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Boosting regulatory T cell function by CD4 stimulation enters the clinic

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Understanding tolerance mechanisms at the cellular and molecular level holds the promise to establish novel immune intervention therapies in patients with allergy or autoimmunity and to prevent transplant rejection. Administration of mAb against the CD4 molecule has been found to be exceptionally well suited for intentional tolerance induction in rodent and non-human primate models as well as in humanized mouse models. Recent evidence demonstrated that regulatory T cells (Treg) are directly activated by non-depleting CD4 ligands and suggests Treg activation as a central mechanism in anti-CD4-mediated tolerance induction. This review summarizes the current knowledge on the role of Treg in peripheral tolerance, addresses the putative mechanisms of Treg-mediated suppression and discusses the clinical potential of harnessing Treg suppressive activity through CD4 stimulation.

Keywords: anti-CD4, cAMP, monoclonal antibody, regulatory T cells, tolerance

REGULATORY T CELLS IN MAINTENANCE OF PERIPHERAL TOLERANCE

The notion of peripheral immune regulation by T cells that shut off other immune cells has been around for many decades (Gershon and Kondo, 1971). Discovery of suitable surface markers (Sakaguchi et al., 1995; Takahashi et al., 1998) and a lineage-specific transcription factor (Hori et al., 2003; Walker et al., 2003) confirmed the existence of a distinct regulatory T cell (Treg) population. Originally identified by their aptitude to hold off autoimmune reactions (Sakaguchi et al., 1995; Takahashi et al., 1998; Wildin et al., 2002; Fontenot et al., 2005), Treg own far-ranging suppressive activity affecting the function, maturation, and survival of all types of immune cells (Thornton and Shevach, 1998; Jonuleit et al., 2001; Grossman et al., 2004; Kojima et al., 2005) in response to self and non-self antigens, including pathogens (Belkaid et al., 2002; Hasenkrug, 2003). Additionally, Treg have been shown to confer regulatory properties upon suppressed T cells implementing a second layer of regulation (Jonuleit et al., 2002; Stassen et al., 2004; Andersson et al., 2008).

Attempts to define the molecular basis of Treg suppression have lead to the description of numerous putative pathways and molecules (Tang and Bluestone, 2008; Shevach, 2009). A majority of studies agreed on cell contact-dependent suppression by Treg *in vitro* (Thornton and Shevach, 1998; Jonuleit et al., 2001) and the observation of persistent contacts between Treg and dendritic cells (DCs) during active suppression by intravital microscopy suggests that cell contact-dependent suppression might also play a role *in vivo* (Tang et al., 2006).

Analyzing the molecular mechanism of contact-dependent Treg suppression by comparison of gene expression in Treg and non-regulatory T cells, we found that Treg up-regulate cAMP in their cytosol upon activation and consign cAMP to conventional $CD4^+$ T cells and DCs (Bopp et al., 2007; Becker et al., 2009; Fassbender et al., 2010) by gap junction intercellular communication (GJIC; Oviedo-Orta et al., 2000). Upon transfer cAMP inhibits the proliferation and differentiation of responder cells, most probably through the induction of *inducible cAMP early repressor* (ICER) expression (Foulkes et al., 1991; Bodor et al., 1996, 2007). Continuative work revealed that cAMP transmission is an essential component of Treg-mediated suppression *in vivo* (Bopp et al., 2007; Becker et al., 2009). Concurrent with stable and persistent Treg–DC interaction (Tang and Bluestone, 2006), transfer of Treg-derived cAMP into conventional T cells *in vivo* was inevitably dependent on the presence of antigen presenting cells (APC) and restricted to the draining lymph node (Bopp et al., 2007). Correspondingly, repression of cAMP accumulation in Treg either by adenylyl cyclase inhibition, application of a cAMP-specific antagonist or phosphodiesterase (PDE) overexpression abrogated Treg suppression (Bopp et al., 2007; Oberle et al., 2007; Becker et al., 2009; Klein et al., 2012; Martin et al., 2012). Inversely, blockade of cAMP degradation by PDE inhibition improved Treg-mediated suppression in a murine asthma model (Bopp et al., 2009).

