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Regulatory T cells – a journey from rodents to the clinic

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1. ABSTRACT

Over the past decade our understanding about a subset of T lymphocytes, now termed regulatory T cells (Tregs) and previously known as suppressor T cells, has increased immensely. Tregs can induce and maintain immune tolerance and have the capacity to facilitate antigen-specific long-term graft survival successfully in animals receiving allogeneic organ transplants. The development of approaches to generate alloantigen reactive Tregs would provide an exciting and effective adjunct or alternative therapy to the life-long program of immunosuppression currently necessary to prevent graft rejection in the clinical setting. This review will focus on how rodent experimental models have helped us to figure out how Tregs could be induced in humans and harnessed to enable long-term transplant acceptance.

2. INTRODUCTION

The rejection of major histocompatibility (MHC)-mismatched grafts is a major obstacle for success in the transplantation field. Currently this immunological barrier is overcome with immunosuppressive drugs. However these drugs have to be given life-long and are accompanied by an increased incidence of serious infections and malignancy. Additionally they can have serious side effects and may inhibit the development of immune tolerance (1). Finally many grafts are rejected after several years even in the presence of these drugs. Therefore the development of antigen-specific tolerance leading to the acceptance of a graft indefinitely is the objective of an increasing number of studies. Manipulating both the host immune system and the graft to induce immune tolerance if successful, would represent a major advance.

Sakaguchi and his colleagues linked T cells with a defined phenotype with regulatory activity by showing that cells expressing CD4 and the interleukin-2 receptor alpha chain (IL2Rα) CD25 (naturally occurring Tregs) contributed to maintaining self-tolerance by down-regulating the immune response to self and non-self antigens (Ags) in an Ag-non-specific manner (2). There is now growing evidence that there are distinct subsets of cells, termed regulatory T cells (Tregs) on the basis of their function (3). These cells can be delineated into two main categories: naturally occurring Tregs that develop in the thymus and are present in naïve, unmanipulated individuals, and Tregs that developr or are induced after antigen stimulation.

Over the past decade researchers have been interested in how these Tregs can prevent the development of autoimmune diseases, and how they modulate the immune response to infections, tumor antigens and after transplantation. The aim of this review is to highlight how rodent models have proved an extremely useful tool for elucidating how Tregs mediate transplant tolerance and how we hope to expand/induce and identify these cells for use in the clinic.

3. EVIDENCE THAT TREGS PLAY A ROLE IN MAINTAINING GRAFT SURVIVAL

Since Sakaguchi's initial experiments in 1995, several groups have demonstrated that immunomodulation of mice and rats with donor antigens, non-depleting anti-CD4 and anti-CD8 monoclonal antibodies (mAb), and costimulatory blockade can induce a robust form of operational tolerance *in vivo* (4-9). T cells from these tolerant animals have been adoptively transferred into secondary syngeneic recipients who, as a result, acquire the ability to prevent grafts from being rejected (eg (10-12)), confirming that graft maintenance is mediated by the subset of T cells transferred.

There is an ongoing debate as to whether naturally occurring $CD25^+CD4^+$ Tregs present in unmanipulated hosts can protect an allograft from rejection. Clearly in normal circumstances naturally occurring Tregs are reactive with self antigens. It is well documented that a T cell receptor specific for a defined MHC+peptide complex may cross react, albeit with different affinity, with other MHC+peptide complexes. Some studies have demonstrated that $CD25^+CD4^+$ naturally occurring Tregs from untreated mice can prevent the rejection of allogeneic grafts when injected into lymphopenic hosts (13). However, whether sufficient numbers are present in unmanipulated recipients to control rejection may depend on a number of factors including the degree of mismatch, immune status of the recipient and the tissue transplanted. Although naturally occurring Tregs cross reactive with alloantigen are present in unmanipulated hosts, exposure to alloantigen increases the frequency and/or potency of Tregs present (14, 15). The origin of the newly acquired Tregs requires further clarification. Data suggesting that the increase in the number of alloantigen reactive Tregs is due to expansion of naturally occurring Tregs have been reported (15). However, on the other hand there are data demonstrating that T cells with regulatory activity can be generated or 'induced' from T cells that before alloantigen exposure did not have any intrinsic regulatory activity (16).

Interesting data have highlighted that the ability to induce Treg-mediated transplant tolerance can be organ-specific in mice. Schenk and colleagues (17) transplanted organs from transgenic B6.H-2bm12 mice into C57BL/6 recipients with a single MHC class II disparity. Heart grafts but not skin grafts were accepted indefinitely. Interestingly, a primary B6.H-2bm12 heart transplant could protect the mice from rejecting a secondary B6.H-2bm12 skin graft. This tolerance was found to be mediated by Tregs infiltrating the donor heart that restricted the expansion of alloreactive T cells. Indeed when Tregs were depleted, B6.H-2bm12 heart allografts are acutely rejected. These data suggest that sufficient induction of Tregs may depend on factors such as the alloantigen load, reflected by the size of the graft and therefore the frequency/number of Treg generated and possibly on the presence of tissue specific peptides, although the latter will require additional studies to confirm.

