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Regulatory T Cells and Human Disease

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Abstract

Naturally occurring CD4^{+} regulatory T cells (Tregs), which specifically express the transcription factor FoxP3 in the nucleus and CD25 and CTLA-4 on the cell surface, are a functionally distinct T cell subpopulation actively engaged in the maintenance of immunological self-tolerance and homeostasis. Recent studies have facilitated our understanding of the cellular and molecular basis of their generation, function, phenotypic and functional stability, and adaptability. It is under investigation in humans how functional or numerical Treg anomalies, whether genetically determined or environmentally induced, contribute to immunological diseases such as autoimmune diseases. Also being addressed is how Tregs can be targeted to control physiological and pathological immune responses, for example, by depleting them to enhance tumor immunity or by expanding them to treat immunological diseases. This review discusses our current understanding of Treg immunobiology in normal and disease states, with a perspective on the realization of Treg-targeting therapies in the clinic.

INTRODUCTION

Naturally occurring CD4^{+} regulatory T cells (Tregs) are an indispensable constituent of the normal immune system for the maintenance of immunological self-tolerance and homeostasis (reviewed in 1–3). Following several findings in the 1970s and 1980s suggesting the existence of T cells capable of suppressing experimental autoimmune diseases in rodents, efforts were made to characterize such autoimmune-suppressive CD4^{+} T cells by reliable molecular markers (reviewed in 1). These efforts culminated in the discovery in the mid-1990s that Tregs constitutively and highly expressed CD25 (IL-2 receptor α chain) (4). Removal of CD25^{+}CD4^{+} T cells, which constituted ∼10% of peripheral CD4^{+} T cells, indeed produced a wide spectrum of autoimmune diseases immunologically similar to human counterparts such as autoimmune gastritis, thyroiditis, and type 1 diabetes (T1D) in otherwise normal mice; reconstitution of CD25^{+}CD4^{+} T cells inhibited disease development. This molecular delineation of natural Tregs revealed that the majority of them were produced by the thymus as a functionally and phenotypically distinct T cell subpopulation (5). The phenotypic characterization has facilitated the elucidation of their function in vivo and in vitro and their roles in antitumor, antimicrobial, and transplantation immunity in animal models, and enabled in the early 2000s the identification of human CD25^{+}CD4^{+} Tregs with an equivalent function and phenotype to those in mice (6–12). Subsequently in 2003, CD25^{+}CD4^{+} natural Tregs in rodents and humans were found to specifically express the transcription factor FoxP3[1] (13–15). Similar to the case of FoxP3-mutant Scurfy mice, which spontaneously succumb to fatal systemic autoimmune/inflammatory disease (16), loss-of-function mutations of the human FOXP3 gene cause severe autoimmune/inflammatory diseases (such as T1D and thyroiditis), allergy, and inflammatory bowel disease (IBD), which together constitute immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, due to deficiency or dysfunction of CD25^{+}CD4^{+} natural Tregs (17; reviewed in 18). Thus, these findings obtained in the past 50 years have firmly established that natural Tregs specifically expressing FOXP3 in the nucleus and CD25 on the cell surface are present in normal individuals and are actively engaged in suppressing aberrant or excessive immune responses against self, microbial, and environmental antigens. Their anomalies can indeed be causative of various immunological diseases such as autoimmune disease, allergy, and immunopathology (19–21). Besides these classical immunological diseases, FoxP3^{+} natural Tregs have been shown to play key roles in a much broader spectrum of diseases and biological conditions, e.g., fetal-maternal tolerance, immunometabolic diseases (such as obesity and atherosclerosis), degenerative diseases with immunological/inflammatory elements, and tissue regeneration (22–28) (**Figure 1**).

Recent years have also witnessed various endeavors to target Tregs to control physiological and pathological immune responses in clinical settings. Treg-up strategies (the expansion of antigen-specific or polyclonal FOXP3^{+} Tregs and strengthening of their suppressive activity) can be effective for treatment of autoimmune and other inflammatory diseases or suppressing undesirable immune responses such as rejection of transplanted organs. On the other hand, Treg-down strategies (reduction of Tregs or their suppressive activity) can be instrumental in evoking antitumor immune responses or enhancing antimicrobial immunity in chronic infection. Biological reagents such as monoclonal antibodies and cytokines and chemical reagents such as inhibitors of cell signaling are envisioned to achieve the aims of these strategies by exploiting immunobiological characteristics allowing differential control of Tregs and conventional T cells (Tconvs).

With these perspectives, this review first briefly considers the cellular and molecular basis of the development and function of FoxP3^{+} Tregs and their functional heterogeneity and adaptability,

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[1]In this review, FoxP3 is used to designate the transcription factor either in rodents alone or when rodents and humans are referred to collectively, and FOXP3 to indicate the transcription factor solely in humans.
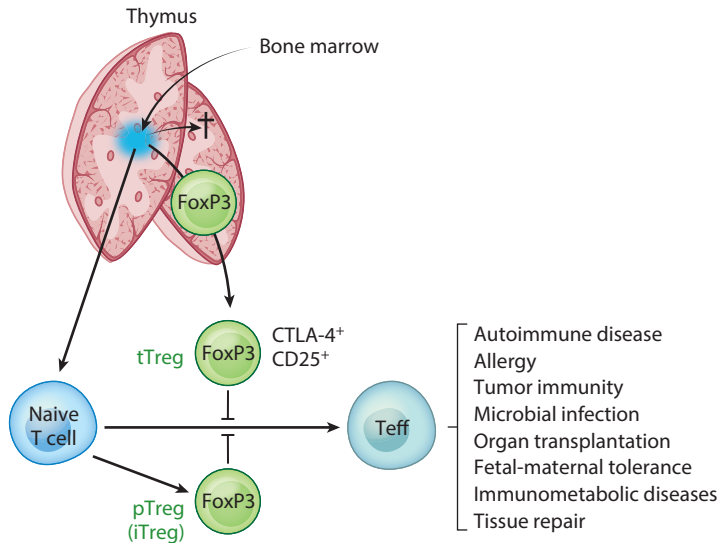
Figure 1

Control of immune responses by FoxP3$^+$ Tregs. The majority of FoxP3$^+$ Tregs in the immune system are produced by the thymus as a functionally distinct and mature population (tTregs). Some T cells differentiate into Tregs in the periphery, especially in the intestinal mucosa (pTregs). Tregs can also be induced in vitro from conventional T cells (iTregs). FoxP3$^+$ Tregs constitutively express CD25 and CTLA-4 on the cell surface at high levels. Numerical or functional anomalies can be causative of a variety of immunological diseases. On the other hand, their expansion or reduction, functional augmentation or attenuation, is able to control physiological and pathological immune responses. The cross indicates death of thymocytes. Abbreviations: iTreg, in vitro–induced Treg; pTreg, peripherally derived Treg; Teff, effector T cell; Treg, regulatory T cell; tTreg, thymus-derived Treg.

in particular, how functional Tregs can be delineated from Tconvs by cell surface, intracellular, or genomic markers. It addresses recent advances in our understanding of the function and phenotype of human Tregs in healthy and disease states, in particular, in autoimmune disease and cancer. It also discusses how to control pathological and physiological immune responses by Treg-up or Treg-down strategies.

DEVELOPMENT AND FUNCTION OF TREGS

Thymus-derived Tregs (tTregs) constitute the majority of FoxP3$^+$ Tregs in the periphery, while some Tconvs in peripheral sites such as the intestinal mucosa gain stable FoxP3 expression and differentiate into peripherally derived Tregs (pTregs) (29). Tconvs can also differentiate in vitro to express FoxP3 under special conditions, forming in vitro–induced Tregs (iTregs).