Regarding cAMP regulation in Treg, Foxp3 has been shown to repress PDE3b expression (Gavin et al., 2006) thereby preventing cAMP degradation. More recently, Huang et al. (2009) showed that the high cAMP content in Treg and their suppressive property depend on Foxp3-mediated repression of the adenylyl cyclase 9 (AC9) regulating miRNA 142-3p. In line with these observations, Lahl et al. (2009) demonstrated that non-functional Treg in Foxp3 mutant scurfy mice harbor significantly reduced levels of cytosolic cAMP. Hence, the transcription factor Foxp3 participates in cAMP buildup by concomitantly regulating the expression of cAMP-generating and degrading enzymes. It is noteworthy that the transmission of cAMP is

actually involved both in the suppression of other T cells (Bopp et al., 2007; Becker et al., 2009; Huang et al., 2009; Klein et al., 2012) and in suppression of DCs (Fassbender et al., 2010). Together these findings classify cAMP as a key component of Treg suppressive mechanism *in vitro* and *in vivo* and disclose cAMP-regulating enzymes as molecular targets for therapeutic intervention with Treg activity in pathological processes like allergy and autoimmunity.

Next to the transfer of cAMP through gap junctions, production of extracellular adenosine has been suggested as an alternative mechanism in cAMP-dependent suppression by Treg (Deaglio et al., 2007). Extracellular nucleotides are anti-inflammatory mediators produced by a variety of cell types including Treg (Deaglio et al., 2007; Mandapathil et al., 2009) and Th17 cells (Chalmin et al., 2012). Physiologically, extracellular nucleotide production represents a protective mechanism in response to tissue injury (Fredholm, 2007). In Treg suppression adenosine formation through the ectoenzymes CD39 and CD73, expressed by murine Treg and a subpopulation of human Treg (Mandapathil et al., 2009), has been assumed to induce cAMP production in conventional T cells or DCs upon binding to the A2A receptor (Deaglio et al., 2007; Ernst et al., 2010). However, the role of adenosine as a major suppressive mechanism employed specifically by Treg is questionable. Blockade of cAMP production in responder T cells by inhibition of adenylyl cyclases does not alter their susceptibility to Treg-mediated suppression (Klein et al., 2012). In addition, A2A receptor expression is detectable on T cells 4 days after stimulation (Deaglio et al., 2007) while T cells are susceptible to Treg suppression exclusively within the first 24 h after stimulation (Hagness et al., 2012). Finally, Blockage of ectonucleotidase activity only slightly abrogates suppression of human T cells by CD39 expressing Treg (Mandapathil et al., 2010). Thus, while nucleotides certainly affect numerous cellular functions – including *de novo* cAMP generation in Treg – their role in Treg suppression is most likely of an indirect nature.

Interestingly, cAMP up-regulation in Treg coincides with another cell contact-dependent mechanism of suppression: Treg constitutively express the two co-inhibitory membrane-bound molecules CTLA-4 and TIGIT (Read et al., 2000; Takahashi et al., 2000) which are believed to provide inhibitory signals. In mice CTLA-4 deficiency (Bachmann et al., 1999), CTLA-4 blockade (Takahashi et al., 2000), and Treg-specific ablation of CTLA-4 (Wing et al., 2008) resulted in spontaneous autoimmunity. Yet, CTLA-4 deficient Treg remain suppressive *in vitro* and *in vivo* (Tang et al., 2004; Read et al., 2006) suggesting additional mechanisms to be involved. Studies on human Treg *in vitro* revealed only a minor role of CTLA-4 in Treg suppression (Birebent et al., 2004) or firmly excluded CTLA-4 as a suppressive mechanism (Baecher-Allan et al., 2001; Jonuleit et al., 2001; Levings et al., 2001). However, discrepancies regarding the importance of CTLA-4 in Treg suppression might in part be due to the use of different target cells. While the role of CTLA-4 in suppression of T cells remains uncertain, it is unequivocally required in the suppression of APC. Suppression of DCs by Treg via CTLA-4 has been shown to induce the downregulation of CD80 and CD86 (Cederbom et al., 2000) preventing effector T cell activation by the APC *in vitro* (Oderup et al., 2006) and *in vivo* (Wing et al., 2008). Notably, elevated cAMP levels in T cells have been shown to increase CTLA-4 expression (Vendetti et al., 2006) and cAMP and CTLA-4 expression are simultaneously up-regulated in Treg upon activation (Becker et al., 2009).