There are now plenty of data demonstrating that Tregs are one of the key mechanisms controlling immune responsiveness to donor antigens and that they have the potential to play a role in both inducing and maintaining tolerance to alloantigens in vivo in a new clinical era where non-specific immunosuppression is minimized. The key issues that need addressing are firstly the cellular and molecular mechanisms by which Tregs prevent rejection, and secondly how the methods for generating Tregs that can prevent rejection of allogeneic transplants in humans can be optimized. Finally the ability to distinguish reliably this group of cells from those mediating rejection and quantitate the functional capacity of the regulatory vs effector populations will allow us to determine whether sufficient donor-specific Tregs have been generated in patients that will maximize the likelihood of achieving graft tolerance.

4. MECHANISMS USED BY TREGS TO PREVENT REJECTION

Both induced and naturally occurring Tregs can prevent rejection by a number of mechanisms including suppression of alloreactive $CD4^+$ and $CD8^+$ effector cells, conversion of other T cells into Tregs, so-called dominant tolerance, and the induction of linked unresponsiveness (1). We now need to identify the molecular mechanisms by which Tregs mediate these effects. The involvement of many proteins in mediating *in vivo* Treg function including costimulatory molecules such as CTLA-4 (14) and cytokines such as IL-10 (18, 19) and TGFβ (1, 20), have been well documented (1) and will not be discussed here. Instead we will focus on some molecules that have recently been linked with Treg function and are likely to be important players in facilitating prevention of graft rejection. A summary of various characterized mechanisms used by Tregs to prevent rejection is illustrated in Figure 1.

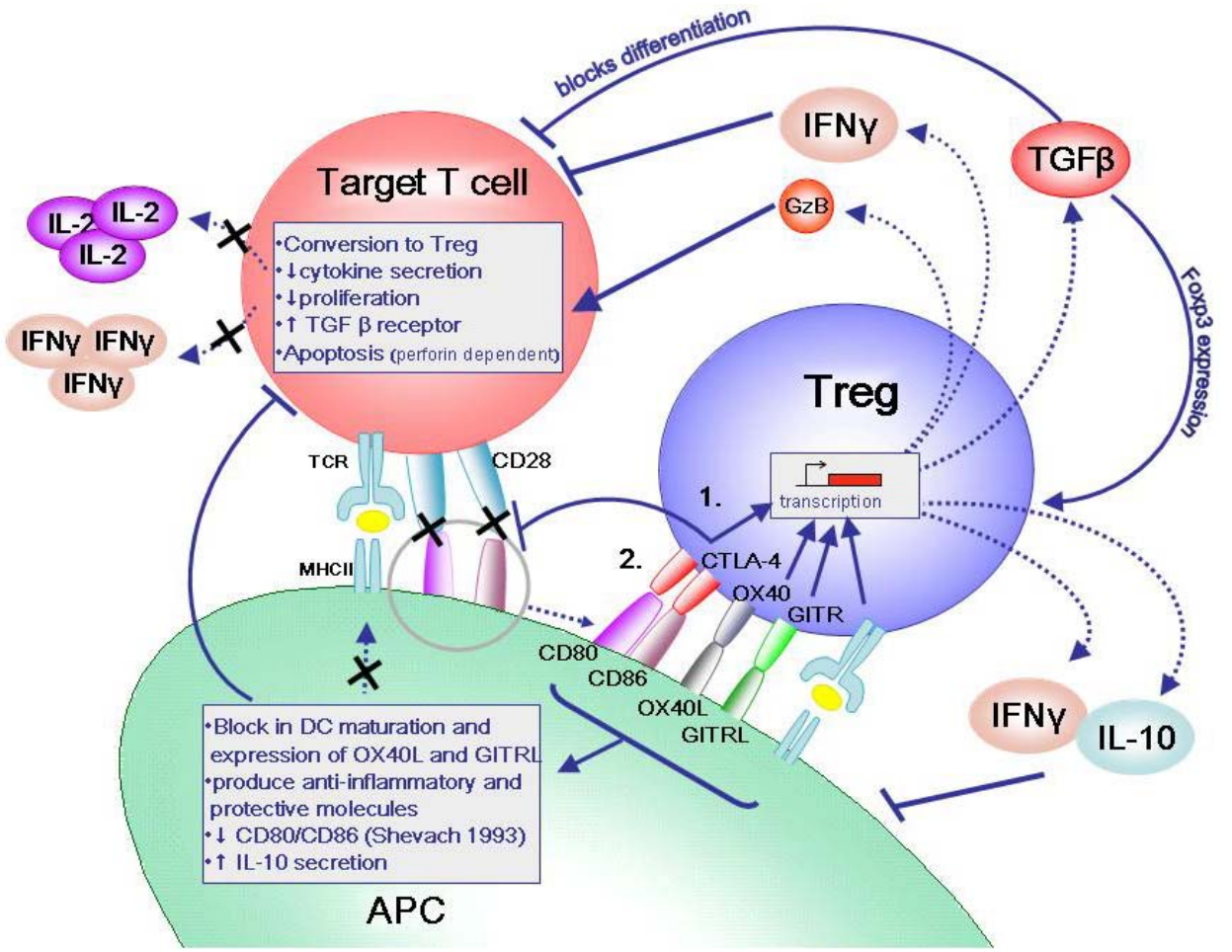

Figure 1. Tregs can inhibit effector T cell function either directly or indirectly (through APCs) in both contact dependent and contact independent mechanisms. TGFβ converts peripheral CD4+CD25- naïve T cells into Tregs (1). IL10 blocks cytokine production and proliferation of CD4+ T cells via its downregulatory effects on APC function (40). IFNγ produced by alloantigen-reactive Tregs may influence the effector function of alloreactive T cells and/or macrophages and DCs (30). CTLA4 is constitutively expressed by CD25+CD4+ Tregs in normal naïve mice and is thought to act in 2 ways: 1) signals from CTLA-4 inducing TGFβ expression; 2) sequestering CD80 and CD86 on the surface of APCs and hence depriving naïve T cells of CD28-mediated costimulation (40). Granzyme B triggers cell contact-mediated suppression in CD4+CD25- effector cells by inducing apoptosis in a perforin-independent manner (23).

