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Current perspectives

Regulatory T-cell therapy for autoimmune and autoinflammatory diseases: The next frontier

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Forkhead box P3–expressing regulatory T (Treg) cells are essential for self-tolerance, with an emerging role in tissue repair and regeneration. Their ability to traffic to tissue and perform complex therapeutic tasks in response to the tissue microenvironment make them an attractive candidate for drug development. Early experiences of Treg cell therapy in patients with graft-versus-host disease, type 1 diabetes, and organ transplantation have shown that it is feasible, safe, and potentially efficacious in some settings. Many ongoing trials in patients with a wide variety of diseases will further enhance our knowledge about the optimal approaches for Treg cell manufacturing and dosing. We review the current preclinical rationale supporting Treg cell therapy in a variety of disease settings ranging from tissue transplantation, autoimmune diseases, and non–immune-mediated inflammatory settings. We point out challenges in development of Treg cell therapy and speculate how synthetic biology can be used to enhance the feasibility and efficacy of Treg cell therapy for autoimmune and autoinflammatory diseases.

Key words: Regulatory T cell; cell therapy; transplant; autoimmune disease; clinical trials; Good Medical Practice manufacturing

Abbreviations used: ACT, Adoptive cell therapy; AIH, Autoimmune hepatitis; DC, Dendritic cell; Dsg, Desmoglein; FOXP3, Forkhead box P3; GvHD, Graft-versus-host disease; HSC, Hematopoietic stem cell; pTreg, Peripheral regulatory T; PV, Pemphigus vulgaris; SLE, Systemic lupus erythematosus; TCR, T-cell receptor; Treg, Regulatory T; tTreg, Thymic regulatory T

Regulatory T (Treg) cells are a small subset (5% to 10%) of peripheral $CD4^+$ T cells that are essential for maintaining immunologic tolerance. Decreased Treg cell numbers or function have been described in the setting of many autoimmune diseases in both patients and animal models.[1,2] Importantly, adoptive transfer of Treg cells has been shown to ameliorate autoimmune disease and prevent transplant rejection in mouse models.[3] These preclinical data have spurred the development of experimental Treg cell therapies, many of which are currently in clinical trials. In this review we will discuss the unique biology of Treg cells and describe both current clinical applications of and emerging approaches to Treg cell therapy, including important outstanding questions in the field (Table I).

Table I Some unknowns in Treg cell therapy

Treg cell biology and genetic engineering	• Relative effect of Treg cells on DCs versus effector T cells in patients • Importance localization (This should read "Importance of localization") of Treg cells to inflamed tissue versus lymph nodes • *In vivo* stability of Treg cell phenotype in patients • Effects of transgenic expression of chimeric antigen receptors or antigen-specific TCRs on Treg cell function in patients
Treg cell product manufacturing	• Optimal starting materials and Treg cell isolation methods • Effect that the patient's disease state has on Treg cell manufacturing outcomes • Optimal culture conditions and length of culture to maximize Treg cell efficacy • Treg cell product phenotypes associated with better clinical outcomes
Treg cell therapy clinical trial design	• Conditions with greatest potential benefit of Treg cell therapy • Optimal Treg cell dosing • Role of other drugs administered with Treg cell therapy • Timing of Treg cell dose(s)

Rationale for Treg cell therapy

Treg cells express the lineage-defining transcription factor forkhead box P3 (FOXP3) and a specific epigenetic signature throughout the genome.[4-6] In particular, demethylation of the Treg cell–specific demethylated region at the Foxp3 locus ensures stable FOXP3 expression and lineage stability of Treg cells.[7,8]

Treg cells develop in the thymus (thymic regulatory T [tTreg] cells) from immature thymocytes in response to self-antigen stimulation during T-cell development. Treg cells can also develop in the periphery from mature T cells (peripheral regulatory T [pTreg] cells) through exposure to specific microenvironments, particularly at mucosal sites in the presence of commensal microbiota.[7,9] Currently, there is no reliable cell-surface marker to distinguish tTreg cells from pTreg cells.

