very effective immunosuppression will not be straightforward.

Despite the above limitations, four specific strategies of immunotherapy have been identified that may be permissive for Treg development and function in a transplant setting. These are: 1. anti-CD3 antibody; 2. anti-thymocyte globulin; 3. anti-CD52 antibody; and 4. mTOR inhibitors.

3.1.1. Anti-CD3 antibody

The early observation that T cells are essential for rejection led to the development of anti-T cell reagents including anti-CD3 antibodies which have a long history in transplantation. These agents were used initially as an anti-rejection therapy [32,33] but evidence began to emerge that they could also induce tolerance in transplant [34,35] and autoimmunity models [36]. Although anti-CD3 antibodies provide an initial period of global immunosuppression due to T cell receptor (TCR) modulation and enhanced effector T cell apoptosis, in the longer term a state of self-tolerance develops which involves the expansion of TGF-β-producing aTreg [37,38]. Anti-CD3 antibodies have been used in Phase I/II trials in recent onset diabetic patients and appear to delay the requirement for exogenous insulin [39]. Importantly, the most benefit was in patients with the highest residual β-cell mass and the least advanced autoimmunity. Thus anti-CD3 antibody therapy could be useful in the transplant setting since the problem of pre-existing