Last year Ley and co-workers established that the Tr1 subset of human Tregs express the serine protease Granzyme B (GZ-B) (21), a molecule that is normally associated with cytotoxic lymphocytes and the mechanism they use to kill virus-infected and tumor cells (22). Since then others have shown that regulatory activity in mice correlates with increased expression of GZ-B and Tregs from GZ-B$^{-/-}$ mice have a reduced ability to suppress as efficiently as Tregs from WT mice (23). GZ-B-mediated Treg suppression is mediated, in part, by the induction of apoptosis in CD4^{+}CD25^{-} effector cells in a perforin-independent manner. In summary, GZ-B has been suggested as one of the key mechanisms through which CD4^{+}CD25^{+} Tregs induce cell contact-mediated suppression.

Glucocorticoid-induced TNFR family related gene (GITR) functions as a costimulator of conventional T cells and is constitutively expressed on mouse Tregs as well as by activated T cells (24, 25). Addition of an agonistic anti-GITR to co-cultures of Tregs and activated T cells results in a loss of suppression suggesting that this gene is required for mediating suppression by Tregs (24, 25). Indeed Tregs isolated on the basis of constitutive GITR expression can prevent development of colitis in an adoptive transfer model regardless of expression of the Treg marker CD25 (26).

The tumor necrosis factor (TNF) receptor family member OX40 (CD134) is transiently expressed on T cells after T-cell receptor (TCR) ligation and is expressed on both naive and activated murine Tregs. Recent work has demonstrated a crucial role for OX40 in the control of tolerance induced by Tregs that is comparable with that of GITR (27). Triggering either GITR or OX40 on Tregs inhibited their capacity to suppress. In a fully allogeneic

C57BL/6>BALB/c bone marrow transplantation, Treg suppression of GVHD was abrogated either by intraperitoneal injection of anti-OX40 or anti-GITR monoclonal antibodies (mAbs) immediately after transfer, or by *in vitro* pre-treatment of Tregs with the same mAbs.

OX40 ligand (OX40L) and GITR ligand (GITRL) are found mainly on activated dendritic cells (DCs). OX40L and GITRL expressed at low levels on immature DCs may be favorable for inducing suppression by Tregs, while activation of the DCs leading to up-regulation of these ligands would allow for inhibition of suppression through the OX40 and GITR molecules expressed on the Treg cells.

Double-negative regulatory T (DN Treg) cells have been identified in both mice and humans. These cells express $\alpha\beta TCR^{+}CD3^{+}NK1.1^{-}$ and can suppress Ag-specific immune responses mediated by $CD8^{+}$ and $CD4^{+}$ T cells (28). In a more widespread approach to identifying molecules that are involved in mediating Treg function, global gene expression differences between these functional DN Tregs and non-functional mutants have been determined. Clues from genes differentially expressed highlight the mechanisms by which DN Tregs down-regulate immune responses and prolong cardiac allograft survival and suggest that increased cell proliferation and survival, immune regulation, and chemotaxis, together with decreased expression of genes for Ag presentation, apoptosis, and protein phosphatases involved in signal transduction (29).

Recently our laboratory has analyzed cytokine expression by alloantigen-reactive Tregs after Ag exposure (30). These data have identified that the production of interferon-gamma (IFNγ) by alloantigen-reactive Tregs is essential for their generation and function *in vivo*. IFNγ may influence the effector function of alloreactive T cells and/or macrophages and DCs.

Interestingly, Tregs stimulated *in vitro* produce little or no IFNγ which highlights the importance of discriminating *in vivo* and *in vitro* data. Studying global gene expression *in vivo* may offer us a more extensive insight into the mechanism of regulation which is likely to differ depending on the Treg subset. These studies should pull out other genes that can also be characterize d that may help us understand how Tregs control the host's immune response to foreign grafts.

5. STRATEGIES FOR GENERATING T REGS *IN VIVO*

Many protocols have been generated and improved in rodents over the past decade in the hope of maximizing the generation of allospecific Tregs for transplantation (1). Here we will highlight some of the recent *in vivo* approaches being assessed, summarized in Figure 2. As well as facilitating the translation of strategies designed to promote transplantation tolerance to the clinic, these techniques also have the potential to benefit the development of novel protocols for the treatment of various autoimmune and immunoproliferative diseases.

It is now well established that Tregs generated *in vivo* are important for inducing and maintaining the long-term acceptance of allogeneic grafts. Pre-treatment of mice with donor alloantigen in the form of a single donor–specific transfusion (DST), combined with anti-CD4 generates $CD25^{+}CD4^{+}$ Tregs that can prevent rejection of organs transplanted several weeks later (31). Tolerance can also be achieved by administering multiple DSTs prior to transplantation without the need for combined anti-CD4 treatment (32). Further development of the DST plus anti-CD4 protocol has demonstrated that additional reactivation with a DST alone one day prior to transplantation also allows Tregs to suppress rejection of third party allografts (33). Replacing the DST in this tolerizing protocol with a soluble protein antigen also allows Tregs to suppress rejection which would allow more flexibility in the design of pre-treatment strategies. If the tolerance achieved using this pre-treatment approach is non-specific, tolerized individuals may also have a reduced capacity to respond to environmental pathogens. Data produced in our laboratory suggest that the induction of dominant tolerance does not necessarily result in attenuated responses to pathogens (34) providing further support for the development of tolerance induction protocols in clinical transplantation.