Development of Thymic Tregs and Their Cell Lineage Stability

The cardinal features of tTregs are that they are not only positively selected by thymic self-peptide/MHC but also driven to differentiate into functionally competent antigen-specific suppressive T cells within the thymus. They are in an antigen-primed or -activated state already in the thymus where only self-peptides are presented, as exemplified by their expression of CD25, CTLA-4, and CD5 (5). It has been indicated by various experiments that a developing T cell with an intermediate-affinity T cell receptor (TCR) for a self-peptide/MHC is driven to the Treg

cell lineage, while a T cell with a high-affinity TCR for the ligand is deleted, and one with a low-affinity TCR differentiates into a naive T cell (reviewed in 30). The events generate a Treg TCR repertoire more strongly skewed in toto toward recognizing thymic self-peptide/MHC ligands than Tconvs selected by the same ligands. It remains to be determined, however, whether a certain range of TCR affinity is sufficient to drive developing T cells into Tregs with higher self-reactivity or, presuming that a TCR signal is an absolute requirement for determining Treg fate (see below), such developing Tregs are subjected to further selection toward higher self-reactivity, for example, by Treg-specific TCR signal alteration (31).

In the periphery, the antigen-primed state of Tregs, together with their self-skewed TCR repertoire, enables their activation and exertion of suppression at a much lower ($\sim$10- to 100-fold) concentration of the peptide/MHC required for activating naive Tconvs recognizing the same peptide/MHC (32). This antigen-sensitive and swift activation of Tregs ensures Treg-dependent dominant self-tolerance upon exposure to self-antigens released from damaged tissues or microbial antigens cross-reactive with self-antigens (33).

In the course of tTreg development, a large portion of Treg-specific enhancers, in particular Treg-specific super-enhancers associated with many Treg signature genes such as *Foxp3*, *Il2ra* (*Cd25*), *Ctla4*, *Ikzf2* (*Helios*), and *Ifzf4* (*Eos*), become gradually activated in parallel at each gene locus at the precursor stage before FoxP3 expression (34). One factor essential in this Treg-specific super-enhancer activation is the genome organizer Satb1 (special AT-rich sequence-binding protein 1), which binds to specific genomic sites from the CD4$^+$CD8$^+$ thymocyte stage to open up the chromatin and activate super-enhancers. The absence of thymic Tregs in T cell–specific Satb1-deficient mice and failure of Satb1-deficient CD25$^+$FoxP3$^-$CD4SP Treg precursors to differentiate into FoxP3$^+$ Tregs in vitro suggest that Satb1-dependent super-enhancer activation is critical for tTreg development. Consistently, Satb1 and MLL4 (myeloid/lymphoid or mixed-lineage leukemia 4), an enzyme involved in enhancer priming, commonly occupy the newly identified conserved enhancer region, designated conserved noncoding sequence 0 (CNS0), at the *Foxp3* locus, with subsequent activation of the enhancers at CNS3 and CNS2, and then the promoter (34, 35). These results support the notion that the TCR signal, depending on its intensity and/or duration, initiates the formation of the specific enhancer landscape at an early stage of thymocyte development, leading to the expression of FoxP3 and other Treg signature genes as well as the induction of Treg-specific DNA demethylation (36). A pioneer factor together with Satb1 may bind to the genomic sites that will develop into super-enhancers of Treg signature genes, priming their expression (34).

Once FoxP3$^+$ Tregs have migrated to the periphery, they are functionally stable as well illustrated by the long-term inhibition of autoimmune disease development in various animal models by adoptive transfer of Tregs (1) and by the occurrence of autoimmune diseases following Treg depletion at any time of life (4, 37, 38). FoxP3$^+$ Tregs, especially effector type Tregs, are continuously in a highly proliferative state in the normal physiological state, presumably due to recognizing self-antigens and antigens derived from commensal microbes (39, 40). These findings, together with the lineage continuity of tTregs from the thymus to the periphery, indicate that epigenetic mechanisms, in particular, DNA methylation/demethylation, which is heritable through cell divisions, play an essential role for stable maintenance of Treg-specific gene expression. Natural Tregs (i.e., tTregs and pTregs) indeed possess Treg-specific hypomethylated regions in the genome, first identified within the *Foxp3* CNS2 region as the Treg-specific demethylated region (TSDR) (41). Such Treg-specific DNA hypomethylation is not limited to the *Foxp3* locus and is found in other Treg signature genes, such as *Il2ra* (*Cd25*), *Ctla4*, *Helios*, and *Eos*, collectively designated Treg-specific demethylated regions (Treg-DRs) (42). The establishment of Treg-specific demethylation is FoxP3 independent as illustrated by FoxP3-deficient Scurfy mice,

which exhibit Treg-specific DNA hypomethylation at *Foxp3* and other Treg signature gene loci in FoxP3⁻CD25⁺CD4⁺ T cells (42, 43). Supporting this notion in humans, FOXP3⁻CD25⁺CD4⁺ T cells from IPEX patients possess demethylation of *FOXP3* CNS2 at normal or even higher levels compared with healthy controls (44). Murine FoxP3-deficient tTregs can indeed acquire Treg-specific demethylation and express Treg signature genes, albeit at slightly lesser levels of gene expression than FoxP3-intact Tregs, in the course of their thymic development (34, 42, 45). Notably, Treg-DRs [∼300 regions assessed by methylated DNA immunoprecipitation (MeDIP) sequencing] and FoxP3-binding sites [∼2,900 sites by chromatin immunoprecipitation (ChIP) sequencing] are not overlapping in the genome, except *Foxp3* CNS2 (32, 46, 47). Treg-DRs were closely associated with Treg upregulated genes such as *Ikzf2*, *Ikzf4*, *Cd25*, and *Ctla4* in a steady state, whereas FoxP3-binding regions had no significant correlation with either up- or downregulated genes in nonactivated Tregs (48). In activated Tregs, however, FoxP3-binding regions showed a strong correlation with downregulated genes such as *Il2*, *Ifng*, and *Zap70*, which notably do not possess Treg-DRs (42, 48). Collectively, Treg-specific DNA hypomethylation, which allows binding of other transcription factors (such as Ets1 and CREB) to Treg-DRs, operates as an enhancer for gene upregulation in steady-state Tregs. On the other hand, FoxP3 is a strong repressor of certain genes such as *Il2*, *Ifng*, and *Zap70* in activated Tregs. It may also be an activator to enhance the expression of Treg-DR-dependent genes by binding to their promoters (together with other transcription factors such as Runx1/CBFβ and Eos) upon Treg activation (49). Posttranslational modification of the FoxP3 protein may contribute to such an activation-dependent gene-control activity of FoxP3 (reviewed in 50). The Treg-specific DNA demethylation pattern similarly occurs in pTregs, which are mainly found in intestinal mucosal tissues and also induced from some Tconvs by homeostatic expansion in a T cell–deficient environment (42). It is, however, not induced in iTregs, which consequently show unstable FoxP3 expression (41, 42).

Thus, a Treg-specific transcription factor network involving FoxP3 and other transcription factors as well as a Treg-specific epigenetic landscape coordinately up- or downregulate Tregspecific gene expression, thereby maintaining Treg functional activity and stability. It is also of note that, despite the expression of common proteins such as CD25 and CTLA-4 by Tregs and activated Tconvs, the epigenomic changes, in particular the Treg-specific CpG hypomethylation pattern, enable clear differentiation between Tregs and activated Tconvs at the genomic level, for example, at the *Cd25* or *Ctla4* locus (42, 51). The Treg-specific epigenomic pattern is therefore instrumental in defining functionally stable Tregs, especially when FoxP3 expression is induced or lost transiently (see below).