While a majority of studies firmly excluded soluble factors in Treg suppression *in vitro*, there is growing evidence that cytokines substantially add to the immune regulatory function of Treg *in vivo*. In particular, transforming growth factor-β (TGF-β) and IL-10 seem to be indispensable for sustained tolerance induction by Treg. A role for TGF-β in maintenance of peripheral tolerance was initially suggested by its importance in infectious tolerance (Chen et al., 2003) particularly its long-lasting production by $CD4^+$ T cells from tolerant mice in long-term acceptance of allografts (Daley et al., 2007). However, in order to exert its biological functions, TGF-β needs to be converted from its latent (bound to latency associated peptide, LAP) into its active conformation by proteolytic cleavage (Khalil, 1999). Yet, there are multiple mechanisms of activating TGF-β from its latency (Lawrence, 2001; Annes et al., 2003) and it is unclear how TGF-β is activated *in vivo*.

Although repeatedly observed in disease models (Nakamura et al., 2001) a direct contribution of TGF-β in Treg suppression remained controversial because anti-TGF-β antibodies and soluble TGF-RII failed to affect the suppressive function of Treg (Andersson et al., 2008). Recently, "glycoprotein A repetitions predominant" (GARP) expressed on the surface of Treg upon activation (Wang et al., 2008, 2009; D'Alise et al., 2011) has been shown to act as a receptor for the TGF-β/LAP complex (Stockis et al., 2009). Reminiscent of infectious Treg suppression (Jonuleit et al., 2002; Stassen et al., 2004) latent TGF-β bound to GARP on the surface of activated Treg has been demonstrated to convert responder T cells into induced Treg (Andersson et al., 2008). Thus, apart from acting as a soluble modulator of immune cells, TGF-β supposedly helps Treg to execute their contact-dependent suppressive activity by binding to GARP (Battaglia and Roncarolo, 2009).

IL-10 has been unequivocally shown to form another important mediator in Treg suppression *in vivo* (Kearley et al., 2005; Collison et al., 2007) particularly in suppression of pathogenic Th17 cells (Chaudhry et al., 2011; Huber et al., 2011). Correspondingly, Treg-specific ablation of IL-10 leads to inflammation (Rubtsov et al., 2008). In contrast to general Treg deficiency, however, Treg-specific IL-10 paucity leads to mucosal but not systemic autoimmunity, suggesting mucosal restriction of IL-10-mediated Treg tolerance induction. This view is supported by our previous observation that human Treg expressing gut-homing β7 integrin preferentially induce IL-10 production in converted secondary T helper suppressor cells (Stassen et al., 2004).

Due to their far-ranging tolerizing capability Treg have become key targets in the development of tolerance-inducing therapies (Wing and Sakaguchi, 2010). Like other T cells, Treg require activation for their function. Attempts to exploit Treg for therapeutic purposes therefore depend on Treg activation, either by antigen or polyclonal stimulation (Jordan et al., 2001). Current efforts to increase the frequency and potency of Treg *in vivo* include the use of cytokines (Tawara et al., 2010), antigen targeting to immature DC (Mahnke et al., 2003), and monoclonal antibodies (mAb) against surface molecules (Belghith et al., 2003). As a whole population Treg are biased toward recognition of self-antigens

(Hsieh et al., 2004), however, because antigenic specificities of Treg in diseases have not been elucidated, potential clinical applications have mainly focused on polyclonal Treg activation methods (Horwitz et al., 2004).

CORECEPTOR ENGAGEMENT AND PERIPHERAL TOLERANCE

T cell surface molecules that participate in T cell receptor-mediated stimulation have a significant influence on T cell function. mAb against coreceptors have been successfully shown to allow intentional tolerance induction in rodent and non-human primate models (Krieger et al., 1996). One particularly well-established regimen of tolerance induction is the administration of anti-CD4 mAb (Waldmann and Cobbold, 1998). Although the mechanisms underlying tolerization by anti-CD4 mAb are not yet fully understood, the activation of Treg has been recognized as the entering wedge to successful tolerance induction (Becker et al., 2009; Kendal et al., 2011; Martin et al., 2012).

