Review

The effects of antibody treatment on regulatory CD4$^+$CD25$^+$ T cells

Huanfa Yi [a,b], Jialin Zhang [c], Yong Zhao [a,b,*]

[a] *Transplantation Biology Research Division, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
[b] *China-U.S. Joint Research Center for Life Sciences, Beijing, China*
[c] *Department of General Surgery and Organ Transplant Unit, First University Hospital, China Medical University, Shenyang, China*

Abstract

Current therapeutic antibodies, at least some, possess the capacity to induce immune tolerance in experimental models with allo-grafts or autoimmune diseases. Clinical application of humanized or chimeric antibodies to treat graft rejection or autoimmune diseases is presently underway. It is now becoming clear that immune tolerance can be acquired in some cases due to the action of regulatory T cells (Tregs), especially CD4$^+$CD25$^+$ Tregs. In addition to their inhibition on immune response, some antibodies could promote tolerance induction in organ transplantation and autoimmune diseases essentially through the induction of Tregs. In this manuscript, we review the recent progress on the effects of therapeutic antibodies on the development, phenotypic changes and functions of CD4$^+$CD25$^+$ Tregs.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Regulatory T cells; Immunosuppression; Antibodies; Immune tolerance; Transplantation

Contents

1. Introduction

Organ transplantation is the preferred treatment modality for end-stage organ failure [1]. At present, though immunosuppressive medicines have significantly improved the short-term graft survival, chronic allograft nephropathy (CAN) and cardiovascular disease remain major challenge for long-term graft survival. So the induction of transplantation tolerance has been one of the major goals of transplant community. Transplantation tolerance can be achieved via central and peripheral tolerance [2]. Peripheral tolerance involve several different mechanisms including T cell clonal deletion, "ignorance", energy and active suppression [3,4]. Accumulating evidence has shown that Tregs play an important role in the induction and maintenance of transplantation tolerance [4,5].

In recent years, it has been demonstrated that monoclonal antibodies (mAbs) can successfully induce long-term immunological tolerance. Though the mechanisms have not been defined completely, it appears that the induction of Tregs and co-stimulation blockade may be the major mechanisms for mAbs to induce transplant tolerance in some experimental models [3,6–11]. Because CD4$^+$CD25$^+$ Tregs are the most regulatory T cell subsets in transplantation tolerance, the goal of this manuscript is to briefly review the current literature about the effects of antibody treatment on the phenotype, development and function of CD4$^+$CD25$^+$ Tregs in experimental models.

2. Antibodies used in animal models and clinical trials

Since the late 1960s, Polyclonal anti-lymphocyte serum (ALS) or anti-thymocyte globulins (ATG) have been using as potent immunosuppressive agents in organ transplantation, and the availability of mAbs in the 1980s provides better practical possibilities. Since then, many groups have demonstrated that a variety of antibodies that interference with T cell-antigen presenting cell (APC) interactions could induce tolerance in some models. At present, mAbs used in transplantation and autoimmune diseases primarily target CD molecules (CD3, CD4, CD8, CD20, CD22, CD28, CD80/86, CD40-CD154, CTLA-4, CD52, CD45RB), chemokines and their receptors (CCR5, CCR1, etc), as well as cytokines (TNF-α, IL-2, etc). At present, must related studies or information were collected in experimental models.

3. Types of Tregs and transplant tolerance

A variety of Treg subsets have been identified according to their surface markers or cytokine products in a number of experimental models, such as CD4$^+$ Treg cells (including natural CD4$^+$CD25$^+$ Tregs [12], IL-10-producing Tr1 cells [13], TGF-β-producing Th3 cells [14]), CD8$^+$ Tregs [15], Veto CD8$^+$ cells [16], γδ T cells, NKT (NK1.1$^+$CD4$^-$CD8$^-$) cells [17], NK1.1$^-$CD4$^-$CD8$^-$ cells [18], and others [19,20]. Co-transfer of purified CD4$^+$CD25$^+$ Tregs (nTregs) along with the CD4$^+$CD25$^-$ T cells significantly delayed graft versus host disease (GVHD) onset caused by the latter cells [21,22] or block MHC-mismatched allogeneic skin graft rejection [23,24]. Tr1 cells have been shown able to prevent GVHD [25] and graft rejection [26].