Recently, researchers have been interested in the idea that Tregs may be generated following APC engagement of apoptotic cells (35), as ingestion of apoptotic cells is an active process of immune tolerance induction. Recent studies have used UVB irradiation (36), or treatment with a photosensitizer followed by UVA irradiation (37), to induce apoptosis and successfully generate syngeneic Tregs in mice. This induction of apoptotic cell-mediated suppressive activity opens up the prospect of pre-treating graft recipients with apoptotic donor cells in order to expand Tregs that mediate tolerance to subsequent transplantation.

An alternative to using whole donor cells as the source of alloantigen is to use donor-specific synthetic peptides. Using HLA class I transgenic mice it has been demonstrated that intrathymic injection of donor HLA class I-derived peptide results in the generation of $CD25^{+}$ $CD4^{+}$ Tregs which induce graft specific tolerance (38). Peptide-mediated Treg generation has also been successfully achieved in lupus prone mice by injecting subnanomolar concentrations of autoepitopes subcutaneously at regular intervals (39). These Treg cells can suppress lupus autoimmunity upon adoptive transfer *in vivo*.

As well as mAb targeting of CD4 during DST pre-treatment, generation of Tregs has been achieved *in vivo* by blocking other molecules such as CD154, LFA-1, ICAM-1 CTLA4 and 4-1BB, at the time of donor-alloantigen delivery (40, 41). This suggests that modifying signals mediated by these surface molecules can promote Treg development. If we can understand the signals mediated by these accessory molecules in Tregs and perhaps identify common mechanisms used by each of the