Treg-Mediated in vivo and in vitro Suppression

Multiple mechanisms have been proposed for Treg-mediated suppression, including cell contact-dependent and humoral factor-mediated ones with involvement of a wide range of molecules, e.g., cell surface molecules (CTLA-4, CD25, TIGIT, CD39, and CD73), cytokines (IL-2, IL-10, TGF-β, and IL-35), and secreted or intracellular molecules (granzyme, cyclic AMP, and IDO) (reviewed in 52, 53). Importantly, ectopic expression of FoxP3 is able to confer Treg-like in vivo and in vitro suppressive activity on Tconvs, indicating that FoxP3 controls the expression of key molecules mediating suppression (13, 15). For weighing the contribution of each mechanism or molecule to Treg-mediated suppression, it can therefore be asked which molecule(s) is directly or indirectly, positively or negatively, controlled by FoxP3 in its expression; whether its deficiency or overexpression is able to cause Scurfy-like autoimmune/inflammatory disease by impairing in vivo suppressive activity; whether reconstitution of the deficiency or overexpression with normal Tregs can prevent disease development; and further, whether Treg-like in vivo and in

vitro suppressive activity can be constructed in Tconvs by deleting or expressing the molecules(s). In this regard, it is known that deficiency of CD25, IL-2, or CTLA-4 causes severe autoimmune/inflammatory diseases similar to Scurfy disease and that normal Tregs are able to suppress the disease development (reviewed in 54, 55).

Since FoxP3^{+} Tregs, especially upon activation, scarcely produce IL-2 because of repression of the *Il2* gene by FoxP3, they are highly dependent on exogenous IL-2 for their survival (56, 57). Tregs are also able to absorb IL-2 as a cytokine sink by their constitutively expressed high-affinity IL-2 receptor, which deprives responder Tconvs of IL-2 and thereby contributes to suppression of their expansion and differentiation into effector T cells, at least, in vitro (58, 59). Although the role of CD25 for suppression by the IL-2 sink is controversial (60), the effectiveness of the mechanism depends in part on the responder T cells. CD8^{+} T cells, in particular self-reactive CD8^{+} T cells, can be driven to anergy and apoptosis in vitro by deprivation of IL-2 and costimulatory signal (see below) (59, 61). IL-2 deprivation can also be effective in suppressing NK cells and hampering secondary expansion of CD8^{+} memory T cells (62, 63). In addition, Treg expression of the high-affinity IL-2 receptor renders Tregs able to quickly sense IL-2 produced by self-reactive Tconvs in the early stages of an immune response and colocalize to suppress their further activation in a dominant manner (64, 65). Further, in active immune responses, IL-2 produced by activated T cells expands Tregs, stabilizes FoxP3 expression, and enhances their suppressive activity in a negative-feedback manner (66).

Treg-specific CTLA-4 deficiency from early life or in adulthood is able to produce autoimmune/inflammatory disease, although the disease is milder in the latter (67–69). As a key mechanism of CTLA-4-mediated suppression, CTLA-4 expressed by Tregs downregulates the expression of CD80 and CD86 by antigen-presenting cells (APCs) such as dendritic cells (DCs) and B cells, thereby depriving responder Tconvs of CD28 costimulatory signals (67, 70–72). Treg CTLA-4 not only competes with CD28 for CD80/CD86 binding but also appears to deplete CD80/CD86 molecules from the cell surface of APCs by transendocytosis (73). Tregs in the steady or activated state may additionally produce soluble CTLA-4, which mediates immune suppression by blocking CD80/CD86 on APCs.

The Treg-dependent blockade of costimulatory signaling to TCR-stimulated CD4^{+} or CD8^{+} Tconvs, together with presumable IL-2 deprivation, can determine the cell fate of the responder T cells in in vitro suppression, depending on the degree of the reduction of costimulatory signal and TCR affinity of the responder T cells. Human self-reactive CD8^{+} T cells with relatively high-affinity TCRs for a self-antigen peptide die by apoptosis when stimulated by the peptide in the presence of natural Tregs downregulating CD80/CD86 expression by APCs; those with intermediate affinities are driven to anergy, while low-TCR-affinity T cells survive and stay dormant (61). Such anergic Tconvs produced in vitro in the presence of Tregs are phenotypically distinct in that they are naive (e.g., CD45RA^{high} and CCR7^{+}) but CTLA-4^{high}. They are also Bcl2^{low}, suggesting their propensity to die by apoptosis later (61). Notably, a substantial proportion of anergic self-reactive T cells with a similar phenotype are present in healthy individuals (61). These results collectively indicate that Treg-mediated suppression is not a whack-a-mole type game of momentary suppression but is instead able to determine the cell fate and survival of responder T cells, especially weakly self-reactive, hence suppression-sensitive T cells, and thereby establish long-term suppression and stable tolerance.

Based on these findings on IL-2/CD25 and CTLA-4, an attempt was made to reconstruct Treg suppressive activity in Tconvs. Abrogation of IL-2 production, constitutive high expression of CTLA-4, and TCR stimulation (which upregulates CD25 and adhesion molecules) together were indeed able to confer significant Treg-like suppressive activity on Tconvs in vivo and in vitro (58). This provides a minimalistic model of suppression in which a combination of molecular

events, not a single one, is able to confer suppressive activity. What is unique in FoxP3$^+$ Tregs and distinct from activated Tconvs is that FoxP3-dependent and Treg-epigenome-dependent control of gene expression robustly induces and maintains IL-2 nonproduction, high CTLA-4 expression, and a TCR-dependent, antigen-primed state.

Assuming the presence of such a core mechanism of suppression that is effective, at least, for prevention of autoimmune disease, Tregs may adaptively employ additional mechanisms of suppression (74). For example, Treg-specific IL-10 deficiency produces IBD and pneumonitis but not autoimmune disease, indicating that Treg-produced IL-10 is not indispensable for self-tolerance but required for the maintenance of immune homeostasis in mucosal tissues (75). Further study is required to determine how cell-contact-dependent and humoral-factor-mediated Treg suppression mechanisms effectively control immune responses depending on the type of responder T cells (their TCR affinity and activation state) or immune cells (DCs, B cells, or other cells), and on the phase, location, and type of inflammation.

Functional Stability, Adaptability, and Tissue-Specific Function of Tregs

In most circumstances Tregs display a high level of lineage stability, retaining their suppressive activity and FoxP3 expression despite various inflammatory stimuli (76). However, it has also been reported that in some cases lineage instability is observed and FoxP3$^+$ cells gain an effector type phenotype concomitant with loss of FoxP3 expression (77–79). It remains to be determined in these cases whether fully epigenetically fixed Tregs can lose stability or FoxP3 unstable cells are representative of the proportion of FoxP3$^+$ cells that lack the full epigenetic program of Tregs and consequently lack lineage stability (42, 80).

In contrast to lineage instability, Tregs may also adapt to their environment, undergoing substantial changes to phenotype while maintaining their suppressive function. A number of studies have demonstrated that Tregs are able to display functional adaptability and gain the expression of transcription factors and chemokine receptors, but not inflammatory cytokines, normally associated with Th1 (81, 82), Th2 (83–85), Th17 (86–88), and T follicular helper cells (89–91). This combination of chemokine receptors but lack of helper function may allow the Tregs to travel to the site of a specific type of inflammation and suppress the corresponding T effector subgroup (92).