Foxp3, as a member of the forkhead/winged-helix family of transcriptional repressors, is still a most specific marker for distinguishing Tregs and non-Treg cells in CD4$^+$T cells now [27]. The precise mechanisms for the immune regulation by these subsets are different. For CD4$^+$CD25$^+$ Tregs, there are at least three mechanisms: First, CD4$^+$CD25$^+$ Tregs suppress the proliferation of effector cells via cell–cell contact style, which may involve in Fas–FasL pathway [28] or Granzyme and/or perforin pathway [29]. Secondly, IL-10 and/or TGF-β may involve in the regulation, but it is controversial. For example, CD4$^+$CD25$^+$ Tregs from IL-10$^{-/-}$ mice are unable to prevent the development of inflammatory bowel disease (IBD) [30]. Anti-TGF-β mAb can attenuate the suppression of CD4$^+$CD25$^+$ Tregs [31], but Piccirillo et al consider that the suppression of CD4$^+$CD25$^+$ Tregs is not correlated with TGF-β [32]. Third, CD4$^+$CD25$^+$ Tregs down-regulate the expression of Immunoglobulin-like transcript 3(ILT3) and ILT4 in DCs [33], then decrease the expression of costimulatory molecules, such as CD40, CD80 and CD86 [15]. Thus, CD4$^+$CD25$^+$ Tregs may perform their suppression via kinds of mechanisms according to different microenvironments, such as intensity and sites of immune response. In addition, other Treg cells have different regulatory mechanisms: CD8$^+$ Treg cells increase IL-4 production [34], NKT cells use costimulatory blockade [17], or DN Treg cells can deplete alloreactive CD8$^+$ T cells [18]. The full significance of other Treg subsets in transplantation tolerance remains to be documented. Much evidence indicates that active immunosuppression by Tregs is essential for induction of peripheral tolerance to both self and foreign antigens in small animal models in vivo. However, no studies in non-human primates or humans specifically using these cells for tolerance induction have been reported so far.

4. The effects of mAbs on CD4$^+$CD25$^+$ Tregs

4.1. ALS or ATG

ALS and ATG are the mixture of antibodies against CD2, CD3, CD45, and HLA molecules, can result in broad T cell depletion via complement-dependent lymphocyte lysis and Fas/

Fas ligand-mediated apoptosis [35], and are used as an integral part of tolerance induction in experimental transplantation [36,37]. ATG treatment resulted in the attenuation from myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, both in a preventive and early therapeutic setting via the expansion of MOG-specific CD4^{+}Foxp3^{+}Tregs [38]. A short course treatment with ALS can effectively expand CD4^{+}CD25^{+} Tregs and significantly prevent onset of type 1 diabetes mellitus in NOD mice [39]. Minamimura et al have recently reported that CD4^{+}CD25^{+} Tregs are spared from T cell-depleting effect of ALS, because of the up-regulation of Bcl-2 in these cells, while post-ALS treatment, CD4^{+}CD25^{-} T cells exhibited transient regulatory activity [40]. Because auto-reactive effector T cells are eliminated by ALS while Tregs survive, this treatment thus creates a long-lasting immunor-egulatory cell-dominant condition [39,41].

4.2. Anti-CD45RB mAb

CD45, which plays a pivotal role in T-cell antigen receptor signal transduction, is recognized as a potential candidate for tolerance induction strategies [8]. As we known, CD4^{+}CD25^{+} Tregs are CD45^{low}, CTLA-4^{high} [42]. Previous report considers that anti-CD45RB mAb treatment leads to a switch from CD45RB^{high} to CD45RB^{low} [43]. In fact, this mAb may selectively deplete CD45RB^{high} effector T cells, thereby enriching Tregs expressing low level of CD45RB, which causes the inversion of CD45RB^{high}/CD45RB^{low} ratio [44,45]. Moreover, this also can explain the up-regulation of CTLA-4 after administration of anti-CD45RB mAb [44,46]. It is reported that anti-CD45RB-treated mice exposed to alloantigens exhib-ited anergic CD4^{+}CD25^{-} effector cells and CD4^{+}CD25^{+} Tregs. Moreover, depletion of CD25^{+}T cells in the peritransplant period significantly prevented anti-CD45RB-mediated engraft-ment of islets [47]. These data indicate that CD4^{+}CD25^{+} Tregs were involved in transplant tolerance induction in this model. Thus compared with those non-selectively T cell-depletion reagents, anti-CD45RB mAb provides a better alternative for transplant tolerance induction.

In addition, anti-CD45 mAb may directly take part in the negative selection and causes the appearance of donor-specific Tregs in thymus [48]. This suggests that thymus is necessary for tolerance induction by anti-CD45RB mAb. Recently, ChA6 mAb, a chimeric anti-CD45RO/RB mAb, can induce the generation of CD4^{+}CD25^{+}Tregs that are phenotypically and functionally equivalent to those of Tr1 cells and CD8^{+} Tregs with low level of CD25 and considerable level of CD28 expression [49].

4.3. Anti-CD25 mAb

Although anti-CD25 mAbs were previously shown to prevent graft rejection via depleting activated T cells in rodent models, they are only used as adjunctive agents to immuno-suppression, and not part of tolerance protocols in the clinic. The presence of anti-CD25 mAb together with anti-CTLA-4 mAb can highly augment lymphokine-activated killer cell activity through the immunosuppression of Tregs [50]. Two anti-human CD25 mAbs (basiliximab and daclizumab) are currently used in clinical organ transplantation and have been shown to prevent acute rejection [51,52]. Recent studies by investigators have shown that PC61, a IgG1 isotype of anti-CD25 mAb, also deplete CD4^{+}CD25^{+} Tregs [53,54], and leads to prevent induction of tolerance [55], to exacerbate of acute GVHD [56] or inhibit tumor growth [57-59]. However, Kohm et al believe that anti-CD25 mAbs, regardless of which isotype, do not deplete CD4^{+}CD25^{+} Tregs, but inactivate the function of CD4^{+}CD25^{+} Tregs via down-regulating the expression of CD25 [60].. However, the depletion of CD4^{+}CD25^{+} Tregs by PC61 is kept for short term, because new CD4^{+}CD25^{+} Tregs are continuously produced from the thymus and enter the periphery.