CD4, a 55-kDa glycoprotein with four extracellular domains (Littman, 1987), recruits the protein kinase $p56^{lck}$ (Rudd et al., 1988; Veillette et al., 1988) to the TCR complex (Holdorf et al., 2002; Kim et al., 2003; Nika et al., 2010) and strengthens the contact between T cells and APCs through its interaction with non-polymorphic regions of MHC class II molecules (Greenstein et al., 1984; Doyle and Strominger, 1987; Konig et al., 1992, 1995). CD4 molecules on T cell surface have been shown to preferentially form disulfide-linked dimers and tetramers (110 and 220 kDa; Li et al., 1998; Moldovan et al., 2002) and mutations disabling dimerization completely abrogate its coreceptor function (Vignali and Vignali, 1999). CD4 expression on mature T cells is uniform with the exception of polarized T helper 2 cells (Itoh et al., 2005) and Treg (Bryl et al., 2001) which both show decreased CD4 expression supposedly entailing altered proximal TCR signaling (Hannier et al., 2002; Itoh et al., 2005; Tsang et al., 2006).

Through its interaction with tyrosine kinase $p56^{lck}$, CD4 engagement alone can induce TCR-independent signaling events in T cells (Zhou and Konig, 2003). Selective engagement of the CD4 coreceptor by certain mAb raises intracellular calcium and IL-2 production (Carrel et al., 1991), whereas other anti-CD4 mAb prime T helper cells to activation-dependent cell death triggered by subsequent TCR/CD3-mediated signals (Newell et al., 1990; Tamma et al., 1997). Comparing mAb against different CD4 epitopes, Baldari and colleagues suggested that the gene-activating and proapoptotic potential of different anti-CD4 mAb may be associated with different epitopes (Baldari et al., 1995; Di Somma et al., 1995; Milia et al., 1997). However, a similar range of divergent responses can be induced through a single CD4 epitope as demonstrated for the CD4-binding (Lasky et al., 1987) human immunodeficiency virus-1 (HIV-1) envelope protein gp120 (Liegler and Stites, 1994; Westendorp et al., 1995; Masci et al., 1999). It is therefore tempting to speculate that the functional outcome of CD4-stimulation might mainly depend on the functional state of the T cell addressed rather than on a specific CD4 epitope. However, the functional state is believed to affect the formation of CD4 oligomers, which, in turn, regulate the activation of the CD4 cytoplasmic tail-associated tyrosine kinase $p56^{lck}$, by *trans*-phosphorylation (Veillette et al., 1989).

Even before the role of the CD4 molecule in T cell activation had been fully recognized, three groups reported that short courses of anti-CD4 mAb application induce long-term tolerance to foreign proteins (Benjamin and Waldmann, 1986; Benjamin et al., 1986; Goronzy et al., 1986; Gutstein et al., 1986). Subsequent studies revealed that anti-CD4-mediated tolerance induction was not based on T cell depletion but rather an activation of regulatory mechanisms (Benjamin et al., 1988; Carteron et al., 1988, 1989; Qin et al., 1990). Further, tolerance could not only be induced to foreign proteins but also to various transplanted allografts (Shizuru et al., 1987; Qin et al., 1989; Davies et al., 1996), demonstrating that the tolerizing potential of anti-CD4 mAb is not restricted to a particular type of antigen. Immunoregulatory mechanisms initially suggested to operate in anti-CD4 induced tolerance include a predisposure of developing T cells to selective deletion, or anergy in the thymus (Arima et al., 1997); immune deviation (Scully et al., 1997); receptor blockade (Fehervari et al., 2002; Harding et al., 2002); modulation of CD4 expression (Portoles et al., 1999); and transmission of negative signals (Chirmule et al., 1999). However, none of these – not mutually exclusive – processes could reasonably explain the "infectious tolerance" phenomenon (Qin et al., 1993). Rather than being submissive, anti-CD4 induced tolerance relied on dominant immune suppression by T cells activated in presence of the antibody. In regard to the dominant suppressive T cell type in charge several functionally and phenotypically different anti-CD4 mAb-induced tolerogenic $CD4^+$ T cell populations have been proposed (Bushell et al., 2003; Chen et al., 2003; Cobbold et al., 2004; Karim et al., 2005). However, whether these had been directly or indirectly induced by anti-CD4 treatment remained undefined at first. The impressive capacity of Treg and their ability to confer regulatory properties upon suppressed T cells (Jonuleit et al., 2002; Stassen et al., 2004; Andersson et al., 2008) in particular, strongly suggested a role of Treg in anti-CD4-mediated "infectious tolerance" induction. In support of this assumption administration of non-depleting anti-CD4 mAb into mice had been shown to result in pre-activation of Treg *in vivo* (Karim et al., 2005; Yang et al., 2007). Eventually, using B6. Foxp3(hCD2) mice to ablate Treg with an anti-hCD2 mAb Kendal et al. (2011) formally demonstrated that Treg are crucial for infectious tolerance induced by non-ablative anti-T cell mAb.