While Tregs are primarily known for their ability to prevent inflammation in the lymphoid organs, recent results have demonstrated that they are also present in healthy tissues and have functions that go beyond suppression of inflammation. For example, Tregs are present in healthy skeletal muscle, visceral adipose tissue, and the hair follicle stem cell niche in the skin. In all of these sites Tregs retain their expected immunosuppressive capacity but also are involved in more specialized functions such as tissue repair and hair regrowth by the production of the growth factor amphiregulin to enhance the regeneration of muscle satellite cells or the notch ligand Jagged1, which induces hair follicle stem cell differentiation (24, 93–95). In the gut mucosa, Tregs can be divided into multiple subsets defined by the expression of GATA3, Helios, and RORγt. For example, GATA3$^+$Helios$^+$ colonic Tregs, which are presumed to be of thymic origin, respond to the alarmin IL-33 produced upon tissue damage, limiting tissue damage during colitis (96). In contrast, RORγt$^+$Helios$^-$ Tregs are pTregs induced by the presence of intestinal microbiota, and the loss of RORγt expression by Tregs results in severe intestinal inflammation (86, 87). Another subset of RORγt$^-$Helios$^-$ Tregs is found to be concentrated in the upper small intestine and acts primarily to prevent allergic responses to food antigens (97). These findings demonstrate that Tregs display a remarkable level of ability to adapt to their environment and contribute to tissue homeostasis not only by controlling inflammation but also via more specialized mechanisms such as the production of growth factors.

HUMAN FOXP3+ T CELLS: THEIR FUNCTION AND PHENOTYPE

While in mice FoxP3 is an almost exclusive marker of Tregs (80), human FOXP3 is relatively easily induced in Tconvs following their activation. However, this expression is transient, at a low level, and does not confer significant in vitro suppressive activity (98). This heterogeneity of FOXP3+ T cells in healthy individuals prompted endeavors to dissect FOXP3+ T cells into functional Tregs and non-Tregs by cell surface markers. The attempts showed that the use of FOXP3, CD25, and CD45RA is able to subdivide circulating FOXP3+CD4+ T cells into three main fractions; that is, fraction I (Fr. I) CD45RA+FOXP3low/CD25low resting or naive Tregs; Fr. II CD45RA−FOXP3high/CD25high effector Tregs; and Fr. III CD45RA−FOXP3low/CD25low cells, the majority of which are not Tregs (40). The classification has a good correlation with Treg suppressive activity, cytokine production, and, importantly, Treg-specific epigenetic changes (**Figure 2**).

Fr. I naive Tregs are stable Tregs with a demethylated TSDR; suppressive capacity; a broad expression of various naive T cell–associated surface markers such as CD45RA, CCR7, and CD62L; and a lack of CD45RO expression (40, 99, 100). They have undergone a relative lack of previous
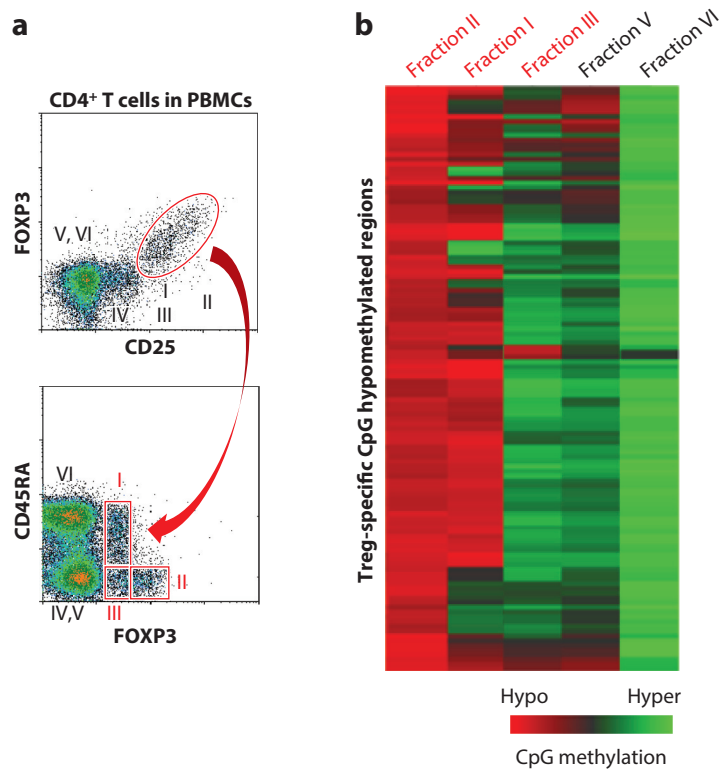

Figure 2

Subpopulations of FOXP3+ T cells in humans. (*a*) CD4+ T cells in the PBMCs of healthy individuals can be dissected into six fractions by the use of CD25, CD45RA, and FOXP3. (*b*) Treg-specific hypomethylated CpG regions (assessed by post-bisulfite adapter tagging sequencing) defined by comparing Fr. II (effector Tregs) and Fr. VI (naive Tconvs) show similarity of Fr. II to Fr. I (naive Tregs) but not to Fr. III, which is more similar to Fr. V (activated Tconvs). Abbreviations: Fr. I, fraction I; Tconv, conventional T cell; Treg, regulatory T cell; PBMC, peripheral blood mononuclear cell.

proliferation in comparison to Fr. II effector Tregs (99). Tregs expressing the recent thymic emigrant marker CD31 are highly enriched within naive Tregs; and both CD31$^+$ and CD31$^-$ naive Treg populations are highly enriched in cord blood and decrease in peripheral blood with aging (40, 99–101).

Upon TCR stimulation, Fr. I naive Tregs differentiate into Fr. II effector Tregs with downregulation of CD45RA (and upregulation of CD45RO), and upregulate the expression of FOXP3, CD25, and CTLA-4. Fr. II effector Tregs are in a highly proliferative state, exhibit strong in vitro suppressive activity, and possess a more profoundly demethylated TSDR. While both Fr. II Tregs and Fr. III FOXP3$^+$ cells are CD45RA$^-$CD45RO$^+$ effector-type cells, the former show higher expression of CD25 and FOXP3 than the latter. It is not always easy, however, to define the exact boundary of CD25 and FOXP3 between these two groups, one highly suppressive and the other nonsuppressive. Comprehensive screening of monoclonal antibodies reactive with human lymphocytes identified CD15s (sialyl Lewis x) as a candidate marker for separating Fr. II and Fr. III cells (102). The study showed that CD15s$^+$CD45RA$^-$FOXP3$^+$ cells, corresponding to Fr. II, had suppressive function, while CD15s$^-$CD45RA$^-$FOXP3$^+$ cells, corresponding to Fr. III, lacked suppressive activity and secreted inflammatory cytokines such as IL-2 and IFN-γ (102). Fr. II effector Tregs also express other molecules such as HLA-DR, TIM-3 and ICOS, as markers of highly suppressive Tregs (103–105), and Helios, which is correlated with a stable Treg phenotype (106).

The majority of Fr. III cells appear to be activated Tconvs. However, recent studies have shown that Fr. III may also include some Tregs (107–109). The CD45RA$^-$CXCR5$^+$CD25^{low} FOXP3^{low} circulating T follicular regulatory (Tfr) group, in particular, is present in Fr. III, constituting around 30% of the cells in Fr. III in the blood of healthy donors (108, 110, 111).

Thus, FOXP3$^+$ T cells are heterogeneous, including naive and effector Tregs and non-Tregs, and show functional and phenotypic differentiation within the Treg population. The dynamics and the composition of the subsets can reflect immunological states of the host succumbing to immunological disease (see below). For differentiating Tregs and non-Tregs among FOXP3$^+$ T cells, cell surface (such as CD25 and CD45RA) and intracellular markers (such as FOXP3) are highly useful but not absolute indicators of Treg identity. Logically, DNA demethylation at Treg-DRs is more accurate than these protein markers in defining functional Tregs because CpG methylation/demethylation is digital information capable of differentiating between Tregs and Tconvs via the mode of gene expression at Treg signature gene loci (36, 42).

