Published in final edited form as:

Nat Rev Rheumatol. 2009 October ; 5(10): 560–565. doi:10.1038/nrrheum.2009.183.

Regulatory T cells as therapeutic targets in rheumatoid arthritis

Jonathan H. Esensten, David Wofsy, and Jeffrey A. Bluestone

Diabetes Center (**J. H. Esensten, J. A. Bluestone**), Division of Rheumatology (**D. Wofsy**), University of California, San Francisco, CA, USA.

Abstract

Regulatory T cells (T_{REG}) are a subset of CD4⁺ T cells with a critical role in the prevention of autoimmunity. Whether defects in T_{REG} contribute to the pathogenesis of rheumatoid arthritis (RA) is unclear. However, a variety of approved and experimental drugs for RA may work, in part, by promoting the function or increasing numbers of T_{REG}. Furthermore, animal studies demonstrate that direct injection of T_{REG} ameliorates a wide range of experimental models of inflammatory and autoimmune diseases. Thus, cell-based therapy with T_{REG} has the potential to produce durable disease remission in patients with RA.

Background

The ultimate goal of therapy for patients with active rheumatoid arthritis (RA) and other autoimmune diseases is to restore normal immune function rather than to achieve broad immunosuppression. Evidence has accumulated over the past decade that regulatory T cells (T_{REG}) could be an ideal target for therapies to induce durable remission of autoimmune and inflammatory disease (reviewed elsewhere¹). T_{REG} are ideal for this purpose because they suppress inflammation in an antigen-specific manner. Furthermore, short-term therapy with T_{REG} can lead to long-term inhibition of autoimmune disease in mouse models, and immunomodulatory agents can affect numbers and functioning of T_{REG} in both mice and humans. Thus, approaches that bolster numbers or functioning of T_{REG} could achieve selective and durable inhibition of pathologic inflammation without blocking protective immune responses against infection. In this Review, we discuss the use of small-molecule drugs, biological agents and direct T_{REG} administration to increase numbers or promote the functions of T_{REG} and to interrupt chronic inflammation in patients with RA (Figure 1).

T_{REG} constitute 5–7% of CD4⁺ T cells in humans.^{2,3} These regulatory cells suppress immune responses through a variety of contact-dependent and contact-independent mechanisms.^{4,5} Importantly, they have an inherently autoreactive T cell receptor (TCR) repertoire, and antigen recognition through the TCR is required to suppress immune responses.^{1,6} The transcription factor forkhead box P3 (FOXP3) is critical for the generation and peripheral maintenance of T_{REG} in both mice⁷ and humans.⁸ Mutations in *FOXP3* or its regulatory regions cause an X-linked syndrome of immune dysregulation, polyendocrinopathy and enteropathy (IPEX) characterized by massive polyclonal T cell activation and tissue infiltration.^{8–11} Immune homeostasis in patients with IPEX can be successfully restored with hematopoietic stem cell transplantation following submyeloablative conditioning, since T_{REG} exert a dominant regulatory effect that prevents

systemic T cell activation and autoimmunity.^{10,12–14} Moreover, other genetic loci that encode molecules involved in T_{REG} function have been linked to autoimmunity in genome-wide association studies.¹⁵ Together, these observations have spurred further work on T_{REG} in patients with common polygenic autoimmune diseases.

Three problems limit the use of FOXP3 expression alone to study T_{REG} in humans. First, FOXP3 is expressed transiently in most activated human T cells, often without conferring a regulatory phenotype.^{16,17} Furthermore, cells that are both FOXP3⁺ and immunosuppressive may lose this suppressive capacity under certain conditions.^{18,19} Second, recent mouse studies have shown that DNA methylation status at the *Foxp3* locus may be a better marker of a stable T_{REG} phenotype than FOXP3 protein expression.^{18,20} Third, FOXP3 is an intracellular protein that cannot be used to isolate T_{REG} for functional studies. Thus, in practice, assessments of T_{REG} function in patients with autoimmune diseases must rely on use of cell surface markers to identify and isolate T_{REG} for *in vitro* studies. Although many cell surface proteins are differentially expressed on T_{REG},²¹ no known cell surface markers are expressed exclusively on T_{REG}. In fact, research on cells identified as T_{REG} has been complicated by the early use of only CD4 and CD25 expression to identify these cells. CD4⁺ T cells that express high levels of CD25 (CD4⁺CD25^{high} cells) are generally FOXP3⁺ and highly immunosuppressive.²² However, the CD4⁺CD25^{high} population also includes effector T cells.²³ This contamination has, in some cases, misled investigators to believe that deficits in T_{REG} function exist where they do not. The best approach, therefore, is to use a constellation of cell surface markers, including folate receptor 4, IL-7 receptor subunit α (CD127), latency-associated peptide (LAP), and glucocorticoid-induced TNF receptor-related gene (GITR) to isolate T_{REG} and study their function.²¹ For instance, our group showed that low levels of CD127 expression in combination with CD4 and CD25 expression can identify more than 95% of FOXP3⁺ T cells that have highly immunosuppressive activity.²

Are T_{REG} defective in RA?