Motivated by the description of activated Treg in murine anti-CD4 tolerance models we previously analyzed the effect of CD4 binding agents on human Treg. Comparing numerous anti-CD4 mAb we found that certain anti-CD4 mAb have the potential to induce the suppressive function of isolated human Treg in a supposedly T cell receptor-independent manner (Becker et al., 2007). In addition, we and others observed that the CD4-binding HIV-1 surface protein gp120 activates the suppressive function of Treg (Nilsson et al., 2006; Kinter et al., 2007) *in vitro* and in two humanized mouse models *in vivo* (Becker et al., 2009; Ji and Cloyd, 2009) signifying that stimulation via the CD4 receptor represents an efficient Treg activating pathway with potential to induce immunological tolerance in humans.

Difference between anti-CD4 mAb to trigger Treg suppressive activity could not be related to a particular CD4 epitopes. However, comparing the Treg activating potential of different anti-CD4

mAb and CD4 binding virus envelopes we observed that one crucial event that separates Treg activating and non-activating CD4 ligands consists in up-regulation of the second messenger cAMP (Becker et al., 2009 and unpublished results). Moreover, the binding affinity of CD4 ligands seems to play a role as suggested by the fact that weak CD4 binding viral envelopes from HIV-2 (gp105) and SIV (gp130) did not activate human Treg *in vitro* and *in vivo*.

However, apart from these general observations the signaling events initiated by separate ligation of CD4 on Treg so far remain unexplored. In particular, it is unclear whether CD4 stimulation of Treg is truly independent of TCR signals, whether and how both pathways resemble or differ from another, and, most important, whether CD4-mediated signals are differently or similarly handled in Treg and conventional $CD4^+$ T effector cells. The latter question is of particular interest since Treg are believed to maintain an activated phenotype through constant stimulation by self antigens, yet, require additional stimulation to become suppressive. Future insights into how TCR and CD4 signaling pathways drive the suppressive activity of Treg will undoubtedly help to understand Treg biology and discover alternative intervention points for functional manipulation of Treg suppressive activity.

As summarized in **Figure 1** at least three different immune mechanisms can be distinguished that help to explain the tolerizing effect of CD4-specific agents: First, a general Treg-independent mechanism that consist in interference with proper CD4 coreceptor function resulting in induction of T cell anergy or T cell depletion (**Figure 1A**). This effect seems to depend either on CD4/MHC class II binding blockade or additional TCR-independent signaling. Second, by modulating antigenic stimulation, individual CD4 mAb induce differentiation of naive T cells into adaptive Tregs (Oliveira et al., 2008), which are suggested to control pathogenic effectors through TGF-β (Oliveira et al., 2011) or IL-10 release (**Figure 1B**). Finally, and crucially important for tolerance induction, CD4-specific mAb activate the suppressive function of Treg (Becker et al., 2007; Kendal et al., 2011), which, upon activation, exert control on pathogenic T cells