TREGS IN AUTOIMMUNE DISEASE

Tregs in IPEX Syndrome and IPEX-Like Monogenic Diseases

In 1982, IPEX syndrome was described as an X-linked immunodeficiency syndrome with severe autoimmune disease, including T1D, enteropathy, and eczema (17). In addition to this classical triad with neonatal onset, IPEX patients develop various autoimmune diseases (e.g., autoimmune thyroiditis, nephritis, arthritis, hepatitis, hemolytic anemia, thrombocytopenia, and alopecia) at various frequencies, and food allergy (18). Among 70 mutations of the *FOXP3* gene so far reported, the most frequent ($\sim$40%) mutations are present in the region encoding the C-terminal forkhead DNA-binding domain and abrogate the expression of functional FOXP3 protein, causing severe clinical manifestations. Other mutations also affect FOXP3 expression in various degrees with variable phenotypes (18, 112). The IPEX syndrome is unequivocal evidence that deficiency or dysfunction of natural Tregs can be a primary cause of not only autoimmune disease but also IBD and allergy in humans, illustrating in humans that Tregs are engaged in suppressing immune responses against self-constituents, commensal microbes, and environmental substances. Some

IPEX patients develop autoimmune disease already in the in utero aseptic environment. Since FOXP3^{+} Tregs are found in the periphery of fetuses from around gestational week 14 onward (113), this indicates that autoimmune disease can occur as a purely intrinsic defect of the immune system without participation of plausible autoimmune-causing environmental factors such as microbial substances cross-reacting with self-antigens. In addition, the development of autoimmune disease in a broad spectrum of organs and tissues in IPEX syndrome suggests that a profound deficiency of Tregs can activate a variety of self-reactive T cell clones that appear to be present in detectable numbers in healthy individuals as well (61, 114, 115).

Mutations of Treg signature genes cause severe autoimmune diseases as seen with FOXP3 mutations. Fatal autoimmune disease develops in individuals with mutations of *IL2RA* (CD25) (116). Autoimmune/inflammatory disease also develops in family groups with heterologous mutations of CTLA-4, which hamper Treg expression of CTLA-4 in resting as well as activated states (117, 118). Patients with a deficiency of LRBA (lipopolysaccharide-responsive and beige-like anchor protein), which is indispensable for CTLA-4 trafficking, also develop severe autoimmunity due to loss of CTLA-4 expression by Tregs (119, 120). These findings confirm the important role of Treg-expressed CTLA-4 and CD25 in human immunity. Other monogenic diseases accompanying Treg anomalies are caused by *BACH2* and *STAT3* mutations, which produce enteropathy and other immunological anomalies with frequent reduction of Tregs in the circulation (121). These "Tregopathies" (121) may include other monogenic autoimmune and immune-deficient diseases such as the DiGeorge (22q11.2 deletion) syndrome and Omenn's syndrome with severe combined immune deficiency, which frequently accompany reduction of thymic and circulating Tregs (122). Notably, APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy) syndrome with various organ-specific autoimmune diseases due to loss-of-function mutations of the *AIRE* gene has decreased numbers of naive Tregs in the circulation (123, 124). The question of how *AIRE* controls central and peripheral self-tolerance including Treg development is under investigation (125).

Tregs in Multifactorial and Polygenic Autoimmune Diseases

It is a key question whether Tregs play any role in the development of multifactorial polygenic common autoimmune diseases [such as T1D, rheumatoid arthritis, and systemic lupus erythematosus (SLE)], which afflict 10% of the population worldwide (126). Since the discovery of an indispensable role of natural Tregs in immunological self-tolerance in humans as illustrated by IPEX syndrome, efforts have been made to search for functional or numerical anomalies, or variations, in natural Tregs in common autoimmune diseases (127–129). The number of FOXP3^{+} Tregs generally increases in inflammation sites of an organ-specific autoimmune disease but does not change much in the peripheral blood. In systemic autoimmune disease, fractionation of circulating FOXP3^{+} cells into Fr. I–III revealed a significant reduction of Fr. II effector Tregs and an increase of Fr. III non-Tregs in patients with active SLE, indicating the expansion of activated Tconvs in the flare of the disease (40, 102). Such findings may reflect the pathophysiology of the disease; however, it is generally difficult to determine whether an anomaly or variation in Treg function or number is a cause or a consequence of an autoimmune response.

Recent genome-wide association studies of common polygenic autoimmune diseases have revealed that ~60% of autoimmune-causal single-nucleotide polymorphisms (SNPs) are mapped to noncoding enhancer regions of immune cells (130–132), including cell type–specific super-enhancers (132), and cell type–specific differentially CpG methylated regions (133, 134) of CD4^{+} T cells. For example, autoimmune SNPs are present in enhancer regions of T cell activation-associated genes such as *IL2RA* and *CTLA4*, which are also Treg function associated (135–137). Yet

it remains unclear whether such T cell–related autoimmune SNPs should affect the development and function of either autoimmune-causing Tconvs as gain-of-function SNPs or autoimmune-suppressing Tregs as loss-of-function SNPs. A key question would be whether autoimmune SNPs are enriched in Treg- or Tconv-specific super-enhancers or, more specifically, in Treg- or Tconv-specific differentially demethylated regions, which may be largely included in super-enhancers.

Particular HLA alleles are associated with common autoimmune diseases, some alleles being autoimmune susceptible, others protective (135). A recent study has shown in Goodpasture disease, an autoimmune kidney disease mediated by Type IV collagen peptide–reactive CD4$^+$ T cells, that Tregs binding the peptide are predominantly increased in individuals with a dominantly protective HLA allele and in those coexpressing the protective and susceptible alleles (138). This finding that HLA polymorphisms contribute to autoimmune disease susceptibility by shaping the number and repertoire of self-antigen-specific Tregs remains to be examined in other autoimmune diseases associated with protective and susceptible HLA alleles (139).

Considering clear demonstrations of the existence of potentially autoimmune-causing self-reactive T cells in the periphery of healthy individuals (61, 114, 115), thymic T cell selection is not an all-or-none pruning of self-reactive TCR repertoire, but rather it controls the frequency of each autoimmune Tconv and Treg clone, depending on HLA and other genetic variations.

TREGS IN TUMOR IMMUNITY

There is ample evidence that FOXP3$^+$ Tregs hinder effective tumor immunity in humans. Cancer-tissue-infiltrating FOXP3$^+$ T cells are predominantly FOXP3^{high} effector Tregs in a majority of cancers (140). They are mostly in an activated and highly proliferative state, compared with Tregs in the blood or non-cancerous tissues, and they express higher levels of T cell activation–associated molecules, such as CD25, CTLA-4, ICOS, OX40, 4-1BB, LAG3, TIGIT, GITR, and PD-1. They also express particular chemokine receptors associated with Th2 cells such as CCR4 and CCR8 at high levels (141–145). An accumulating number of studies have demonstrated that a high frequency of tumor-infiltrating effector Tregs, especially a high ratio of FOXP3$^+$ cells to CD8$^+$ T cells, in various solid tumors is correlated significantly with poor prognosis (146–151). In colorectal cancer (CRC), however, there are several reports that increased FOXP3 expression in tumor tissue is associated with a better prognosis, while other reports show its association with poor prognosis (152, 153). This discrepancy can be attributed to dominant infiltration of effector Tregs or FOXP3$^+$ non-Tregs (145). In effector Treg–predominant CRCs, patients with high-*FOXP3* mRNA in cancer tissues showed significantly poorer prognosis than those with low-*FOXP3* mRNA. In contrast, Fr. III non-Treg-predominant CRCs showed the opposite prognosis, i.e., a better prognosis of the high-*FOXP3* mRNA group compared with the low-*FOXP3* mRNA group. The expansion of FOXP3$^+$ non-Tregs, apparently activated Tconvs, was correlated with mRNA levels of proinflammatory cytokine genes such as *TNF* and *IL12* and depended on the presence of particular species of intestinal bacteria, such as *Fusobacterium nucleatum*. The results indicate the contribution of cancer tissue microenvironments, especially microbial inflammation, to local composition of FOXP3$^+$ subpopulations (145).