But Game et al find that basiliximab does not influence the immunosuppressive function of CD4^{+}CD25^{+} Tregs in vitro, despite of abolishing the CTLA-4, HLA-DR and CD62L expression on CD4^{+}CD25^{+} Tregs [61]. Basiliximab can reduce apoptosis of CD4^{+}CD25^{+} Tregs possibly via increasing the expression of PTEN [61]. Consistently, another CD25 mAb, daclizumab, does not change the number and function of CD4^{+}CD25^{+} Tregs in renal transplantation patients [62].

4.4. Anti-CD20 mAb

Because CD20 is solely expressed on B cells, not B progenitors, CD20 mAb (such as rituximab) is mainly used to deplete B cells in the treatment of several autoimmune or lympho-proliferative diseases [63–65]. Interestingly, a recent study showed that rituximab therapy could significantly increase the frequency and the number of CD4^{+}CD25^{+} Tregs with the recovery of B cells in systemic lupus erythematosus (SLE) patients [66]. The reason is still unclear; perhaps due to expansion or emigrant of thymic-derived CD4^{+}CD25^{+} Tregs, conversion of CD4^{+}CD25^{-} T cells or decreased traffic of Tregs to inflammatory sites following the remission of disease.

4.5. Anti-CD4 mAb

Anti-CD4 mAbs are potential therapeutic agents for the prevention of autoimmune diseases and the treatment of graft rejection by restoring tolerance to self-antigens and inducing tolerance to antigens introduced under the cover of the Ab therapy [7,67]. Though some studies propose that depleting anti-CD4 mAb can inhibit CD4^{+} T cell proliferation triggered by TCR/CD3 ligation [68] and induce Tregs [69] as well as non-depleting anti-CD4 mAbs do, Several lines of evidence strongly support that non-depleting anti-CD4 mAbs appear to be more effective than depleting ones. It has been shown that a short-course treatment with non-depleting anti-CD4 mAb and donor antigen can significantly induce donor antigen specific-CD4^{+}CD25^{+} Tregs [[23,70–73]. It have been confirmed that these CD4^{+}CD25^{+} Tregs are transformed from CD4^{+}CD25^{-} precursors in periphery [74]. However, the possibility that some CD4^{+}CD25^{+} Tregs develop in thymus can not be excluded.

4.6. Anti-CD52 mAb (alemtuzumab)

CD52 is an exceptionally abundant antigen which is expressed specifically on human lymphocytes, monocytes, eosinophils. Alemtuzumab(rat IgG2b) and its humanized form cause extensive T and B lymphocyte depletion, which results in a prolonged lymphopenia, particularly of CD4^{+} cells. It has been clinically used to treat lympho-proliferative disorders (leukemia and lymphoma) [75,76] and autoimmune diseases [77,78], and to deplete lymphocytes in organ and bone marrow transplantation [79–82].

It was reported that the recognition of CD52 on T lymphocytes by alemtuzumab led to the activation of cell proliferation via the CD2 pathway and the expression of interleukin -2 receptors [83]. Recently, a report shows that the interaction between 4C8 antigen (it is recently determined that it is CD52, too [84]) and its mAb provides a co-stimulatory signaling even stronger than that of anti-CD28, which then leads to the production of CD4^{+}CD25^{+} Tregs from CD4^{+}CD25^{−} T cells that perform their suppressive function via a cell contact-dependent fashion [85]. So at least in vitro, costimulation of CD4^{+} T cells by alemtuzumab indeed leads to the induction of certain Treg cell subset which inhibits polyclonal or allogeneic response of CD4^{+} and CD8^{+}T cells. CD52-costimulation slightly increased FOXP3 expression in CD4^{+}CD25^{−}T cells in the protein level. In addition, anti-CD52 mAb-induced CD4^{+}CD25^{+} Tregs can be expanded more than 20-fold with IL-2, and these expanded Tregs do not lost their immunosuppressive function and can prevent GVHD-like pathology in SCID mice injected with human PBMCs. But some experiments need to be done to decide whether alemtuzumab can expand Tregs.

4.7. Anti-CD40L mAb (CD154)

Interruption of co-stimulation signaling pathway can result in the suppression of immune response and possibly same in immune tolerance. Combining with donor antigens [86] or other blockers [87–89], nondepleting antagonists to CD154 have been used in animal models or non-human primates models [90–92]. Despite these mAbs can induce the apoptosis of alloreactive T cells, it appears that Tregs play more important roles in the induction of infectious tolerance. CD4^{+}CD25^{+} Tregs harvested from C3H mice having received donor-specific blood transfusion from DBA/2 plus anti-CD40L mAb exhibited a much more powerful suppressive phenotype, ensuring the long-term survival of DBA/2 skin allografts, even when co-transferred at equal numbers with CD4^{+}CD25^{−} T cells [93,94]. But at present, it is unclear that these CD4^{+}CD25^{+} Tregs come from expanded inherent CD4^{+}CD25^{+} Tregs or induced from aggressive T effector cells.