Whether T_{REG} defects are present in patients with RA is not clear. One study reported that the number of CD4⁺CD25^{high} T_{REG} in the peripheral blood of patients with RA is elevated as compared with that of healthy individuals,²⁴ whereas other studies suggest no differences in peripheral blood T_{REG} numbers between these two groups.^{25–27} Several studies have reported that CD4⁺CD25^{high} T cells are present in the synovial fluid of patients with RA and that their function is normal *in vitro*.^{24,26,28,29} However, these *in vitro* assays are not antigen-specific and the accumulation of polyclonal T_{REG} in inflamed tissues might be a general phenomenon.^{30,31} Thus, a critical question is whether numbers of antigen-specific T_{REG} differ in healthy and affected individuals.

Two groups have reported that peripheral blood T_{REG} isolated from patients with RA and from control individuals showed no difference in their ability to suppress effector T cell proliferation.^{24,25} However, another group reported a striking defect in the capacity of T_{REG} from patients with RA to suppress effector T cell proliferation.³² These divergent results could reflect differences in the populations of patients, the methods used to purify T_{REG}, or how the suppression assays were performed. Some evidence suggests that T_{REG} from RA patients are defective in their ability to suppress the production of two principal proinflammatory cytokines—interferon (IFN)- γ and tumor necrosis factor (TNF)—by effector T cells.²⁵ This defect is associated with decreased surface expression of cytotoxic T-lymphocyte antigen 4 (CTLA-4) on T_{REG} and can be reversed by increasing CTLA-4 expression with phorbol ester treatment *in vitro*.²⁷ Importantly, effector T cells can become resistant to suppression by T_{REG} in patients with autoimmune disease, and this observation can influence interpretation of the above results.³³ Overall, whether the reported defects in

T_{REG} function in patients with RA are the cause or result of chronic inflammation is not yet clear.

Effects of approved drugs on T_{REG}

Although much still remains to be clarified about how T_{REG} defects might contribute to the pathogenesis of RA, approaches that specifically boost T_{REG} activity could be useful in the treatment of RA. High concentrations of TNF can block the immunosuppressive functions of T_{REG} *in vitro*.³² In this context, a notable observation is that treatment of RA patients with anti-TNF drugs bolsters T_{REG} suppression of proinflammatory cytokine production.²⁵ For example, infliximab treatment restores the ability of T_{REG} to suppress the production of IFN- γ and TNF by effector T cells, an effect that could be attributable to either improved T_{REG} function or increased sensitivity of effector T cells to such suppression. However, several additional findings suggest that TNF has direct effects on T_{REG} that can be inhibited by infliximab. First, restoration of T_{REG} function was accompanied by increased numbers of CD4⁺CD25^{high} T cells in the blood of patients after anti-TNF treatment. Second, further work from the same group showed that these cells are induced T_{REG} that express low levels of L-selectin (CD62L) and suppress effector cell cytokine production via transforming growth factor β and IL-10.³⁴ Finally, anti-TNF treatment decreased expression of TNF receptor 2 and GITR, and increased FOXP3 expression, within the CD4⁺CD25^{high} population.³²

Key points

- FOXP3⁺ regulatory T cells (T_{REG}) control autoimmunity in humans
- Some evidence suggests that patients with rheumatoid arthritis have defects in T_{REG}, but whether these defects are the cause or result of chronic inflammation is not clear
- Several approved and experimental drugs promote function or increase numbers of T_{REG}, and these effects may be responsible for these drugs' efficacy in treating RA
- Cellular therapy with autologous *ex vivo* expanded T_{REG} may prove effective as a treatment for patients with RA