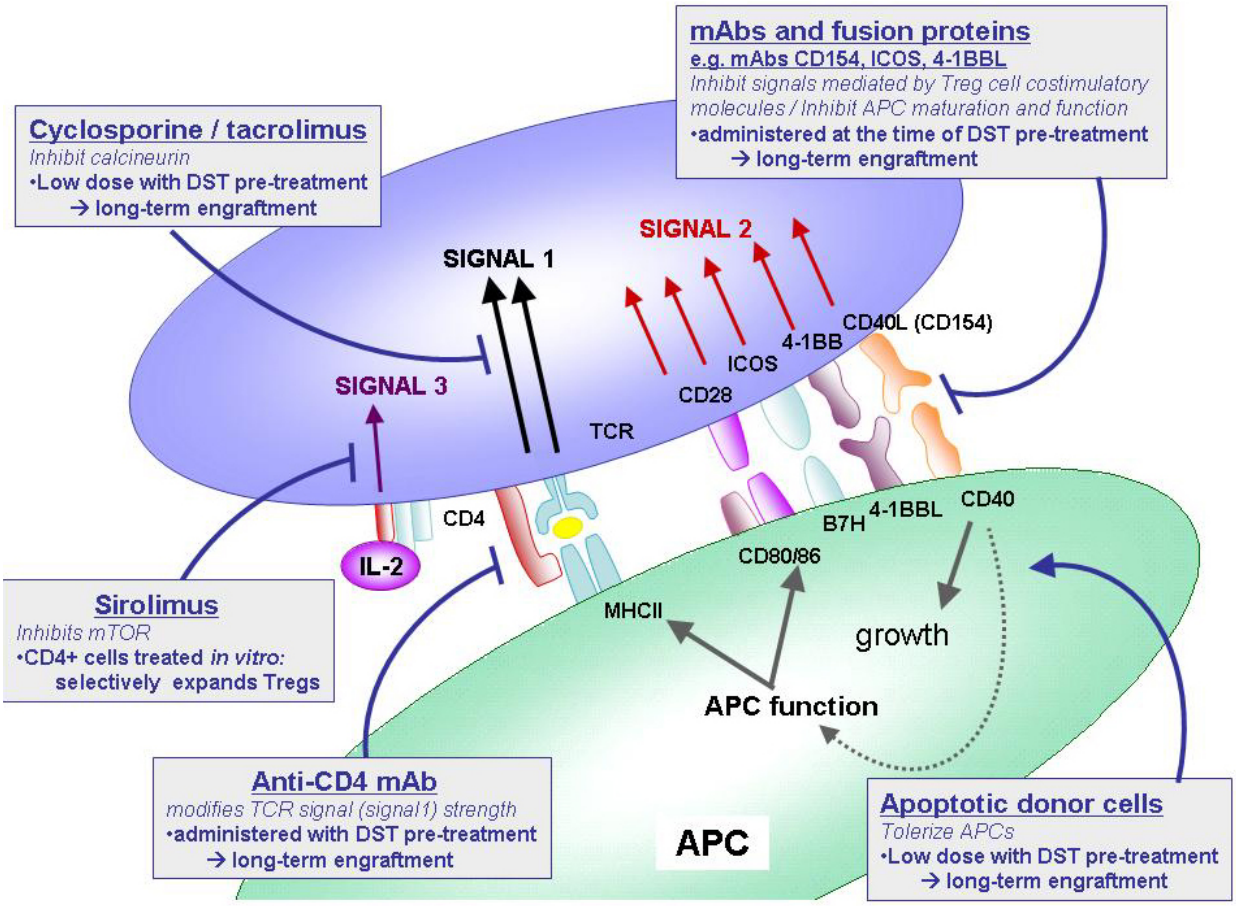

Figure 2. A number of methods have been developed to expand antigen-specific Treg cells from either naturally occurring Tregs or CD25-CD4+ T cells both *in vivo* and *in vitro*. In general, T cells are exposed to either donor-specific or non-specific antigen, together with modification of T cell signal intensity. This can be achieved by blocking signals via the TCR (Cyclosporine A, tacrolimus, anti-CD4 mAb), via costimulatory molecules (mAbs and fusion proteins) or cytokine receptors (sirolimus), or by blocking the maturation and activation of APCs (apoptotic donor cells, mAbs and fusion proteins).

stimuli, it may be possible to bypass the peripheral signaling events in future and target the effector pathways using immunosuppressive drugs. This would provide a cost-effective way of inducing Tregs.

One common worry with post-transplant immune suppression is that immunosuppressive drugs such as Cyclosporine A (CsA) and tacrolimus may somehow compromise the production of organ-specific Tregs (42). CsA suppresses T cell activation by preventing the nuclear translocation and activation of NFAT transcription factors (43). Indeed $CD25^{+}CD4^{+}$ T cells from $NFATc2^{-/-}$ $c3^{-/-}$ double knockout (DKO) mice lack regulatory capacity with the exception of a small subpopulation that highly expresses GITR and CD25 (44). *In vivo* studies in mice have also shown that CsA inhibits the induction of unresponsiveness that can be achieved using costimulation blockade. In this case, CsA therapy at the time of costimulation blockade was found to prevent apoptosis of alloantigen reactive However, in contrast, other data have provided a new insight into tolerogenic potential of CsA and rapamycin aimed at inducing Tregs has recently been published. Zheng et al (45) have shown that the combined administration of IL-2- and IL-15-related cytolytic fusion proteins along with sirolimus facilitates long-term engraftment in mice. This protocol selectively destroys cytopathic T cells without compromising the regulatory $CD25^{+}$ $CD4^{+}$ T cell subset. Through the use of this strategy NOD mice can be rendered tolerant to allogeneic tissues and undergo long-term engraftment of islet allografts. Kitade and colleagues have previously used a rat cardiac allograft model (46), to induce donor-specific Treg-mediated tolerance by pre-treatment with DST. This model has recently been adapted to include administration of low doses of CsA together with DST (47). In contrast to high-dose CsA alongside pretransplant DST which abrogates the generation of Tregs, a lower dose of CsA was found to promote Treg development either in synergy with perioperative DST or alone. This suggests that subtle modifications to cell signals required for inducing Tregs could be successfully achieved using CsA. Tacrolimus has also been shown to selectively expand murine naturally occurring Tregs *in vitro* (48) suggesting a use for this drug in ex vivo cellular therapy. Appropriate use of these drugs

could provide a general treatment regardless of the genetic background of the donor and recipient, and offers an exciting prospect of generating Tregs *in vivo*.

The recent emergence of information about how molecules such as OX40 and GITR are essential mediators of Treg function has highlighted how the activation status of dendritic cells (DCs) may dictate Treg activity. Immature DCs appear to be favorable for inducing suppression by Tregs, while activation of DCs leading to up-regulation of ligands such as OX40L and GITRL may inhibit of suppression. This has raised the potential that modification of DCs might facilitate the induction of Tregs. Indeed studies have shown that differentiating bone-marrow cells under conditions that promote the development of DC, such as in the presence of antibodies directed against different CD200R family members, augments the ability to generate DCs that can induce functional Tregs. CD200 is a family of transmembrane proteins that can deliver immunoregulatory signals after engagement of CD200R. DCs produced in the presence of anti-CD200R2/3 monoclonal antibodies (mAbs), induce Tregs that function in a TGF-β- and CTLA4-dependent manner (49).

Tomasoni and colleagues (50) have used an adenoviral vector encoding for a kinase defective form of IKK2 (dnIKK2) to block NF-κB activation of bone marrow-derived rat DC and found that dnIKK2-transfected DCs were immature, induced the formation of hyporesponsive Tregs when cultured with allogeneic T cells *in vitro*, and were capable of prolonging kidney allograft survival when infused *in vivo* in rat recipients before transplantation, without the need for any immunosuppressive therapy. In this system indefinite survival could not be achieved which may be due to the short half-life of engrafted allogeneic DC. Overall, it appears that the ability to modify DCs may bring with it the possibility of using this source of antigen presenting cells as an alternative approach to generate of Tregs *in vivo*.

6. FUTURE PERSPECTIVES

The success of generating graft tolerance using a particular protocol can vary between different strains of mice. This indicates that regardless of the type of therapy developed in rodent models, transferring the approach to humans will require an understanding of the genetic factors that influence individual donor-recipient interactions. For this reason it will be vital to develop a technique to reliably identify whether sufficient donor-specific Tregs have been generated, in order to confidently predict that graft tolerance will be achieved. Therefore understanding which markers or behavioral phenotypes that reliably distinguish regulatory T cells from other T cell types is an important step that is still required in order to progress Treg therapy to human transplant patients.