Recently, a report proposes that recruitment of CD4^{+}CD25^{+} Tregs into grafts might be an alternative mechanism for tolerance induction in this model, because CCR4 and CCL22 are up-regulated in grafts coinciding with the increasing expression of Foxp3. Tolerance induction by anti-CD154 mAb could not be achieved in CCR4^{−/−} recipients, indicating that recruitment of Foxp3^{+}Tregs to allograft tissue is dependent on CCR4 and critical for tolerance induction by this approach [95].

4.8. Anti-CD3 mAb

Although they were used initially as non-specific immuno-suppressants in transplantation, CD3-specific mAbs have elicited renewed interest owing to their ability to induce immune tolerance. In addition to the rapid depletion of T cells and a decrease of CD4/CD8 ratio [96–98], Different types of Tregs can be produced according to CD3 mAb isoforms or administration routes.

The proportions of CD4^{+}CD25^{+} Tregs in anti-CD3 mAb treated mice were significantly increased in pancreatic and mesenteric lymph nodes, but not in the spleen of in adult overtly diabetic NOD mice [99,100]. These cells are fully unresponsive to antigen-specific or mitogen stimulation and produce high levels of TGF-β, indicating that the induced CD4^{+}CD25^{+} Tregs in this model are distinct from naturally occurring Tregs [100]. Interestingly, changing the mAb administration route perhaps produce distinct Treg subpopulations. Oral administration of anti-CD3 mAb can prominently increase the latency-associated peptide (LAP)[+]CD4^{+}CD25^{−} Tregs in mesenteric lymph nodes or spleens. LAP^{+}CD4^{+}CD25^{−} Tregs from mice that have been orally administrated CD3 mAb have excellent immuno-suppressive activity in vitro and in vivo through a TGF-β-dependent mechanism [14]. It is unclear why it can induce CD4^{+}CD25^{−}LAP^{+} Tregs in this model yet.

Besides CD4^{+} Tregs could be induced by anti-CD3 mAbs, Bisikirska et al show that hOKT3γ1 (Ala-Ala), a modified anti-human CD3 mAb, can induce CD8^{+}CD25^{+}Foxp3^{+}Tregs in patients with type 1 diabetes. These induced CD8^{+}CD25^{+}Foxp3[+−] Treg cells inhibit the responses of CD4^{+} cells to antigens in a cell-contact dependent fashion [101].

4.9. Anti-CD28 mAb

CD28 is the most prominent co-stimulatory receptor, it interacts with its ligands, B7 molecules (CD80 or CD86) expressed on APCs, to provide "secondary" signaling for T cell activation. Though the expression of CD28 has similar levels in both CD25^{+} and CD25^{−} populations, CD28/B7 co-stimulatory pathway appears to be more important for the development, homeostasis, peripheral maintenance and function of nTregs than for CD25^{−} T cells, because CD28 or B7-deficient mice present a profound decrease of nTregs. Moreover, CD4^{+}CD25^{+} Tregs from CD28^{−/−} mice have a functional deficit on CD25^{−}T cells [102]. CD28 may support the survival and self-renewal of peripheral Tregs via regulating IL-2 production by conventional T cells and CD25 expression on nTregs themselves [103]. The combination of suboptimal DST with anti-CD28 mAb induces donor-specific tolerance that correlates with enhanced numbers of regulatory T-cells [104]. In addition, JJ316, a stimulatory anti-CD28 mAb known to promote Th2 function and the expansion of Treg cells, can efficiently prevented the inflammatory process of adjuvant arthritis in rats [105].

Recently, Beyersdorf et al find superagonistic anti-CD28 mAb, a new CD28 mAb with more potent co-stimulation effect independently of TCR signaling, can effectively abate or prevent the symptoms of autoimmune diseases [106,107,111]. Tregs are preferentially expanded and activated in vivo over CD25$^-$ T cells in this model [106,107]. Lin et al also provide the evidence that superagonistic anti-CD28 mAb can expand Tregs in vivo and in vitro [108]. On the other hand, Tregs expanded by superagonistic anti-CD28 mAb reveal a dose-dependent increase in suppressive activity [106,107]. But the severe toxic response of CD28 superagonist in clinical trials limits its application in the future.

4.10. Other antibodies and CD4$^+$CD25$^+$ Tregs

It has been reported that the anti-inflammatory extracellular matrix protein, thrombospondin-1, promoted the generation of human peripheral Treg cells through the ligation of one of its receptor, CD47 [109]. CD47 stimulation by mAb induced naive or memory CD4$^+$CD25$^-$ T cells to become immunosuppressive [109]. Triggering either GITR or OX40 (CD134) on CD4$^+$CD25$^+$ Tregs using agonist mAbs significantly inhibited their capacity of immunosuppression [110]. Strikingly, the suppression of Tregs on GVHD was abrogated either by intraperitoneal injection of anti-OX40 or anti-GITR mAbs [110]. The results suggest that OX40 directly controls Tregs-mediated suppression.