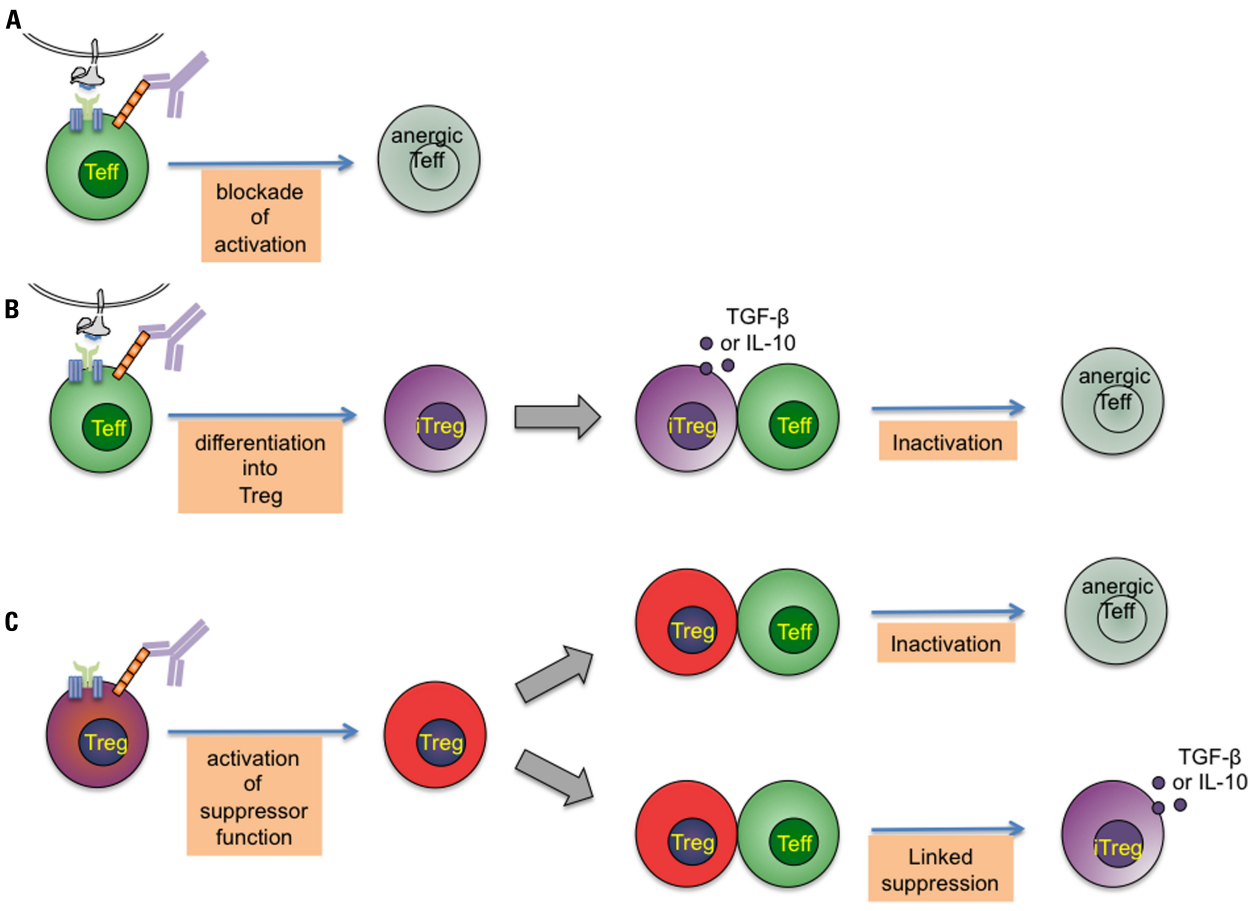

FIGURE 1 | Potential modes of tolerance induction by CD4-specific monoclonal antibodies. A short-term treatment with non-depleting CD4-specific mAb induces dominant tolerance to foreign proteins and transplanted allografts. This figure represents the different immune mechanisms that have been proposed to explain the tolerizing effect of CD4-specific mAb. Three intervening points can be distinguished: **(A)** CD4 binding by non-depleting CD4-specific mAb modulates antigenic stimulation through the T-cell receptor complex resulting in induction of T cell anergy. **(B)** By modulating antigenic stimulation, CD4-specific mAb induce differentiation of naive T cells into adaptive regulatory T cells, which control pathogenic effectors through transforming growth factor-β (TGF-β) and IL-10 release. **(C)** Crucially important for tolerance induction, CD4-specific mAb activate the (cAMP-dependent) suppressive function of Treg, which, upon activation, exert control on pathogenic T cells by direct and linked suppression.

by direct and linked suppression (**Figure 1C**). These different effects of CD4 stimulation are intrinsic functions of individual anti-CD4 mAb.