Many groups currently use markers such as CD25 and Foxp3 (forkhead box transcription factor P3) to detect and purify Tregs. In addition to the findings that regulatory activity is found in $CD25^-$ $CD4^+$ cells (13), there is also the potential to confuse Tregs with recently activated T cells expressing CD25 (1). Additionally despite a well characterized link between the transcription factor Foxp3 and mouse Tregs (51), mouse antigen-driven IL-10 producing Tregs do not express Foxp3, but they do inhibit the proliferation of $CD25^-$ $CD4^+$ T cells with a similar efficiency as $CD25^+$ $CD4^+$ naturally occurring Tregs (52). Recently of GFP-Foxp3 fusion protein reporter knockin mice have been generated (53) and demonstrate that Foxp3 expression is restricted to T cells with suppressor activity, suggesting that it has the potential to be an excellent candidate marker to identify these cells in mice. But because Foxp3 is not expressed on the cells surface, it is likely that other markers will be needed for purification of murine Tregs *in vitro* whose Treg function can then be confirmed by Foxp3 staining. These may comprise Foxp3-regulated cell surface molecules.

Recent evidence that Foxp3 expression in humans, unlike mice, may not be specific for cells with a regulatory phenotype and may be a consequence of activation status (54), and the detection of CD25+CD8+ regulatory cells (55) make it likely that a panel of markers will be needed to identify Tregs in a clinical setting. With this in mind, the emergence of genechip technology, which enables the characterization of changes in global gene expression, may facilitate the detection of Tregs in the absence of a Treg master gene. Generation and identification of these suppressor cells will be vital to successfully induce Treg-mediated graft tolerance in the future. The next decade should see much more progress with these studies in rodents which should allow us to delineate the most favorable method of inducing Tregs and will hopefully permit the transfer of Treg therapy to humans.

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7. REFERENCES

1. Wood, K. J. & S. Sakaguchi: Regulatory T cells in transplantation tolerance. *Nature Reviews. Immunology*, 3, 199-210 (2003)
2. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh & M. Toda: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *Journal of Immunology*, 155, 1151-64 (1995)
3. Karim, M., A. R. Bushell & K. J. Wood: Regulatory T cells in transplantation. *Current Opinion in Immunology*, 14, 584-91 (2002)
4. Slavin, S., S. Strober, Z. Fuks & H. S. Kaplan: Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-