5. Summary

Antibody therapeutics offer promising approaches for the induction of immune tolerance. T cell tolerance induced by mAbs may be achieved by expansion, induction and recruitment of CD4$^+$CD25$^+$ Tregs or even other types of Tregs. The ideal antibody therapeutics should do not interfere with, but rather promote, Tregs while still controlling innate and adaptive immunity against the graft. For those antibodies for tolerance induction to become clinically applicable, there is a need to know how the various antibody-based regimens affect the power of Tregs when combined with immunosuppressive medicines.

Acknowledgements

References

[1] Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation — how much of the promise has been realized? Nat Med 2005;11:605–13.

[2] Sykes M. Mixed chimerism and transplant tolerance. Immunity 2001;14:417–24.

[3] Lechler RI, Garden OA, Turka LA. The complementary roles of deletion and regulation in transplantation tolerance. Nat Rev Immunol 2003;3:147–58.

[4] Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol 2003;3:199–210.

[5] Walsh PT, Taylor DK, Turka LA. Tregs and transplantation tolerance. J Clin Invest 2004;114:1398–403.

[6] Waldmann TA. A novel means of favorably tipping the balance between cytopathic and regulatory T cells. Immunity 2003;19:465–6.

[7] Waldmann H, Cobbold S. How do monoclonal antibodies induce tolerance? A role for infectious tolerance? Annu Rev Immunol 1998;16:619–44.

[8] Luke PP, O'Brien CA, Jevnikar AM, Zhong R. Anti-CD45RB monoclonal antibody-mediated transplantation tolerance. Curr Mol Med 2001;1:533–43.

[9] Chatenoud L. CD3-specific antibody-induced active tolerance: from bench to bedside. Nat Rev Immunol 2003;3:123–32.

[10] Cobbold SP. T cell tolerance induced by therapeutic antibodies. Philos Trans R Soc Lond B Biol Sci 2005;360:1695–705.

[11] Graca L, Le Moine A, Cobbold SP, Waldmann H. Antibody-induced transplantation tolerance: the role of dominant regulation. Immunol Res 2003;28:181–91.

[12] Sakaguchi S, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. Int Rev Immunol 2005;24:211–26.

[13] Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997;389:737–42.

[14] Ochi H, Abraham M, Ishikawa H, Frenkel D, Yang K, Basso AS, et al. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4+ CD25- LAP+ T cells. Nat Med 2006;12:627–35.

[15] Chang CC, Ciubotariu R, Manavalan JS, Yuan J, Colovai AI, Piazza F, et al. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat Immunol 2002;3:237–43.

[16] George JF, Thomas JM. The molecular mechanisms of veto mediated regulation of alloresponsiveness. J Mol Med 1999;77:519–26.

[17] MacDonald HR. Development and selection of NKT cells. Curr Opin Immunol 2002;14:250–4.

[18] Zhang ZX, Yang L, Young KJ, DuTemple B, Zhang L. Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. Nat Med 2000;6:782–9.

[19] Mills KH. Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 2004;4:841–55.

[20] Sutmuller RP, Offringa R, Melief CJ. Revival of the regulatory T cell: new targets for drug development. Drug Discov Today 2004;9:310–6.

[21] Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J Exp Med 2001;193:1311–8.

[22] Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4 (+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J Exp Med 2002;196:389–99.

[23] Graca L, Thompson S, Lin CY, Adams E, Cobbold SP, Waldmann H. Both CD4(+)CD25(+) and CD4(+)CD25(−) regulatory cells mediate dominant transplantation tolerance. J Immunol 2002;168:5558–65.

[24] Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, et al. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. J Immunol 2001;166:3789–96.

[25] Roncarolo MG, Gregori S, Levings M. Type 1 T regulatory cells and their relationship with CD4+CD25+ T regulatory cells. Novartis Found Symp 2003;252:115–27 discussion 127–131, 203–110.

[26] Zelenika D, Adams E, Humm S, Lin CY, Waldmann H, Cobbold SP. The role of CD4+ T-cell subsets in determining transplantation rejection or tolerance. Immunol Rev 2001;182:164–79.

[27] Zhang L, Zhao Y. The regulation of Foxp3 expression in regulatory CD4 (+)CD25(+)T cells: multiple pathways on the road. J Cell Physiol 2007;211:590–7.

[28] Watanabe T, Yoshida M, Shirai Y, Yamori M, Yagita H, Itoh T, et al. Administration of an antigen at a high dose generates regulatory CD4+ T cells expressing CD95 ligand and secreting IL-4 in the liver. J Immunol 2002;168:2188–99.

[29] Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 2005;174:1783–6.

[30] Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 1999;190:995–1004.

[31] Shevach EM, McHugh RS, Piccirillo CA, Thornton AM. Control of T-cell activation by CD4+ CD25+ suppressor T cells. Immunol Rev 2001;182:58–67.

[32] Piccirillo CA, Letterio JJ, Thornton AM, McHugh RS, Mamura M, Mizuhara H, et al. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. J Exp Med 2002;196:237–46.

[33] Cederbom L, Hall H, Ivars F. CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. Eur J Immunol 2000;30:1538–43.

[34] Zhou J, Carr RI, Liwski RS, Stadnyk AW, Lee TD. Oral exposure to alloantigen generates intragraft CD8+ regulatory cells. J Immunol 2001;167:107–13.