Tocilizumab, an antibody that blocks the human IL-6 receptor, has shown efficacy in the treatment of RA^{35–38} and has been approved by European regulators for this indication. IL-6 is a proinflammatory cytokine produced by a variety of hematopoietic cells, including dendritic cells. IL-6 can block the immunosuppressive activity of T_{REG} in mice.³⁹ By contrast, blockade of the IL-6 receptor with a monoclonal antibody in mice attenuates the severity of graft-versus-host disease and increases the absolute number of T_{REG} in the spleens of treated mice through conversion of peripheral CD4⁺ T cells to T_{REG}.⁴⁰ IL-6 prevents FOXP3 upregulation in human T cells *in vitro*.^{41,42} Thus, blockade of the IL-6 receptor may benefit patients with RA, at least in part, through augmenting the conversion of peripheral effector T cells to T_{REG}, perhaps by preventing IL-6 from driving CD4⁺ cells toward a type 17 T-helper cell (T_H17) phenotype.⁴³ Importantly, IL-6 reportedly has no effect on the immunosuppressive capacity of T_{REG} from the synovial fluid of patients with RA.⁴⁴ More work needs to be done, therefore, to elucidate the effects of IL-6 receptor blockade on the balance between T_{REG} and T_H17 differentiation.

Another drug class that may affect T_{REG} function in patients with RA includes the FDA-approved CD28 co-stimulation blocker, abatacept (a CTLA4-Ig fusion protein), and its higher-affinity derivative, belatacept. These drugs bind to the CD28 ligands CD80 and CD86 with a higher affinity than does the CD28 receptor, which prevents T-cell co-

stimulation and interferes with T-cell-driven autoimmune processes.⁴⁵ Long-term belatacept treatment as part of an immunosuppressive regimen after kidney transplant had no effect on the percentage of circulating CD4⁺FOXP3⁺ T cells but increased the percentage of FOXP3⁺ T cells in biopsy samples from kidneys undergoing acute rejection.⁴⁶ However, in mice, complete blockade of CD28 binding to CD80 and CD86 results in a precipitous loss of T_{REG}.⁴⁷ These apparently contradictory results are probably related to the subsaturating doses of belatacept that were used in the human studies,⁴⁶ which resulted in selective loss of effector T cells and an increased proportion of T_{REG} in inflamed tissue.^{48,49} Determining the optimal dose of CTLA4-Ig in patients with RA and other diseases will be critical if effector T cells are to be selectively inhibited while T_{REG} function is preserved.

Rapamycin is an immunosuppressive small-molecule drug with a wide variety of effects on cells of both the innate and adaptive immune systems.⁵⁰ Importantly, rapamycin and other mTOR (mammalian target of rapamycin) inhibitors promote human T_{REG} survival and differentiation and block effector T cell proliferation.^{51,52} Patients with RA who were treated with both the rapamycin derivative, everolimus, and methotrexate showed a greater response rate than patients treated with methotrexate alone. Thus, mTOR inhibition may ameliorate autoimmunity in part by promoting T_{REG} function. However, to what extent the clinical efficacy of rapamycin results from its effects on T_{REG} is not known.

Experimental drugs that affect T_{REG}

Toll-like receptors (TLRs) are expressed on cells of the immune system and respond to the presence of microbial products as well as to human self-molecules such as RNA and DNA.⁵³ T_{REG} express multiple TLRs, and different TLR ligands regulate the immunosuppressive capacity of murine T_{REG} *in vitro* and *in vivo* (reviewed elsewhere^{54,55}). Mice deficient in TLR9 have elevated numbers of T_{REG} in gut-associated lymphoid tissue, which highlights the importance of TLRs to T_{REG} homeostasis.⁵⁶ Furthermore, synovial fluid from patients with RA contains TLR3 ligands, which promote inflammatory cytokine production in RA synovial fibroblasts.⁵⁷ In fact, hydroxychloroquine, a drug currently used to treat RA, may work in part by blocking TLR signaling.⁵⁸ IRS 954, an experimental antagonist of TLR7 and TLR9, reduces titers of antibodies to antinuclear antigens and decreases glomerulonephritis in lupus-prone (NZB × NZW F1) mice.⁵⁹ These results suggest that TLR antagonists could have therapeutic effects in patients with RA as a result of directly or indirectly boosting T_{REG} function.

Trichostatin A is a small-molecule inhibitor of histone deacetylases (HDACs) that increases numbers of T_{REG} in normal mice by increasing thymic output of T_{REG}.⁶⁰ A recent study demonstrated that the HDAC inhibitors MS-275 and vorinostat (suberoylanilide hydroxamic acid) induce FOXP3 expression and immunosuppressive capacity in human T cells activated *in vitro*.⁶¹ Trichostatin A may work by increasing the acetylation and functioning of FOXP3,⁶⁰ or by preventing T_{REG} from producing IL-17.⁶² Thus, promotion of T_{REG} differentiation might be a general result of HDAC inhibition, which suggests that HDAC inhibitors may, in future, have a role in therapy for autoimmune and inflammatory diseases. One HDAC inhibitor, vorinostat, has been approved by the FDA for the treatment of cutaneous T cell lymphoma,⁶³ and could be tested in patients with autoimmune disease.