FOXP3 can also be induced in mature $CD4^+$ T cells *in vitro* by means of T-cell receptor (TCR) stimulation in the presence of IL-2 and TGF-β.[10] In this review we will be focusing primarily on freshly isolated Treg cells from peripheral blood, which are used in most clinical trials and consist predominantly of tTreg cells.

Treg cells exert dominant tolerance

A small number of Treg cells with limited TCR specificities can control diverse effector cells in a given tissue environment, a property termed dominant tolerance.[11] For example, a few thousand Treg cells specific for a mouse islet-specific hybrid peptide[12] can reverse diabetes after onset in nonobese diabetic mice despite the many antigens that are targeted by $CD4^{+}$ and $CD8^{+}$ T cells in this disease.[13,14]

Treg cells exert dominant tolerance through several mechanisms, including their constitutive expression of the high-affinity IL-2 receptor, which serves as a sink for IL-2 that controls effector cell expansion[14,15]; stimulating degradation of tryptophan to kynurenines (through indoleamine 2, 3-dioxygenase), which inhibit effector T cells[16]; and direct effects on antigen presentation by dendritic cells (DCs) by reducing expression of CD80/86 costimulatory molecules. This last activity depends on expression of cytotoxic T lymphocyte–associated antigen 4 on Treg cells, which binds with high affinity to CD80/CD86 and modulates their expression through transendocytosis.[17-19] These Treg cell functions are enhanced by TCR stimulation and exposure to inflammation. Finally, Treg cells can be activated to secrete anti-inflammatory cytokines, such as IL-10, TGF-β, and IL-35; express CD39 and CD73 to degrade proinflammatory extracellular ATP to immunosuppressive adenosine; and directly kill antigen-presenting cells in a perforin- and granzyme-dependent manner.[20] IL-2 signals are required for maximal Treg cell survival and suppressive function by signaling through signal transducer and activator of transcription 5b, enhancing Treg cell interactions with DCs, and increasing suppressive function.[15] TCR signaling enhances Treg cell–DC interactions and interferon regulatory factor 4 expression, which contributes to T cell–suppressive function.[21] The versatility of their suppressive functions makes Treg cells effective guardians of immune homeostasis.

Treg cell localization is critical for function

Treg cells suppress locally through direct contact and paracrine actions in the tissue in which they reside. Thus their ability to traffic to and accumulate in specific tissue is vital to their function, deploying different combinations of suppressive activities in response to tissue microenvironments. Resting Treg cells express CD62 ligand (This should be "CD62L" as this molecule is never referred to as "CD62 ligand") and CCR7 to home to secondary lymphoid tissue to control activation and clonal expansion of other T cells.[22] Depending on the context of their activation, Treg cells can mimic the phenotypes of $T_{H}1$, $T_{H}2$, $T_{H}17$, and follicular helper T effector cells through expression of the transcription factors T-bet, GATA-3, retinoic acid–related orphan receptor γT, and B-cell lymphoma 6, which drive the differentiation of these effector cells.[23-28] This additional layer of effector transcriptional program endows Treg-, $T_{H}1$-, $T_{H}2$-, and $T_{H}17$-like properties through expression of chemokine receptors and adhesion molecules to "shadow" distinct effectors to suppress inflammation in the target tissue (Fig 1). Importantly, adoptive cell therapy (ACT) of Treg cells could potentially exploit subsets of Treg cells to improve targeting of Treg cells to specific tissues and organs.