[35] Genestier L, Fournel S, Flacher M, Assossou O, Revillard JP, Bonnefoy-Berard N. Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. Blood 1998;91:2360–8.

[36] Hale DA, Gottschalk R, Maki T, Monaco AP. Determination of an improved sirolimus (rapamycin)-based regimen for induction of allograft tolerance in mice treated with antilymphocyte serum and donor-specific bone marrow. Transplantation 1998;65:473–9.

[37] Kawai T, Cosimi AB, Colvin RB, Powelson J, Eason J, Kozlowski T, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. Transplantation 1995;59:256–62.

[38] Chung DT, Korn T, Richard J, Ruzek M, Kohm AP, Miller S, et al. Anti-thymocyte globulin (ATG) prevents autoimmune encephalomyelitis by expanding myelin antigen-specific Foxp3+ regulatory T cells. Int Immunol 2007;19:1003–10.

[39] Ogawa N, Minamimura K, Kodaka T, Maki T. Short administration of polyclonal anti-T cell antibody (ALS) in NOD mice with extensive insulitis prevents subsequent development of autoimmune diabetes. J Autoimmun 2006;26:225–31.

[40] Minamimura K, Gao W, Maki T. CD4+ regulatory T cells are spared from deletion by antilymphocyte serum, a polyclonal anti-T cell antibody. J Immunol 2006;176:4125–32.

[41] Ogawa N, List JF, Habener JF, Maki T. Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. Diabetes 2004;53:1700–5.

[42] Yi H, Zhen Y, Jiang L, Zheng J, Zhao Y. The phenotypic characterization of naturally occurring regulatory CD4+CD25+ T cells. Cell Mol Immunol 2006;3:189–95.

[43] Basadonna GP, Auersvald L, Khuong CQ, Zheng XX, Kashio N, Zekzer D, et al. Antibody-mediated targeting of CD45 isoforms: a novel immunother-apeutic strategy. Proc Natl Acad Sci U S A 1998;95:3821–6.

[44] Luke PP, Deng JP, Lian D, O'Connell PJ, Garcia B, Jevnikar AM, et al. Prolongation of allograft survival by administration of anti-CD45RB monoclonal antibody is due to alteration of CD45RBhi: CD45RBlo T-cell proportions. Am J Transplant 2006;6:2023–34.

[45] Luke PP, Deng JP, O'Brien CA, Everest M, Hall AV, Chakrabarti S, et al. Alteration in CD45RBhi/CD45RBlo T-cell ratio following CD45RB monoclonal-antibody therapy occurs by selective deletion of CD45RBhi effector cells. Transplantation 2003;76:400–9.

[46] Fecteau S, Basadonna GP, Freitas A, Ariyan C, Sayegh MH, Rothstein DM. CTLA-4 up-regulation plays a role in tolerance mediated by CD45. Nat Immunol 2001;2:58–63.

[47] Salvalaggio PR, Camirand G, Ariyan CE, Deng S, Rogozinski L, Basadonna GP, et al. Antigen exposure during enhanced CTLA-4 expression promotes allograft tolerance in vivo. J Immunol 2006;176:2292–8.

[48] Deng S, Moore DJ, Huang X, Mohiuddin M, Lee MKt, Velidedeoglu E, et al. Antibody-induced transplantation tolerance that is dependent on thymus-derived regulatory T cells. J Immunol 2006;176:2799–807.

[49] Gregori S, Mangia P, Bacchetta R, Tresoldi E, Kolbinger F, Traversari C, et al. An anti-CD45RO/RB monoclonal antibody modulates T cell

[50] Okita R, Yamaguchi Y, Emi A, Matsuura K, Toge T. Enhancement of lymphokine-activated killer cell induction using anti-CD25 and anti-CTLA-4 monoclonal antibodies. Oncol Rep 2007;17:1429–35.

[51] Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K, Soulillou JP. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. CHIB 201 International Study Group. Lancet 1997;350:1193–8.

[52] Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med 1998;338:161–5.

[53] Stephens LA, Anderton SM. Comment on "Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ T regulatory cells". J Immunol 2006;177:2036 author reply 2037–2038.

[54] Zelenay S, Demengeot J. Comment on "Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ T regulatory cells". J Immunol 2006;177:2036–7 author reply 2037–2038.

[55] Bigenzahn S, Blaha P, Koporc Z, Pree I, Selzer E, Bergmeister H, et al. The role of non-deletional tolerance mechanisms in a murine model of mixed chimerism with costimulation blockade. Am J Transplant 2005;5:1237–47.

[56] Martin PJ, Pei J, Gooley T, Anasetti C, Appelbaum FR, Deeg J, et al. Evaluation of a CD25-specific immunotoxin for prevention of graft-versus-host disease after unrelated marrow transplantation. Biol Blood Marrow Transplant 2004;10:552–60.

[57] Sutmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med 2001;194:823–32.

[58] Jones E, Dahm-Vicker M, Simon AK, Green A, Powrie F, Cerundolo V, et al. Depletion of CD25+ regulatory cells results in suppression of melanoma growth and induction of autoreactivity in mice. Cancer Immun 2002;2:1.