IL-2 is a principal survival factor for T_{REG}⁶⁴ and, in mice, its absence may contribute to defective T_{REG} function in inflamed tissues.³¹ Mice treated with a stabilized form of IL-2 demonstrate increased T_{REG} proliferation and function.^{31,65} IL-2, also known as aldesleukin, is currently used in cancer therapy, and some evidence suggests that it might also boost T_{REG} numbers in humans.⁶⁶ Thus, administration of IL-2 could prove useful in the treatment of human autoimmune disease.

Cellular therapy moving to the clinic

First attempts to use cell-based approaches as therapy for inflammatory arthritis involved hematopoietic stem cell transplantation (HSCT) to treat active, destructive, refractory RA^{67–69} and juvenile idiopathic arthritis.^{70,71} Although this treatment was curative for some patients, prolonged immunosuppression after HSCT was associated with a significant risk of infection, which limited the potential of this therapy.⁷² However, data from these trials implicated T_{REG} as a critical component of the regulatory state of the newly reconstituted immune system after transplant. In patients with juvenile idiopathic arthritis treated with HSCT, post-transplant reconstitution of CD4⁺CD25^{high} T_{REG} was more rapid than that of CD4⁺CD25[–] cells. This preferential recovery of T_{REG} could result from the relative resistance of T_{REG} to apoptosis after genotoxic stress.⁷³ These results imply that other approaches to shifting the balance of the immune system away from inflammation and toward regulation, for example those that selectively bolster T_{REG} numbers or function, could prove to be successful and less risky than HSCT.

One approach to boost numbers of T_{REG} without myeloablative conditioning is to isolate T_{REG} from patients, expand them in culture, and then re-infuse the cells.⁷⁴ Since T_{REG} are only a small fraction of peripheral CD4⁺ T cells (5–7%), the most effective approach to T_{REG}-based immunotherapy will require *in vitro* expansion of natural T_{REG} under carefully controlled conditions to ensure the preservation of their regulatory capacity.^{3,75} *Ex vivo* T_{REG} expansion and re-infusion has successfully prevented or reversed a number of autoimmune diseases in mouse models, including models of inflammatory arthritis.^{76–80} In the future, several new techniques may be incorporated into T_{REG}-based immunotherapies to improve the efficacy and safety of this approach. For example, T_{REG} may be targeted to inflamed tissues by transducing polyclonal T_{REG} with antigen-specific T cell receptors, an approach previously used to modify effector T cells used in cancer immunotherapy.⁸¹ Alternatively, antigen-specific T_{REG} could be selected from a polyclonal population by stimulation with relevant peptides.⁸²

Pitfalls of cellular therapy

Several pitfalls are possible in the treatment of patients with T_{REG}. First, incomplete lineage commitment enables some human CD4⁺FOXP3⁺ cells to express nuclear factor ROR- γ t and to develop into IL-17-producing effector cells.^{62,83,84} Moreover, recent data from mice suggest that some fully differentiated T_{REG} may be unstable and able to trans-differentiate *in vivo* into effector memory T cells that produce pathogenic cytokines such as IFN- γ and IL-17.⁹⁵ Contamination of therapeutic T_{REG} with these unstable T_{REG}, or even with true autoreactive effector T cells, could lead to exacerbation rather than attenuation of the autoimmune process. Second, T_{REG} may be less effective in suppressing effector T cells in an autoimmune setting, and may even augment IL-17 production *in vitro*.^{86,87} Third, it is unclear whether a large polyclonal population of T_{REG} would be able to interrupt an autoimmune process that targets antigens found in specific tissues. Fourth, polyclonal T_{REG} therapy could result in generalized immunosuppression and increased susceptibility to infection. Finally, some patients with autoimmune disease may have poorly appreciated intrinsic defects in T_{REG} function that cannot be overcome by infusion of large numbers of these defective cells. In spite of these potential concerns, however, clinical trials are underway to test the therapeutic potential of T_{REG} in graft-versus-host disease.^{91,92}

Outlook

Although existing drugs significantly reduce the morbidity and mortality associated with RA, these therapies are not curative. Several drugs that affect T_{REG} numbers or function have shown efficacy in the treatment of RA. These results imply that direct administration of T_{REG} could be an ideal therapy to induce durable remission of RA, as these cells persist *in*

vivo and act in an antigen-specific manner. Thus, approaches that bolster T_{REG} numbers and functions could be a fruitful means of selectively and durably inhibiting pathologic inflammation without blocking protective immune responses against infection.