Since Tregs can be found both circulating in blood and resident in tissues, it is unclear whether tumor-infiltrating Tregs originate in the tumor-associated tissue or instead infiltrate from the circulation. The immune signature of a tumor is linked to its tissue of origin, suggesting a link between tumor- and tissue-resident Tregs (154, 155). Additionally, comparison of Tregs taken from the blood, tissue, or tumor demonstrates that, while tissue Tregs and tumor Tregs have greater similarity due to an activated phenotype, the three groups remain relatively distinct (142). While these findings suggest a relationship between tissue- and tumor-resident Tregs, analysis of the

TCR repertoire of these populations finds little overlap (144, 154). It is likely that they are not expanded in response to the same antigens and that tumor-resident Tregs may have been initially recruited from the wider pool of Tregs in the lymphoid tissue and blood. Once in situ they may then gain some phenotypic and functional features of tissue-resident Tregs due to the relative similarity of the tumor and its tissue of origin.

Collectively, the key features of tumor-infiltrating Tregs are that they are highly activated and terminally differentiated effector Tregs. In some tumors, such as CRCs, however, the FOXP3^{+} population harbors a significant proportion of FOXP3^{+} Tconvs. Tumor-infiltrating effector Tregs are therefore a key target in enhancing tumor immunity in cancer tissues (see below).

TREG-UP STRATEGIES FOR IMMUNE SUPPRESSION

In vivo Expansion of Natural Tregs and Treg Adoptive Cell Therapy

Due to their capacity to induce stable immune tolerance, therapies that expand or generate FOXP3^{+} Tregs or enhance their suppressive function have been of significant interest as approaches to treat autoimmune and other immunological diseases and prevent rejection of organ transplants. Due to their expression of the high-affinity IL-2 receptor, Tregs are highly sensitive to changes in IL-2 availability (65, 156). Correspondingly, administration of IL-2 is an effective method of inducing in vivo expansion of Tregs and prevention of autoimmunity in a number of mouse models (157). A more targeted approach is to attempt to focus the effect of the IL-2 on Tregs by blocking the ability of the IL-2 to interact with IL-2Rβ, common on CD8 cells, causing its preferential targeting to Tregs expressing CD25 (IL-2Rα). This can be achieved by binding IL-2 in complex to specific anti-IL-2 antibodies that block the IL-2Rβ binding site (158). In humans, low doses of IL-2 are used to expand natural Tregs while avoiding complications from IL-2 signaling on NK cells or effector T cells. This approach has been demonstrated to successfully expand Tregs in patients with graft-versus-host disease (GVHD), T1D, alopecia areata, and hepatitis C virus–induced vasculitis (159). In addition, Tregs, especially effector Tregs, express TNF receptor 2 (TNFR2) at much higher levels than activated Tconvs (160). Stimulation of TNFR2 by specific antibodies or agonists preferentially expanded natural Tregs in vivo and in vitro and has been shown to be effective in treating GVHD (161). Besides these ways to expand natural Tregs, pTregs can be induced in rodents by targeting antigen to tolerogenic DCs, which are low in CD80/CD86 expression and capable of secreting TGF-β (162).

Another approach to immune suppression is Treg-based adoptive cell therapy for autoimmune disease: that is, to purify circulating Tregs from a patient, expand them in vitro, and transfer them back to the patient. Naive Tregs can be expanded 500- to 2,000-fold, making it feasible to generate clinically usable numbers of cells from limited quantities of blood (163). This is achieved by expansion of purified Tregs using polyclonal stimuli such as TCR and CD28 costimulation, delivered either by antibody-coated beads or by artificial APCs, in the presence of IL-2 (163–165). Antigen stimulation of naive Tregs with alloantigen is able to expand antigen-specific Tregs and has proven effective in the prevention of GVHD or graft rejection in transplantation settings (166, 167). It remains to be seen whether these antigen-specific approaches will be more effective or whether it is better to use polyclonal stimuli to generate the largest possible numbers of expanded Tregs. In these Treg expansions, Fr. I naive Tregs are a suitable target for expansion because they have higher proliferative capacity than the terminally differentiated Fr. II and also because they avoid contamination with potential non-Tregs in Fr. III (168). Transferred Tregs have been demonstrated to persist in recipients for at least one year, and phase 1 trials of autologous Treg transfer have already demonstrated that this approach is feasible and safe in patients with T1D or GVHD (164, 165).

A problem of the use of naturally present Tregs for adoptive cell therapy is that purification and expansion of Tregs is limited by their relative scarcity in human blood and their slow rate of in vitro expansion. To bypass these issues, one strategy is to generate CAR (chimeric antigen receptor)-Tregs, which express an antibody Fab region specific for a particular alloantigen or self-antigen and strongly suppress the alloimmune or autoimmune responses (169–171).

Conversion of Tconvs into Tregs

Besides therapeutic use of natural Tregs, iTregs generated by antigen stimulation of naive Tconvs in the presence of TGF-β and IL-2 have been extensively studied in experimental settings (172; reviewed in 173). Induction of iTregs, for example, by retinoic acid and short-chain fatty acids, also requires TGF-β. In addition, activation of the AKT signaling pathway impairs the development of natural Tregs; and inhibitors of the pathway, for example, rapamycin, can induce FoxP3 when combined with premature termination of TCR signaling (174, 175). These iTregs in humans and mice, however, lack Treg-type epigenomic changes, especially Treg-specific demethylation, and hence are functionally unstable and difficult to generate from activated or effector T cells, or in the presence of inflammatory cytokines.

An ideal strategy for antigen-specific immune suppression is to convert in vivo and in vitro not only naive Tconv and Fr. III non-Tregs but also effector/memory Tconvs mediating autoimmune disease into functionally stable FOXP3$^+$ Tregs. Chemical inhibition of cyclin-dependent kinases (CDK) 8/19, which reversibly associate with the Mediator complex and control transcription positively and negatively, is able to induce FoxP3 in antigen-stimulated effector/memory as well as naive CD4$^+$ and CD8$^+$ T cells in vitro (176). Furthermore, in vivo administration of a CDK8/19 inhibitor along with antigen immunization generated functionally stable (i.e., epigenetically Treg type) antigen-specific FoxP3$^+$ Tregs, which effectively suppressed autoimmune disease in animal models. The in vitro induction was dependent on STAT5 activation, independent of TGF-β, and not affected by inflammatory cytokines. The results indicate that a TCR-derived signal through CDK8/19 is physiologically repressing FoxP3 expression in activated Tconvs and that release from the repression suffices to induce FOXP3 in Tconvs. Thus, FOXP3 can be induced in Tconvs by targeting distinct signaling pathways (e.g., TGF-β-SMADs, AKT-mTOR, and TCR-CDK8/19 pathways). A combination of targeting these pathways, together with the installation of a Treg-type epigenome, is envisioned to generate better iTreg preparations for adoptive Treg therapy.