[59] Nagai H, Horikawa T, Hara I, Fukunaga A, Oniki S, Oka M, et al. In vivo elimination of CD25+ regulatory T cells leads to tumor rejection of B16F10 melanoma, when combined with interleukin-12 gene transfer. Exp Dermatol 2004;13:613–20.

[60] Kohm AP, McMahon JS, Podojil JR, Begolka WS, DeGutes M, Kasprowicz DJ, et al. Cutting Edge: Anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ T regulatory cells. J Immunol 2006;176:3301–5.

[61] Game DS, Hernandez-Fuentes MP, Lechler RI. Everolimus and basiliximab permit suppression by human CD4+CD25+ cells in vitro. Am J Transplant 2005;5:454–64.

[62] Kreijveld E, Koenen HJ, Klasen IS, Hilbrands LB, Joosten I. Following Anti-CD25 Treatment, A Functional CD4+CD25+ Regulatory T-Cell Pool Is Present in Renal Transplant Recipients. Am J Transplant 2007;7:249–55.

[63] Eisenberg R. Update on rituximab. Ann Rheum Dis 2005;64(Suppl 4): iv55–7.

[64] Sfikakis PP, Boletis JN, Tsokos GC. Rituximab anti-B-cell therapy in systemic lupus erythematosus: pointing to the future. Curr Opin Rheumatol 2005;17:550–7.

[65] Thatayatikom A, White AJ. Rituximab: a promising therapy in systemic lupus erythematosus. Autoimmun Rev 2006;5:18–24.

[66] Vallerskog T, Gunnarsson I, Widhe M, Risselada A, Klareskog L, van Vollenhoven R, et al. Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. Clin Immunol 2007;122:62–74.

[67] Schulze-Koops H, Lipsky PE. Anti-CD4 monoclonal antibody therapy in human autoimmune diseases. Curr Dir Autoimmun 2000;2:24–49.

[68] Pullar CE, Morris PJ, Wood KJ. Altered proximal T-cell receptor signalling events in mouse CD4+ T cells in the presence of anti-CD4

monoclonal antibodies: evidence for reduced phosphorylation of Zap-70 and LAT. Scand J Immunol 2003;57:333–41.

[69] Pearson TC, Madsen JC, Larsen CP, Morris PJ, Wood KJ. Induction of transplantation tolerance in adults using donor antigen and anti-CD4 monoclonal antibody. Transplantation 1992;54:475–83.

[70] Saitovitch D, Bushell A, Mabbs DW, Morris PJ, Wood KJ. Kinetics of induction of transplantation tolerance with a nondepleting anti-Cd4 monoclonal antibody and donor-specific transfusion before transplantation. A critical period of time is required for development of immunological unresponsiveness. Transplantation 1996;61:1642–7.

[71] Saitovitch D, Morris PJ, Wood KJ. Recipient cells expressing single donor MHC locus products can substitute for donor-specific transfusion in the induction of transplantation tolerance when pretreatment is combined with anti-Cd4 monoclonal antibody. Evidence for a vital role of Cd4+ T cells in the induction of tolerance to class I molecules. Transplantation 1996;61:1532–8.

[72] Bushell A, Morris PJ, Wood KJ. Transplantation tolerance induced by antigen pretreatment and depleting anti-CD4 antibody depends on CD4+ T cell regulation during the induction phase of the response. Eur J Immunol 1995;25:2643–9.

[73] Kingsley CI, Karim M, Bushell AR, Wood KJ. CD25+CD4+ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. J Immunol 2002;168:1080–6.

[74] Karim M, Kingsley CI, Bushell AR, Sawitzki BS, Wood KJ. Alloantigen-induced CD25+CD4+ regulatory T cells can develop in vivo from CD25−CD4+ precursors in a thymus-independent process. J Immunol 2004;172:923–8.

[75] Osterborg A, Dyer MJ, Bunjes D, Pangalis GA, Bastion Y, Catovsky D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. J Clin Oncol 1997;15:1567–74.

[76] Countouriotis A, Moore TB, Sakamoto KM. Cell surface antigen and molecular targeting in the treatment of hematologic malignancies. Stem Cells 2002;20:215–29.

[77] Isaacs JD, Watts RA, Hazleman BL, Hale G, Keogan MT, Cobbold SP, et al. Humanised monoclonal antibody therapy for rheumatoid arthritis. Lancet 1992;340:748–52.

[78] Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H. Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. Lancet 1993;341:1620–2.

[79] Hale G, Jacobs P, Wood L, Fibbe WE, Barge R, Novitzky N, et al. CD52 antibodies for prevention of graft-versus-host disease and graft rejection following transplantation of allogeneic peripheral blood stem cells. Bone Marrow Transplant 2000;26:69–76.

[80] Calne R, Moffatt SD, Friend PJ, Jamieson NV, Bradley JA, Hale G, et al. Campath IH allows low-dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. Transplantation 1999;68:1613–6.

[81] Kirk AD, Hale DA, Mannon RB, Kleiner DE, Hoffmann SC, Kampen RL, et al. Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). Transplantation 2003;76:120–9.

[82] Knechtle SJ, Pirsch JD, Fechner Jr JH, Becker BN, Friedl A, Colvin RB, et al. Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. Am J Transplant 2003;3:722–30.