Review criteria

Papers cited in this Review were identified by searching the PubMed database using the search terms “T_{REG}” and “rheumatoid arthritis” and by using the reference lists from those papers to identify other relevant publications. Additional papers were suggested by colleagues or identified by searching PubMed using specific terms relevant to the various topics discussed in this Review. The reference list was last updated in July 2009.

Acknowledgments

The authors thank Todd Brusko and Amy Putnam for helpful discussions and for assistance with figure design.

References

1. Tang Q, Bluestone JA. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol. Rev.* 2006; 212:217–237. [PubMed: 16903917]
2. Liu W, et al. CD127 expression inversely correlates with FOXP3 and suppressive function of human CD4⁺ T_{REG} cells. *J. Exp. Med.* 2006; 203:1701–1711. [PubMed: 16818678]
3. Putnam AL, et al. Expansion of human regulatory T-cells from patients with type 1 diabetes. *Diabetes.* 2009; 58:652–662. [PubMed: 19074986]
4. Shevach EM. Mechanisms of Foxp3⁺ T regulatory cell-mediated suppression. *Immunity.* 2009; 30:636–645. [PubMed: 19464986]
5. Andre S, Tough DF, Lacroix-Desmazes S, Kaveri SV, Bayry J. Surveillance of antigen-presenting cells by CD4⁺ CD25⁺ regulatory T cells in autoimmunity: immunopathogenesis and therapeutic implications. *Am. J. Pathol.* 2009; 174:1575–1587. [PubMed: 19349365]
6. Hsieh CS, et al. Recognition of the peripheral self by naturally arising CD25⁺ CD4⁺ T cell receptors. *Immunity.* 2004; 21:267–277. [PubMed: 15308106]
7. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat. Immunol.* 2003; 4:330–336. [PubMed: 12612578]
8. Bacchetta R, et al. Defective regulatory and effector T cell functions in patients with *FOXP3* mutations. *J. Clin. Invest.* 2006; 116:1713–1722. [PubMed: 16741580]
9. Wildin RS, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 2001; 27:18–20. [PubMed: 11137992]
10. Mazzolari E, et al. A new case of IPEX receiving bone marrow transplantation. *Bone Marrow Transplant.* 2005; 35:1033–1034. [PubMed: 15778724]
11. Dorsey MJ, Petrovic A, Morrow MR, Dishaw LJ, Sleasman JW. FOXP3 expression following bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. *Immunol. Res.* 2009; 44:179–184. [PubMed: 19471859]
12. Baud O, et al. Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. *N. Engl. J. Med.* 2001; 344:1758–1762. [PubMed: 11396442]
13. Rao A, et al. Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. *Blood.* 2007; 109:383–385. [PubMed: 16990602]
14. Seidel MG, et al. Selective engraftment of donor CD4⁺25^{high} FOXP3-positive T cells in IPEX syndrome after nonmyeloablative hematopoietic stem cell transplantation. *Blood.* 2009; 113:5689–5691. [PubMed: 19478054]
15. Wang J, Wicker LS, Santamaria P. IL-2 and its high-affinity receptor: genetic control of immunoregulation and autoimmunity. *Semin. Immunol.* doi:10.1016/j.smim.2009.04.004.

16. Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human CD4⁺FOXP3 T cells by T-cell receptor stimulation is transforming growth factor- β dependent but does not confer a regulatory phenotype. *Blood*. 2007; 110:2983–2990. [PubMed: 17644734]
17. Miyara M, et al. Functional delineation and differentiation dynamics of human CD4⁺ T cells expressing the FOXP3 transcription factor. *Immunity*. 2009; 30:899–911. [PubMed: 19464196]
18. Floess S, et al. Epigenetic control of the *FOXP3* locus in regulatory T cells. *PLoS Biol*. 2007; 5:e38. [PubMed: 17298177]
19. Zhou L, Chong MM, Littman DR. Plasticity of CD4⁺ T cell lineage differentiation. *Immunity*. 2009; 30:646–655. [PubMed: 19464987]
20. Wieczorek G, et al. Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. *Cancer Res*. 2009; 69:599–608. [PubMed: 19147574]
21. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol. Rev*. 2008; 223:371–390. [PubMed: 18613848]
22. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4⁺CD25^{high} regulatory cells in human peripheral blood. *J. Immunol*. 2001; 167:1245–1253. [PubMed: 11466340]
23. Michel L, et al. Patients with relapsing–remitting multiple sclerosis have normal T_{REG} function when cells expressing IL-7 receptor α -chain are excluded from the analysis. *J. Clin. Invest*. 2008; 118:3411–3419. [PubMed: 18769633]
24. van Amelsfort JM, Jacobs KM, Bijlsma JW, Lafeber FP, Taams LS. CD4⁺CD25⁺ regulatory T cells in rheumatoid arthritis: differences in the presence, phenotype, and function between peripheral blood and synovial fluid. *Arthritis Rheum*. 2004; 50:2775–2785. [PubMed: 15457445]
25. Ehrenstein MR, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF- α therapy. *J. Exp. Med*. 2004; 200:277–285. [PubMed: 15280421]
26. Mottonen M, et al. CD4⁺ CD25⁺ T cells with the phenotypic and functional characteristics of regulatory T cells are enriched in the synovial fluid of patients with rheumatoid arthritis. *Clin. Exp. Immunol*. 2005; 140:360–367. [PubMed: 15807863]
27. Flores-Borja F, Jury EC, Mauri C, Ehrenstein MR. Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. *Proc. Natl Acad. Sci. USA*. 2008; 105:19396–19401. [PubMed: 19036923]
28. Cao D, et al. Isolation and functional characterization of regulatory CD25^{bright}CD4⁺ T cells from the target organ of patients with rheumatoid arthritis. *Eur. J. Immunol*. 2003; 33:215–223. [PubMed: 12594850]
29. Lawson CA, et al. Early rheumatoid arthritis is associated with a deficit in the CD4⁺CD25^{high} regulatory T cell population in peripheral blood. *Rheumatology (Oxford)*. 2006; 45:1210–1217. [PubMed: 16571607]
30. Lazarski CA, Hughson A, Sojka DK, Fowell DJ. Regulating T_{REG} cells at sites of inflammation. *Immunity*. 2008; 29:511–512. [PubMed: 18957259]
31. Tang Q, et al. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity*. 2008; 28:687–697. [PubMed: 18468463]
32. Valencia X, et al. TNF downmodulates the function of human CD4⁺CD25^{hi} T-regulatory cells. *Blood*. 2006; 108:253–261. [PubMed: 16537805]
33. Schneider A, et al. The effector T cells of diabetic subjects are resistant to regulation via CD4⁺ FOXP3⁺ regulatory T cells. *J. Immunol*. 2008; 181:7350–7355. [PubMed: 18981158]
34. Nadkarni S, Mauri C, Ehrenstein MR. Anti-TNF- α therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF- β . *J. Exp. Med*. 2007; 204:33–39. [PubMed: 17200409]
35. Emery P, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann. Rheum. Dis*. 2008; 67:1516–1523. [PubMed: 18625622]
36. Maini RN, et al. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum*. 2006; 54:2817–2829. [PubMed: 16947782]

37. Jones G, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. *Ann. Rheum. Dis.* doi:ard.2008.105197v1.
38. Genovese MC, et al. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum.* 2008; 58:2968–2980. [PubMed: 18821691]
39. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4⁺CD25⁺ T cell-mediated suppression by dendritic cells. *Science.* 2003; 299:1033–1036. [PubMed: 12532024]
40. Chen X, et al. Blockade of interleukin-6 signaling augments regulatory T cell reconstitution and attenuates the severity of graft versus host disease. *Blood.* 2009; 114:891–900. [PubMed: 19491393]
41. Yang L, et al. IL-21 and TGF- β are required for differentiation of human T_H17 cells. *Nature.* 2008; 454:350–352. [PubMed: 18469800]
42. Dominitzki S, et al. Cutting edge: trans-signaling via the soluble IL-6R abrogates the induction of FoxP3 in naive CD4⁺CD25⁺ T cells. *J. Immunol.* 2007; 179:2041–2045. [PubMed: 17675459]
43. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* 2007; 8:942–949. [PubMed: 17676045]
44. van Amelsfort JM, et al. Proinflammatory mediator-induced reversal of CD4⁺, CD25⁺ regulatory T cell-mediated suppression in rheumatoid arthritis. *Arthritis Rheum.* 2007; 56:732–742. [PubMed: 17328044]
45. Linsley PS, Nadler SG. The clinical utility of inhibiting CD28-mediated costimulation. *Immunol. Rev.* 2009; 229:307–321. [PubMed: 19426230]
46. Bluestone JA, et al. The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation. *Am. J. Transplant.* 2008; 8:2086–2096. [PubMed: 18828769]
47. Tang Q, et al. Cutting edge: CD28 controls peripheral homeostasis of CD4⁺CD25⁺ regulatory T cells. *J. Immunol.* 2003; 171:3348–3352. [PubMed: 14500627]
48. Genovese MC, et al. Efficacy and safety of the selective co-stimulation modulator abatacept following 2 years of treatment in patients with rheumatoid arthritis and an inadequate response to anti-tumour necrosis factor therapy. *Ann. Rheum. Dis.* 2008; 67:547–554. [PubMed: 17921185]
49. Genovese MC, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor α inhibition. *N. Engl. J. Med.* 2005; 353:1114–1123. [PubMed: 16162882]
50. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. *Nat. Rev. Immunol.* 2009; 9:324–337. [PubMed: 19390566]
51. Battaglia M, et al. Rapamycin promotes expansion of functional CD4⁺CD25⁺FOXP3⁺ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J. Immunol.* 2006; 177:8338–8347. [PubMed: 17142730]
52. Strauss L, Czystowska M, Szajnik M, Mandapathil M, Whiteside TL. Differential responses of human regulatory T cells (T_{REG}) and effector T cells to rapamycin. *PLoS One.* 2009; 4:e5994. [PubMed: 19543393]
53. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* 2004; 5:987–995. [PubMed: 15454922]
54. Conroy H, Marshall NA, Mills KH. TLR ligand suppression or enhancement of T_{REG} cells? A double-edged sword in immunity to tumours. *Oncogene.* 2008; 27:168–180. [PubMed: 18176598]
55. van Maren WW, Jacobs JF, de Vries IJ, Nierkens S, Adema GJ. Toll-like receptor signalling on T_{REG}s: to suppress or not to suppress? *Immunology.* 2008; 124:445–452. [PubMed: 18540962]
56. Hall JA, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity.* 2008; 29:637–649. [PubMed: 18835196]
57. Brentano F, Schorr O, Gay RE, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via Toll-like receptor 3. *Arthritis Rheum.* 2005; 52:2656–2665. [PubMed: 16142732]