term survival of allogeneic bone marrow and skin grafts. *Journal of Experimental Medicine*, 146, 34-48 (1977)
5. Yamada & M. H. Sayegh: The CD154-CD40 costimulatory pathway in transplantation. *Transplantation*, 73, S36-9 (2002)
6. James, T., M. X. Jin, N. C. Chowdhury & S. F. Oluwole: Tolerance induction to rat islet allografts by intrathymic inoculation of donor spleen cells. *Transplantation*, 56, 1148-52 (1993)
7. Sykes, M., G. L. Szot, K. A. Swenson & D. A. Pearson: Induction of high levels of allogeneic hematopoietic reconstitution and donor-specific tolerance without myelosuppressive conditioning. *Nature Medicine*, 3, 783-7 (1997)
8. Jonker, M., Ossevoort, Ma & M. Vierboom: Blocking the CD80 and CD86 costimulation molecules: lessons to be learned from animal models. *Transplantation*, 73, S23-6 (2002)
9. Larsen, C. P., E. T. Elwood, D. Z. Alexander, S. C. Ritchie, R. Hendrix, C. Tucker-Burden, H. R. Cho, A. Aruffo, D. Hollenbaugh, P. S. Linsley, K. J. Winn & T. C. Pearson: Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature*, 381, 434-8 (1996)
10. Quigley, R. L., K. J. Wood & P. J. Morris: Mediation of antigen-induced suppression of renal allograft rejection by a CD4 (W3/25+) T cell. *Transplantation*, 47, 684-8 (1989)
11. Oluwole, S. F., K. Reemtsma & M. A. Hardy: Characteristics and function of suppressor T lymphocytes in immunologically unresponsive rats following pretreatment with UV-B-irradiated donor leukocytes and peritransplant cyclosporine. *Transplantation*, 47, 1001-7 (1989)
12. Qin, S., S. P. Cobbold, H. Pope, J. Elliott, D. Kioussis, J. Davies & H. Waldmann: "Infectious" transplantation tolerance. *Science*, 259, 974 - 977 (1993)
13. Graca, L., A. Le Moine, C. Y. Lin, P. J. Fairchild, S. P. Cobbold & H. Waldmann: Donor-specific transplantation tolerance: the paradoxical behavior of CD4+CD25+ T cells. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 10122-6 (2004)
14. Kingsley, C. I., M. Karim, A. R. Bushell & K. J. Wood: CD25+CD4+ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. *Journal of Immunology*, 168, 1080-6 (2002)
15. Nishimura, E., T. Sakihama, R. Setoguchi, K. Tanaka & S. Sakaguchi: Induction of antigen-specific immunologic tolerance by in vivo and in vitro antigen-specific expansion of naturally arising Foxp3+CD25+CD4+ regulatory T cells. *International Immunology*, 16, 1189 - 1202 (2004)
16. Karim, M., C. I. Kingsley, A. Bushell, B. Sawitzki & K. J. Wood: Alloantigen-induced CD25+CD4+ regulatory T cells can develop in vivo from CD25-CD4+ precursors in a thymus independent process. *Journal of Immunology*, 172, 923 - 928 (2004)
17. Schenk, S., D. D. Kish, C. He, T. El-Sawy, E. Chiffoleau, C. Chen, Z. Wu, S. Sandner, A. V. Gorbachev, K. Fukamachi, P. S. Heeger, M. H. Sayegh, L. A. Turka & R. L. Fairchild: Alloreactive T cell responses and acute rejection of single class II MHC-disparate heart allografts are under strict regulation by CD4+ CD25+ T cells.[erratum appears in J Immunol. 2005 Apr 15;174 (8):5135 Note: Chen, Chuangqui [corrected to Chen, Chuangqi]]. *Journal of Immunology*, 174, 3741-8 (2005)
18. Hara, M., C. I. Kingsley, M. Niimi, S. Read, S. E. Turvey, A. R. Bushell, P. J. Morris, F. Powrie & K. J. Wood: IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. *Journal of Immunology*, 166, 3789-96 (2001)
19. Kohyama, M., D. Sugahara, S. Sugiyama, H. Yagita, K. Okumura & N. Hozumi: Inducible costimulator-dependent IL-10 production by regulatory T cells specific for self-antigen. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 4192-7 (2004)
20. Nakamura, K., A. Kitani & W. Strober: Cell contact-dependent immunosuppression by CD4 (+)CD25 (+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *Journal of Experimental Medicine*, 194, 629-44 (2001)
21. Grossman, W. J., J. W. Verbsky, B. L. Tollefsen, C. Kemper, J. P. Atkinson & T. J. Ley: Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood*, 104, 2840-8 (2004)
22. Trapani, J. A. & V. R. Sutton: Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Current Opinion in Immunology*, 15, 533-43 (2003)
23. Gondek, D. C., L. F. Lu, S. A. Quezada, S. Sakaguchi & R. J. Noelle: Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *Journal of Immunology*, 174, 1783-6 (2005)
24. McHugh, R. S., M. J. Whitters, C. A. Piccirillo, D. A. Young, E. M. Shevach, M. Collins & M. C. Byrne: CD4 (+)CD25 (+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor.[see comment]. *Immunity*, 16, 311-23 (2002)
25. Shimizu, J., S. Yamazaki, T. Takahashi, Y. Ishida & S. Sakaguchi: Stimulation of CD25 (+)CD4 (+) regulatory T cells through GITR breaks immunological self-tolerance.[see comment]. *Nature Immunology*, 3, 135-42 (2002)
26. Uraushihara, K., T. Kanai, K. Ko, T. Totsuka, S. Makita, R. Iiyama, T. Nakamura & M. Watanabe: Regulation of murine inflammatory bowel disease by CD25+ and CD25- CD4+ glucocorticoid-induced TNF receptor family-related gene+ regulatory T cells.[erratum appears in J Immunol. 2004 Jan 1;172 (1):717]. *Journal of Immunology*, 171, 708-16 (2003)
27. Valzasina, B., C. Guiducci, H. Dislich, N. Killeen, A. D. Weinberg & M. P. Colombo: Triggering of OX40 (CD134) on CD4 (+)CD25+ T cells blocks their inhibitory activity: a novel regulatory role for OX40 and its comparison with GITR. *Blood*, 105, 2845-51 (2005)
28. Young, K. J., B. DuTemple, M. J. Phillips & L. Zhang: Inhibition of graft-versus-host disease by double-negative regulatory T cells. *Journal of Immunology*, 171, 134-41 (2003)
29. Lee, B. P., E. Mansfield, S. C. Hsieh, T. Hernandez-Boussard, W. Chen, C. W. Thomson, M. S. Ford, S. E. Bosinger, S. Der, Z. X. Zhang, M. Zhang, D. J. Kelvin, M. M. Sarwal & L. Zhang: Expression profiling of murine double-negative regulatory T cells suggest mechanisms for prolonged cardiac allograft survival. *Journal of Immunology*, 174, 4535-44 (2005)
30. Sawitzki, B., C. I. Kingsley, V. Oliveira, M. Karim, M. Herber & K. J. Wood: Interferon gamma production by