[83] Valentin H, Gelin C, Coulombel L, Zoccola D, Morizet J, Bernard A. The distribution of the CDW52 molecule on blood cells and characterization of its involvement in T cell activation. Transplantation 1992;54:97–104.

[84] Watanabe T, Masuyama J, Sohma Y, Inazawa H, Horie K, Kojima K, et al. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. Clin Immunol 2006;120:247–59.

[85] Masuyama J, Kaga S, Kano S, Minota S. A novel costimulation pathway via the 4C8 antigen for the induction of CD4+ regulatory T cells. J Immunol 2002;169:3710–6.

[86] Parker DC, Greiner DL, Phillips NE, Appel MC, Steele AW, Durie FH, et al. Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. Proc Natl Acad Sci U S A 1995;92:9560–4.

[87] Zheng XX, Markees TG, Hancock WW, Li Y, Greiner DL, Li XC, et al. CTLA4 signals are required to optimally induce allograft tolerance with combined donor-specific transfusion and anti-CD154 monoclonal antibody treatment. J Immunol 1999;162:4983–90.

[88] Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996;381:434–8.

[89] Honey K, Cobbold SP, Waldmann H. CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. J Immunol 1999;163:4805–10.

[90] Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Buchanan K, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med 1999;5:686–93.

[91] Kenyon NS, Chatzipetrou M, Masetti M, Ranuncoli A, Oliveira M, Wagner JL, et al. Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. Proc Natl Acad Sci U S A 1999;96:8132–7.

[92] Graca L, Honey K, Adams E, Cobbold SP, Waldmann H. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. J Immunol 2000;165:4783–6.

[93] Zheng XX, Sanchez-Fueyo A, Domenig C, Strom TB. The balance of deletion and regulation in allograft tolerance. Immunol Rev 2003;196:75–84.

[94] Sanchez-Fueyo A, Weber M, Domenig C, Strom TB, Zheng XX. Tracking the immunoregulatory mechanisms active during allograft tolerance. J Immunol 2002;168:2274–81.

[95] Lee I, Wang L, Wells AD, Dorf ME, Ozkaynak E, Hancock WW. Recruitment of Foxp3+ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. J Exp Med 2005;201:1037–44.

[96] Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002;346:1692–8.

[97] Herold KC, Burton JB, Francois F, Poumian-Ruiz E, Glandt M, Bluestone JA. Activation of human T cells by FcR nonbinding anti-CD3 mAb, hOKT3gamma1(Ala-Ala). J Clin Invest 2003;111:409–18.

[98] Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 2005;54:1763–9.

[99] Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. J Immunol 1997;158:2947–54.

[100] Belghith M, Bluestone JA, Barriot S, Megret J, Bach JF, Chatenoud L. TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. Nat Med 2003;9:1202–8.

[101] Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC. TCR stimulation with modified anti-CD3 mAb expands CD8+ T cell population and induces CD8+CD25+ Tregs. J Clin Invest 2005;115:2904–13.

[102] Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity 2000;12:431–40.

[103] Tang Q, Henriksen KJ, Boden EK, Tooley AJ, Ye J, Subudhi SK, et al. Cutting edge: CD28 controls peripheral homeostasis of CD4+CD25+ regulatory T cells. J Immunol 2003;171:3348–52.

[104] Urakami H, Ostanin DV, Hunig T, Grisham MB. Combination of donor-specific blood transfusion with anti-CD28 antibody synergizes to prolong graft survival in rat liver transplantation. Transplant Proc 2006;38:3244–6.

[105] Rodriguez-Palmero M, Franch A, Castell M, Pelegri C, Perez-Cano FJ, Kleinschnitz C, et al. Effective treatment of adjuvant arthritis with a stimulatory CD28-specific monoclonal antibody. J Rheumatol 2006;33:110–8.

[106] Beyersdorf N, Hanke T, Kerkau T, Hunig T. Superagonistic anti-CD28 antibodies: potent activators of regulatory T cells for the therapy of autoimmune diseases. Ann Rheum Dis 2005;64(Suppl 4):iv91–5.

[107] Schmidt J, Elflein K, Stienekemeier M, Rodriguez-Palmero M, Schneider C, Toyka KV, et al. Treatment and prevention of experimental autoimmune

neuritis with superagonistic CD28-specific monoclonal antibodies. J Neuroimmunol 2003;140:143–52.

[108] Lin CH, Hunig T. Efficient expansion of regulatory T cells in vitro and in vivo with a CD28 superagonist. Eur J Immunol 2003;33:626–38.

[109] Grimbert P, Bouguermouh S, Baba N, Nakajima T, Allakhverdi Z, Braun D, et al. Thrombospondin/CD47 interaction: a pathway to generate regulatory T cells from human CD4+ CD25- T cells in response to inflammation. J Immunol 2006;177:3534–41.

[110] Valzasina B, Guiducci C, Dislich H, Killeen N, Weinberg AD, Colombo MP. 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 2005;105:2845–51.

[111] Adams AB, Shirasugi N, Durham MM, Strobert E, Anderson D, Rees P, et al. Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. Diabetes 2002;51:265–70.