58. Kyburz D, Brentano F, Gay S. Mode of action of hydroxychloroquine in RA—evidence of an inhibitory effect on Toll-like receptor signaling. *Nat. Clin. Pract. Rheumatol.* 2006; 2:458–459. [PubMed: 16951696]
59. Barrat FJ, Meeker T, Chan JH, Guiducci C, Coffman RL. Treatment of lupus-prone mice with a dual inhibitor of TLR7 and TLR9 leads to reduction of autoantibody production and amelioration of disease symptoms. *Eur. J. Immunol.* 2007; 37:3582–3586. [PubMed: 18034431]
60. Tao R, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat. Med.* 2007; 13:1299–1307. [PubMed: 17922010]
61. Lucas JL, et al. Induction of Foxp3⁺ regulatory T cells with histone deacetylase inhibitors. *Cell. Immunol.* 2009; 257:97–104. [PubMed: 19358983]
62. Koenen HJ, et al. Human CD25^{high}FOXP3⁺ regulatory T cells differentiate into IL-17-producing cells. *Blood.* 2008; 112:2340–2352. [PubMed: 18617638]
63. Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *Oncologist.* 2007; 12:1247–1252. [PubMed: 17962618]
64. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3⁺ CD25⁺ CD4⁺ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J. Exp. Med.* 2005; 201:723–735. [PubMed: 15753206]
65. Webster KE, et al. *In vivo* expansion of T_{REG} cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. *J. Exp. Med.* 2009; 206:751–760. [PubMed: 19332874]
66. Wei S, et al. Interleukin-2 administration alters the CD4⁺FOXP3⁺ T-cell pool and tumor trafficking in patients with ovarian carcinoma. *Cancer Res.* 2007; 67:7487–7494. [PubMed: 17671219]
67. Verburg RJ, et al. High-dose chemotherapy and autologous hematopoietic stem cell transplantation in patients with rheumatoid arthritis: results of an open study to assess feasibility, safety, and efficacy. *Arthritis Rheum.* 2001; 44:754–760. [PubMed: 11315914]
68. Teng YK, et al. Long-term followup of health status in patients with severe rheumatoid arthritis after high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation. *Arthritis Rheum.* 2005; 52:2272–2276. [PubMed: 16052541]
69. Snowden JA, et al. Autologous hemopoietic stem cell transplantation in severe rheumatoid arthritis: a report from the EBMT and ABMTR. *J. Rheumatol.* 2004; 31:482–488. [PubMed: 14994391]
70. De Kleer IM, et al. Autologous stem cell transplantation for refractory juvenile idiopathic arthritis: analysis of clinical effects, mortality, and transplant related morbidity. *Ann. Rheum. Dis.* 2004; 63:1318–1326. [PubMed: 15361393]
71. Brinkman DM, et al. Autologous stem cell transplantation in children with severe progressive systemic or polyarticular juvenile idiopathic arthritis: long-term follow-up of a prospective clinical trial. *Arthritis Rheum.* 2007; 56:2410–2421. [PubMed: 17599770]
72. Snowden JA, Kapoor S, Wilson AG. Stem cell transplantation in rheumatoid arthritis. *Autoimmunity* 1. doi:10.1080/08916930802198550.
73. Komatsu N, Hori S. Full restoration of peripheral Foxp3⁺ regulatory T cell pool by radioresistant host cells in scurfy bone marrow chimeras. *Proc. Natl Acad. Sci. USA.* 2007; 104:8959–8964. [PubMed: 17494743]
74. Brusko T, Bluestone J. Clinical application of regulatory T cells for treatment of type 1 diabetes and transplantation. *Eur. J. Immunol.* 2008; 38:931–934. [PubMed: 18395864]
75. Peters JH, et al. Clinical grade T_{REG}: GMP isolation, improvement of purity by CD127 depletion, T_{REG} expansion, and T_{REG} cryopreservation. *PLoS ONE.* 2008; 3:e3161. [PubMed: 18776930]
76. Masteller EL, et al. Expansion of functional endogenous antigen-specific CD4⁺CD25⁺ regulatory T cells from nonobese diabetic mice. *J. Immunol.* 2005; 175:3053–3059. [PubMed: 16116193]
77. Tang Q, et al. *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* 2004; 199:1455–1465. [PubMed: 15184499]