For stable reestablishment of self-tolerance in patients with autoimmune disease or establishment of stable graft tolerance in individuals with organ transplants, the effectiveness of the above ways of adoptive cell transfer may depend on expansion of antigen-specific natural Tregs originally present in the host in the manner of infectious tolerance (**Figure 3**).

TREG-DOWN STRATEGIES FOR IMMUNE ENHANCEMENT

Treg depletion effectively evokes antitumor immunity in experimental models (151, 177). Systemic depletion of Tregs, however, may elicit autoimmunity as an adverse effect due to their indispensable roles in immunological self-tolerance. It is therefore the key issue in establishing Treg-targeting cancer immunotherapy to determine how tumor-infiltrating Tregs can be targeted without affecting tumor-reactive CD4$^+$ or CD8$^+$ Tconvs, or Tregs in other tissues, and how biologicals, such as monoclonal antibodies (mAbs), and small molecules are able to achieve these goals.
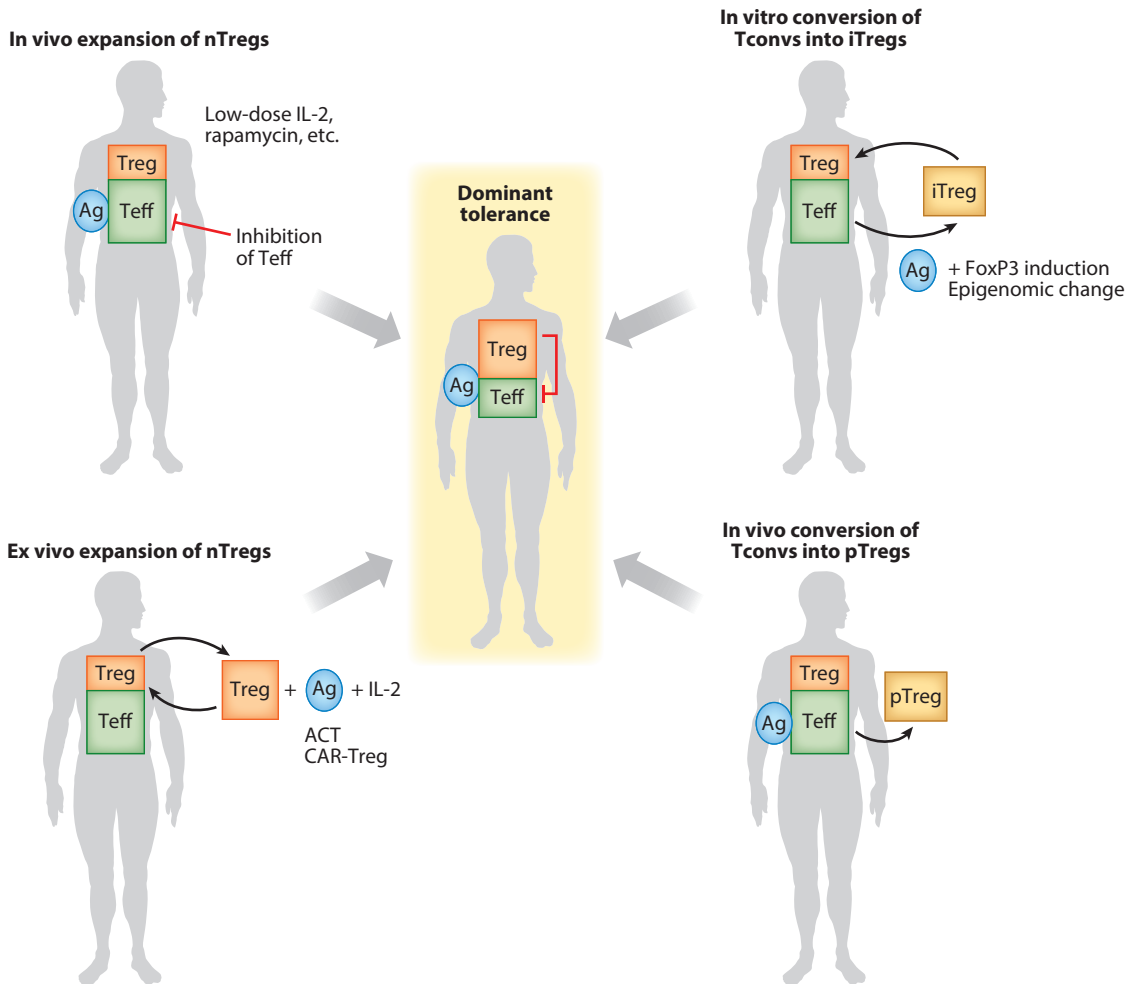
Figure 3

Establishment of Treg-mediated dominant tolerance to treat immunological diseases. nTregs can be expanded in an antigen-specific or nonspecific (polyclonal) fashion in vivo or in vitro. Tconvs can also be converted into pTregs in vivo or into iTregs in vitro. Abbreviations: ACT, adoptive cell therapy; Ag, antigen; CAR, chimeric antigen receptor; iTreg, in vitro–induced Treg; nTreg, natural Treg; pTreg, peripherally derived Treg; Tconv, conventional T cell; Teff, effector T cell; Treg, regulatory T cell.

Depletion of Tregs by Monoclonal Antibodies

Cell surface molecules selectively enriched, if not exclusively, on tumor-infiltrating Tregs can be good targets for antibody-mediated Treg depletion. They include CD25, CTLA-4, GITR, 4-1BB, OX-40, LAG3, and TIGIT, and some chemokine receptors such as CCR4 and CCR8 (141, 143, 178–180) (**Figure 4**). Although many surface molecules are similarly upregulated or downregulated by both Tconvs and Tregs, some molecules, such as CTLA-4, show differential levels and kinetics of expression between the two populations. For example, CTLA-4 is only expressed by Tconvs upon activation, while naive and effector Tregs in the periphery constitutively express CTLA-4, and the expression by Tregs is further upregulated in tumors, often to the highest extent among tumor-infiltrating lymphocytes (180). Anti-CTLA-4 mAb (ipilimumab) was initially
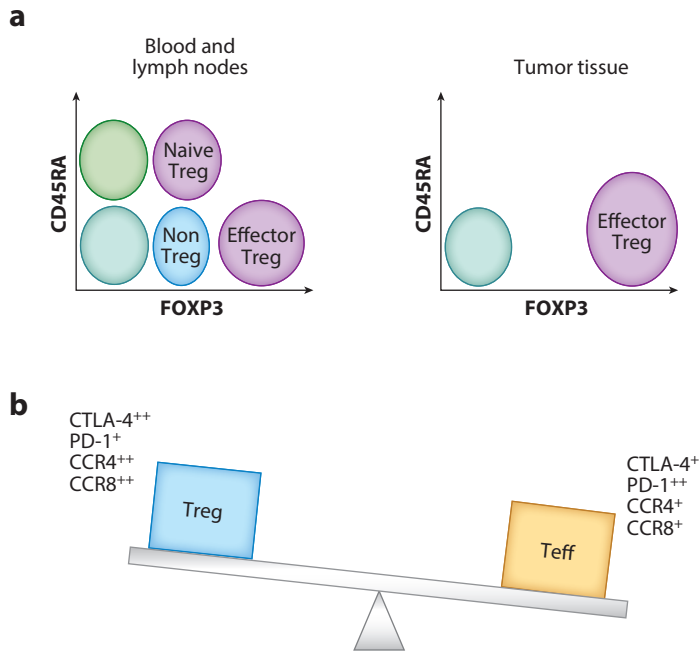
Figure 4