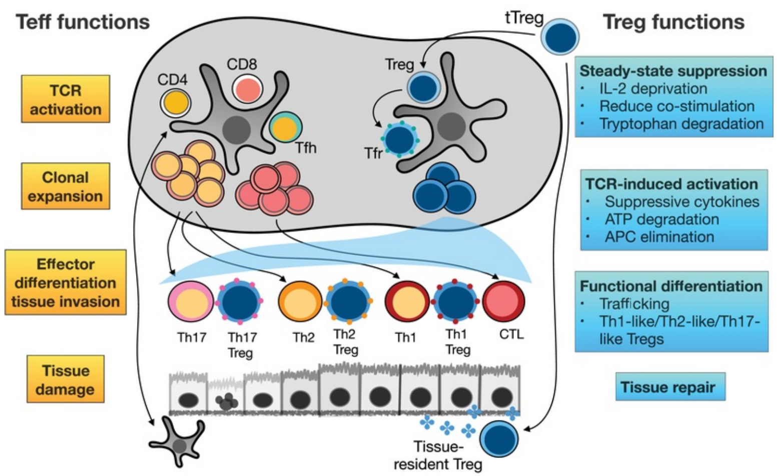

Fig 1 Treg cell mechanisms of action. Treg cells control inflammation and effector T-cell function through a variety of mechanisms both in lymph nodes and tissues. Treg cells mirror the phenotype of the effector cells that they regulate. In addition to controlling effector T-cell function, Treg cells contribute to tissue repair.

Tissue repair

In addition to their role in suppressing effector immunity, Treg cells also contribute to tissue homeostasis and repair.[29,30] For example, Treg cells produce the epidermal growth factor receptor ligand amphiregulin in damaged tissue. Amphiregulin deficiency does not affect Treg cell–suppressive function; however, Treg cell–derived amphiregulin protects the lungs of mice from damage during influenza virus infection.[31] This function of Treg cells is elicited through IL-18 and IL-33 in Treg cells that express IL-18 receptor and the IL-33 receptor ST2.[31] Treg cell–derived amphiregulin has also been implicated in the repair of damaged muscle and in patients with colitis (This should read: "...in the repair of damaged muscle and may be protective in models of colitis.").[32-35] ST2-expressing Treg cells are also enriched in adipose tissue, where their deficiency is associated with high-fat diet–induced obesity.[36-39] Skin-resident Treg cells have been implicated, facilitating (This should read: "Skin-resident Treg cells facilitate epithelial stem cell differentiation...") epidermal stem cell differentiation in hair regeneration and epidermal (delete: "epidermal") wound healing.[39,40] Similarly, Treg cells can directly promote oligodendrocyte differentiation from progenitor cells to promote myelination in the central nervous system.[41]

Taken together, these properties make Treg cells an attractive therapeutic candidate for diseases that are difficult to treat with conventional small-molecule and biologic drugs.

Reported clinical trials of Treg cell therapy

Fifty clinical trials of ACT of polyclonal Treg cell therapy have been completed or are ongoing in immune and nonimmune inflammatory disease settings (Fig 2, as listed at clinicaltrials.gov; also see Gliwinski et al[42] for a table). This work has been accomplished largely with *ex vivo*–expanded Treg cells, which are isolated based on cell-surface receptor expression (generally $CD4^{+}CD25^{+}CD127^{-}$ cells) and then expanded by using polyclonal activation through the TCR.[43,44] The methods of separation and expansion vary from site to site and patient populations,[43-46] yet overall, the published results suggest continued manufacturing success and excellent safety profiles in patients treated with as many as 2.5 billion Treg cells. In a subset of the studies, there is some suggestion of efficacy, although rigorous evidence is still lacking at this early stage.

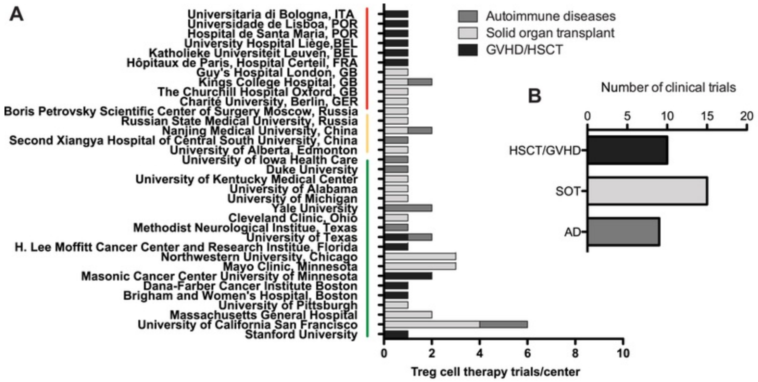

Fig 2 Ongoing Treg cell clinical trials. Clinical trials involving Treg cell therapy were identified on clinicaltrials.gov and categorized by indication and location. Data were collected in August 2018. *AD*, Autoimmune disease; *HSCT*, hematopoietic stem cell transplantation; *SOT*, solid organ transplantation.