alloantigen reactive $CD25^{+}CD4^{+}$ regulatory T cells is important for their regulatory function in vivo. *Journal of Experimental Medicine*, 201, 1925 - 1935 (2005)
31. Pearson, T. C., J. C. Madsen, C. P. Larsen, P. J. Morris & K. J. Wood: Induction of transplantation tolerance in adults using donor antigen and anti-CD4 monoclonal antibody. *Transplantation*, 54, 475-83 (1992)
32. Bushell, A., M. Karim, C. I. Kingsley & K. J. Wood: Pretransplant blood transfusion without additional immunotherapy generates CD25+CD4+ regulatory T cells: a potential explanation for the blood-transfusion effect. *Transplantation*, 76, 449-55 (2003)
33. Karim, M., G. Feng, K. J. Wood & A. Bushell: CD25+CD4+ regulatory T cells generated by exposure to a model protein antigen prevent allograft rejection: antigen specific reactivation in vivo is critical for bystander regulation. *Blood*, 105, 4871 - 4877 (2005)
34. Bushell, A., E. Jones, A. Gallimore & K. J. Wood: The generation of CD25+CD4+ regulatory T cells that prevent allograft rejection does not compromise immunity to a viral pathogen. *Journal of Immunology*, 174, 3290 - 3297 (2005)
35. Albert, M. L.: Death-defying immunity: do apoptotic cells influence antigen processing and presentation? *Nature Reviews. Immunology*, 4, 223-31 (2004)
36. Schwarz, A., A. Maeda, M. K. Wild, K. Kernebeck, N. Gross, Y. Aragane, S. Beissert, D. Vestweber & T. Schwarz: Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. *Journal of Immunology*, 172, 1036-43 (2004)
37. Maeda, A., A. Schwarz, K. Kernebeck, N. Gross, Y. Aragane, D. Peritt & T. Schwarz: Intravenous infusion of syngeneic apoptotic cells by photopheresis induces antigen-specific regulatory T cells. *J Immunol*, 174, 5968-76 (2005)
38. Tamura, S., Y. Beck, Y. Ando & H. Tahara: Intrathymic inoculation of donor HLA class I-derived peptide generates donor-specific CD4+CD25+ regulatory T cells. *Transplant Proc*, 37, 40-2 (2005)
39. Kang, H. K., M. A. Michaels, B. R. Berner & S. K. Datta: Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. *Journal of Immunology*, 174, 3247-55 (2005)
40. Feng, G. & K. Wood: Regulatory T cells--an emerging role in transplantation. *Yonsei Medical Journal*, 45, 968-77 (2004)
41. Lee, J., E. N. Lee, E. Y. Kim, H. J. Lee, H. J. Park, C. L. Sun, S. K. Lee, J. W. Joh, K. W. Lee, G. Y. Kwon & S. J. Kim: 4-1BB promotes long-term survival in skin allografts treated with anti-CD45RB and anti-CD40L monoclonal antibodies. *Transplant Proc*, 37, 123-5 (2005)
42. Uraushihara, K., T. Kanai, K. Ko, T. Totsuka, S. Makita, R. Iiyama, T. Nakamura & M. Watanabe: Regulation of murine inflammatory bowel disease by CD25+ and CD25- CD4+ glucocorticoid-induced TNF receptor family-related gene+ regulatory T cells.[erratum appears in J Immunol. 2004 Jan 1;172 (1):717]. *Journal of Immunology*, 171, 708-16 (2003)
43. Crabtree, G. R. & E. N. Olson: NFAT signaling: choreographing the social lives of cells. *Cell*, 109 Suppl, S67-79 (2002)
44. Bopp, T., A. Palmetshofer, E. Serfling, V. Heib, S. Schmitt, C. Richter, M. Klein, H. Schild, E. Schmitt & M. Stassen: NFATc2 and NFATc3 transcription factors play a crucial role in suppression of CD4+ T lymphocytes by CD4+ CD25+ regulatory T cells. *Journal of Experimental Medicine*, 201, 181-7 (2005)
45. Zheng, X. X., A. Sanchez-Fueyo, M. Sho, C. Domenig, M. H. Sayegh & T. B. Strom: Favorably tipping the balance between cytopathic and regulatory T cells to create transplantation tolerance.[see comment]. *Immunity*, 19, 503-14 (2003)
46. Kitade, H., M. Kawai, T. Koshiba, A. Giulietti, L. Overbergh, O. Rutgeerts, D. Valckx, M. Waer, C. Mathieu & J. Pirenne: Early accumulation of interferon-gamma in grafts tolerized by donor-specific blood transfusion: friend or enemy? *Transplantation*, 78, 1747-55 (2004)
47. Kawai, M., H. Kitade, C. Mathieu, M. Waer & J. Pirenne: Inhibitory and Stimulatory Effects of Cyclosporine A on the Development of Regulatory T Cells In Vivo. *Transplantation*, 79, 1073-1077 (2005)
48. Battaglia, M., A. Stabilini & M.-G. Roncarolo: Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood*, 443 - 4748, (2005)
49. Gorczynski, R. M., L. Lee & I. Boudakov: Augmented induction of CD4+CD25+ Treg using monoclonal antibodies to CD200R. *Transplantation*, 79, 488-91 (2005)
50. Tomasoni, S., S. Aiello, L. Cassis, M. Noris, L. Longaretti, R. A. Cavinato, N. Azzollini, A. Pezzotta, G. Remuzzi & A. Benigni: Dendritic Cells Genetically Engineered with Adenoviral Vector Encoding dnIKK2 Induce the Formation of Potent CD4+ T-Regulatory Cells. *Transplantation*, 79, 1056-61 (2005)
51. Ramsdell, F: Foxp3 and natural regulatory T cells: key to a cell lineage? *Immunity*, 19, 165-8 (2003)
52. Vieira, P. L., J. R. Christensen, S. Minaee, E. J. O'Neill, F. J. Barrat, A. Boonstra, T. Barthlott, B. Stockinger, D. C. Wraith & A. O'Garra: IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. *Journal of Immunology*, 172, 5986-93 (2004)
53. Fontenot, J., J. Rasmussen, L. Williams, J. Dooley, A. Farr & A. Rudensky: Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity*, 22, 329 - 341 (2005)
54. Morgan, M., J. van Bilsen, A. Bakker, B. Heemskerk, M. Schilham, F. Hartgers, B. Elferink, L. van der Zanden, R. de Vries, T. Huizinga, T. Ottenhoff & R. Toes: Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. *Human Immunology*, 66, 13 - 20 (2005)
55. Bienvenu, B., B. Martin, C. Auffray, C. Cordier, C. Becourt & B. Lucas: Peripheral CD8+CD25+ T lymphocytes from MHC class II deficient mice exhibit regulatory activity. *Journal of Immunology*, 175, 246 - 253 (2005)

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