Targeting Tregs in tumor tissues to augment tumor immunity. (*a*) Compared with blood or lymph nodes, key features of tumor tissues are predominant expansion of FOXP3^{high} effector Tregs and the presence of effector/memory type Tconvs and few naive Tconvs or Tregs. (*b*) In tumor tissues, effector Tregs and Tconvs express various cell surface molecules at different levels, allowing differential depletion by cell-depleting monoclonal antibodies specific for these molecules. Abbreviations: Tconv, conventional T cell; Teff, effector T cell; Treg, regulatory T cell.

suggested to block a negative signal via CTLA-4 into effector CD8$^+$ T cells and thereby to enhance antitumor immune responses. Recent studies, however, have demonstrated that ipilimumab predominantly kills CTLA-4-expressing Tregs in tumor tissues by antibody-dependent cellular cytotoxicity (ADCC), at least in mouse models (178, 181, 182). Fc-engineered ipilimumab (ART-Fc anti-CTLA-4 mAb) with ~1,000-fold higher affinity for the activating FcγRIIIa, thus possessing potent ADCC activity, selectively depleted effector Tregs and enhanced tumor-antigen-specific CD8$^+$ T cell responses in vitro for humans and in vivo in mice (180). Clinical responders among ipilimumab-treated melanoma patients showed a significant reduction of tumor Tregs (183). In addition, melanoma patients possessing a high-affinity genetic variant of Fc-receptor (V158 variant of FcγRIIIa) with an enhanced ADCC activity showed significantly better responses and better overall survival upon imatinib treatment when compared with patients with a low-affinity variant (F158) (184).

Considering the different levels and kinetics of CTLA-4 expression by Tregs and Tconvs, the timing and the sequence of antibody treatment and tumor-antigen vaccination are critical for eliciting effective antitumor immune responses. For example, simultaneous treatment with vaccination and ART-Fc anti-CTLA-4 mAb with high ADCC activity resulted in depletion of both Tregs and antigen-stimulated CTLA-4-upregulating CD8$^+$ T cells, thus hindering antitumor immune responses (180). In contrast, delaying vaccination several days from the antibody treatment only depleted Tregs and effectively expanded antigen-specific CD8$^+$ T cells with resulting

enhancement of antitumor immune responses in vitro for humans as well as in tumor-bearing mice (180). The results illustrate the importance of Treg reduction prior to tumor-antigen vaccination for effective augmentation of antitumor immunity.

Tregs also express PD-1 at a low level in the blood and at a high level in tumors. In contrast to CTLA-4, PD-1 expression in tumors is equivalent or higher on CD8^{+} and CD4^{+} T cells than Tregs. Antibody-mediated PD-1 blockade enhances the effector function of PD-1-expressing CD8^{+} and CD4^{+} Tconvs; however, the antibody also expands and enhances the suppressive activity of PD-1-expressing Tregs (185, 186). Mice with Treg-specific PD-1 deficiency show significant enhancement of tumor growth with Treg expansion and more potent suppressive activity. In humans, recent reports have shown that ~10% of cancer patients treated with anti-PD-1 antibody develop hyperprogressive disease, which is characterized by rapid cancer progression and high infiltration of proliferating Tregs in tumor tissues (186, 187). Treg-specific depletion prior to, or in combination with, anti-PD-1 antibody treatment may prevent hyperprogressive disease and enhance the effectiveness of anti-PD-1 therapy.

Treg–Depleting Small Molecules

Not only antibodies, but also small molecules are able to selectively target effector Tregs and reduce them by exploiting Treg-specific immunobiological properties. For example, imatinib is a tyrosine kinase inhibitor of the oncogenic BCR-ABL fusion protein specifically expressed in chronic myelogenous leukemia (CML) cells. It is known that imatinib-treated CML patients, in particular those in remission, often develop effective T cell responses against leukemic cells (188–190). Notably, long-term imatinib-treated CML patients in complete molecular remission with no detectable *BCR-ABL* mRNA in the blood showed selective depletion of FoxP3^{+} effector Tregs, whereas those who failed in molecular remission did not (191). The former group saw a general increase in effector- or memory-type CD8^{+} T cells producing multiple cytokines. Similarly, in vitro, imatinib induced apoptosis predominantly in effector Tregs, augmenting CD8^{+} T cell responses against various tumor antigens in healthy individuals and cancer patients. Imatinib also reduced Tregs in tumor-bearing mice, inhibiting tumor growth. As a plausible mechanism of imatinib-induced Treg depletion, the drug inhibits tyrosine phosphorylation of lymphocyte-specific protein tyrosine kinase (LCK), a T cell–specific signaling molecule, as an off-target effect. Because of FoxP3-dependent gene repression (see above), the expression of LCK and its downstream signaling molecule ZAP-70 is maintained at low levels in Tregs compared with CD4^{+} or CD8^{+} Tconvs. A combination of these low LCK and ZAP-70 levels and LCK inhibition by imatinib reduces the TCR proximal signal more severely in activated Tregs than in activated Tconvs, causing the former to die by apoptosis at a therapeutic range of imatinib for CML. Treg-specific deficiency of other signaling molecules, such as PI3Kδ, c-Rel, or CARMA1, also impairs Treg function and augments tumor immunity in mice (192–194). It remains to be determined whether inhibitors of these signaling molecules selectively affect Tregs while leaving CD8^{+} T cells intact at a particular dose range or specifically in tumor tissues.

Autoimmunity Versus Tumor Immunity

While differential control of Tregs versus Tconvs is important for augmenting tumor immunity as discussed above, control of tumor Tregs versus tissue or circulating Tregs is also essential to prevent autoimmunity while inducing effective tumor immunity. One way of achieving this aim is to selectively deplete tumor Tregs while preserving Tregs in other tissues including lymphoid organs and in the circulation (reviewed in 151). Given that activated Fr. II effector Tregs are predominant

in tumor tissues, Fr. II Treg-depleting antibodies discussed above, such as anti-CTLA-4, -CCR4, or -CCR8 mAb, may preserve Fr. I naive Tregs and less-activated Fr. II tissue-resident Tregs in other tissues to prevent autoimmunity. Another way to induce differential control of tumor immunity and autoimmunity is to adjust the degree and duration of Treg depletion. It generally requires more thorough and longer Treg depletion to induce autoimmune disease than to evoke antitumor immune responses, presumably because tumor antigens including neoantigens may well be more immunogenic than immunologically intact self-antigens. A third way to induce differential control is to deplete Tregs locally in tumor tissues, rather than systemically, e.g., by intratumoral injection of Treg-depleting antibody (195, 196). The use of a photoactivated Treg-depleting antibody, such as anti-CD25, engineered to exert killing activity only after light exposure also enables specific depletion of tumor Tregs locally at light-exposed tumor tissue (197). Additionally, assessment of treated patients for their genetic susceptibility to autoimmune disease (see above) would allow the prediction of autoimmunity due to systemic Treg reduction/depletion.

CONCLUSIONS AND PERSPECTIVE

There is now increasing evidence that Tregs play significant roles in various immunological and inflammatory diseases. This review has discussed the roles of FoxP3$^+$ Tregs, natural or induced, mainly in autoimmune disease and cancer because the former represents the diseases in which immunological tolerance needs to be reestablished, the latter representing those in which tolerance needs to be breached to allow the evocation of effective immune responses. Treg-up strategies could be common in treating autoimmune disease, allergy, immunopathology (such as IBD), and other diseases requiring immune suppression, and also in controlling graft rejection (as reviewed in 21, 198, 199). Treg-down strategies have the potential to be highly effective in treating cancer and chronic microbial diseases (as reviewed in 151, 200). It is hoped that these strategies, with further refinement, will be extended to prevention of immunological diseases.

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