CLINICAL APPROACHES TO Treg-MEDIATED TOLERANCE INDUCTION

Current immunosuppressive therapies are efficient in preventing acute transplant rejection and dampening inflammation in autoimmune diseases such as rheumatoid arthritis or lupus. Nevertheless, immune suppression remains inadequate, as it comprises significant side effects such as organ toxicity and hypersuppression disabling protective immune responses against pathogens and enhancing the risk of chronic infections. Hence, there is a clinical need for novel immunotherapeutic drugs with the ability to rebalance the immunologic tolerance network without persistently affecting immune function. In contrast to pharmacological immune suppression, re-induction of tolerance through the exploitation of evolutionarily established tolerance mechanisms is expected to offer a parentally operative cure. Among mechanisms operative in self-tolerance, the immune-suppressive activity of Treg appears to be exceptionally well suited for therapeutic exploitation for several reasons: First, activated Treg dampen the function of a wide range of immune cells including T cells (Pandiyan et al., 2007), B cells (Lim et al., 2005), DC (Misra et al., 2004; Larmonier et al., 2007), and monocytes (Taams et al., 2005) and affect a broad range of immune contexts including cardiovascular disease (Ait-Oufella et al., 2006) and obesity-induced insulin resistance (Feuerer et al., 2009). Second, the activation of Treg is antigen-specific defined by the selected T cell receptor repertoire in the thymus. However, once activated the suppressive mechanisms of Treg operate in an antigen-non-specific manner, sidestepping the need to identify disease-specific antigens to affect a particular Treg population. Prime examples of the Treg immune dampening potential are experiments demonstrating that Treg can be expanded and re-infused to limit immune responses (Hoffmann et al., 2002) preventing GvHD induction without causing toxicity. While persistent polyclonal Treg activation would lead to general immune hyporesponsiveness, a short-term Treg activation – as established for tolerance induction with non-depleting anti-CD4 mAb in mice – is expected to induce (or re-induce) antigen-specific regulatory networks that maintain antigen-specific tolerance when Treg activity has returned to normal levels.

Based on the evidence for Treg activation by CD4 ligands as outlined above, anti-CD4 mAb seem to represent ideal compounds for Treg-mediated tolerance induction. However, although animal studies have provided a compelling basis for clinical application of anti-CD4-mediated tolerance induction, this approach has been remarkably unsuccessful when transferred to the clinic. Although short interventions with particular mAb have been shown to offer quick symptomatic relief, improvements supposedly caused by inactivation and depletion of $CD4^+$ T cells (Kon et al., 2001; Choy et al., 2002) remained transient. Failure to establish an anti-CD4-based tolerogenic therapy in humans is most likely due to difficulties in translating the timing and dosage used in animal models for human application. Importantly, in contrast to animal models, mAb are administrated at late disease stages in clinical studies. Whereas the immature immune system seems to dependably allow tolerance induction with anti-CD4 mAb, it seems more difficult to tolerize the experienced immune system in patients, in part due to the presence of effector and memory T cells resistant to the suppressive action of Treg (Yang et al., 2007). In fact, Treg-based therapies have been found to be generally less effective in models of autoimmune diseases. Wehrens et al. (2011) for example observed that functionally active Treg failed to control hyperactivated T effector cells in rheumatoid arthritis patients with ongoing inflammation but prevented autoaggressive immune responses in non-inflammatory arthritis. Impaired Treg suppression under inflammatory conditions has been mainly ascribed to the influence of TNF-α, IL-1, and IL-6, which turn effector T cells resistant to Treg-mediated suppression (Walker, 2009; Goodman et al., 2011). Certainly, resistance to Treg-mediated suppression can be overcome by blockade of IL-6 (Chen et al., 2009) and supposedly, the beneficial effects of anti-TNF-α treatment include a similar effect too (Ehrenstein et al., 2004; Valencia et al., 2006). Thus, provided Treg can be sufficiently activated in the host, their suppressive efficiency might depend on the disease stage, which strongly argues for a combination of Treg enhancing strategies with biologicals that reverse Treg resistance in autoaggressive T effector cells. As exemplified with anti-CD3 mAb already in the clinic evacuation of T effectors cells and concomitant enhancement of Treg activity can form a very effective treatment (Chatenoud and Bluestone, 2007).

With regard to anti-CD4-mediated tolerance induction in humans, it is important to emphasize again that anti-CD4 mAb vary in their capacity to activate Treg (Becker et al., 2007) and antibodies used in clinical trials so far have not been analyzed with regard to their Treg activating potential. However, clinical trials with Treg enhancing agents such as the anti-CD4 mAb Tregalizumab in rheumatoid arthritis have been initiated to investigate the efficacy of Treg-based anti-CD4-mediated tolerance induction in patients with autoimmune diseases.

CONCLUDING REMARKS

In summary, polyclonal activation of Treg through their surface molecules by biologicals that enhance their intracellular cAMP level are effective to induce the suppressive function of Treg for re-induction of tolerance in small animal models and in humanized mice. It is therefore expected that polyclonal Treg activation forms a rational for tolerance induction in humans. However, both the exact conditions, efficiency in different stages of disease and cooperation with additional treatment regiments to diminish T effector cells need to be thoroughly explored. Moreover differential signals in Treg versus T effector cells are far from being clear. In addition to deepening our understanding of Treg biology investigation of the latter holds the key to define alternative entry points for therapeutic manipulation of Treg function.

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