78. Scalapino KJ, Tang Q, Bluestone JA, Bonyhadi ML, Daikh DI. Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of *ex vivo* expanded regulatory T cells. *J. Immunol.* 2006; 177:1451–1459. [PubMed: 16849451]
79. Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4⁺CD25⁺ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J. Immunol.* 2002; 169:4712–4716. [PubMed: 12391178]
80. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4⁺CD25⁺ regulatory T cells. *J. Immunol.* 2003; 170:3939–3943. [PubMed: 12682220]
81. Morgan RA, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science.* 2006; 314:126–129. [PubMed: 16946036]
82. Arbour N, et al. A new clinically relevant approach to expand myelin specific T cells. *J. Immunol. Meth.* 2006; 310:53–61.
83. Beriou G, et al. IL-17-producing human peripheral regulatory T cells retain suppressive function. *Blood.* 2009; 113:4240–4249. [PubMed: 19171879]
84. Voo KS, et al. Identification of IL-17-producing FOXP3⁺ regulatory T cells in humans. *Proc. Natl Acad. Sci. USA.* 2009; 106:4793–4798. [PubMed: 19273860]
85. Zhou X, et al. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells *in vivo*. *Nat. Immunol.* doi:10.1038/ni.1774.
86. Annunziato F, et al. Phenotypic and functional features of human T_H17 cells. *J. Exp. Med.* 2007; 204:1849–1861. [PubMed: 17635957]
87. Evans HG, Suddason T, Jackson I, Taams LS, Lord GM. Optimal induction of T helper 17 cells in humans requires T cell receptor ligation in the context of Toll-like receptor-activated monocytes. *Proc. Natl Acad. Sci. USA.* 2007; 104:17034–17039. [PubMed: 17942669]
88. Riley JL, June CH, Blazar BR. Human T regulatory cell therapy: take a billion or so and call me in the morning. *Immunity.* 2009; 30:656–665. [PubMed: 19464988]
89. Umbilical cord blood T-regulatory cell infusion followed by donor umbilical cord blood transplant in treating patients with high-risk leukemia or other hematologic diseases. *ClinicalTrials.gov.* 2009 [online] <http://clinicaltrials.gov/ct2/show/NCT00376519>.

Figure 1.

Effects on T_{REG} of various therapies for RA. Several immunomodulatory agents that are or may be effective in the treatment of RA boost numbers or function of T_{REG}. Cytokine-based therapies, such as IL-2 (aldesleukin) or agents that block TNF or the IL-6 receptor, may restore the regulatory capacity of T_{REG} and increase peripheral T_{REG} production and/or survival. TLR antagonists (for example, IRS 954) may restore the regulatory capacity of T_{REG} by decreasing production of inflammatory cytokines from APCs. Effector T-cell co-stimulation is blocked via competitive binding of CTLA4-Ig to CD28 ligands on APCs. Administration of CTLA4-Ig also increases the percentage of T_{REG} in inflamed tissue. HDAC inhibitors (for example, MS-275, vorinostat) may promote T_{REG} stability by increasing FOXP3 expression. Abbreviations: APC, antigen presenting cell; CTLA-4, cytotoxic T-lymphocyte antigen 4; FOXP3, forkhead box P3; HDAC, histone deacetylase; IL, interleukin; RA, rheumatoid arthritis; TNF, tumor necrosis factor; T_{REG}, regulatory T cells.