ACT of Treg cells to prevent graft-versus-host disease

Preventing graft-versus-host disease (GvHD) after allogeneic stem cell transplantation was one of the first preclinical demonstrations of efficacy of Treg cell therapy.[47,48] To date, 4 phase I trials, 1 phase II trial, and 1 case study in hematopoietic stem cell (HSC) transplant recipients have been reported.

In one setting umbilical cord blood−derived Treg cells were expanded before infusion with HSCs from cord blood of a third-party donor. Despite the suboptimal dose and limited survival of the allogeneic Treg cells, the investigators observed a trend of delaying GvHD onset when compared with historical controls.[49-51]

In another setting, haploidentical donor-derived Treg cells were infused directly after isolation without *ex vivo* expansion, and patients received HSCs along with a high dose of donor T cells from the same donor without immunosuppression. Only a few cases of mild GvHD were observed, and none had chronic GvHD.[52,53] The investigators concluded that infusion of donor Treg cells enabled infusion of a high dose of conventional T cells for the prevention of cancer relapse, improved immune reconstitution, can be associated with lower CMV disease risk, and did not increase GvHD.

In a third small trial, a separate group of investigators infused *ex vivo*–isolated Treg cells into 5 treatment-refractory patients with chronic GvHD.[54] This study is consistent with a previous case report showing improvement of chronic GvHD with Treg cell infusion.[55]

ACT of polyclonal and alloantigen-specific Treg cells to prevent solid-organ transplant rejection

Treg cell therapy for the prevention of transplant rejection and induction of transplantation tolerance has a long history and rich preclinical data to support its efficacy.[3] Currently, 2 phase I trials in kidney transplantation have been reported. The first pilot trial treated subclinical inflammation present on 6-month surveillance biopsy with *ex vivo*–expanded Treg cells. The study showed that it is feasible to expand Treg cells from immunosuppressed patients. Moreover, the pharmacokinetics of the infused Treg cells, which were monitored by using a novel deuterium labeling approach, were similar to those of nonimmunosuppressed patients with type 1 diabetes.[56]

In a separate phase I trial, patients receiving living donor kidney transplants received alemtuzumab induction, followed by infusion of up to 5×10^9 Treg cells 60 days later. A 5- to 20-fold increase in the percentage of Treg cells in all subjects up to 1 year after transplantation was observed.[57]

Liver transplantation offers a clinical setting in which the efficacy of Treg cell therapy can be tested by withdrawing immunosuppressive drugs. A trial in Japan enrolled 10 living donor liver transplant recipients who received an autologous Treg cell–enriched cell preparation 13 days after liver transplantation and cyclophosphamide induction. Seven of the 10 patients were successfully withdrawn from immunosuppression.[58] Although the trial was not controlled and the product was a complex mixture of different cell types (including Treg cells), this result strongly suggests efficacy compared with a historic rate of 13% successful cessation of immunosuppression within 2 years of liver transplantation.

Extensive preclinical data in mice demonstrate that alloantigen-specific Treg cells have superior efficacy when compared with polyclonal Treg cells, thus potentially achieving more targeted suppression, reducing the dose, and leading to safer therapies with less chance of off-target activities. Donor alloantigen–reactive Treg cells exist at a high precursor frequency,[59-61] making it feasible to expand adequate cell numbers in short-term *ex vivo* culture. This approach provided the first opportunity to study antigen-specific Treg cell therapy in human subjects.[44] There are currently several ongoing transplant studies using alloantigen-reactive Treg cell populations.

ACT of Treg cells for autoimmune diseases

Treg cells have been shown to be impaired in a variety of autoimmune settings with documented reductions in Treg cell numbers, function, and survival; responsiveness to IL-2; and effector T-cell resistance to Treg cell suppression.[1] In some cases these findings are directly linked to genetic defects in Treg cells, such as mutations in the *FOXP3* gene, the master Treg cell transcription factor, resulting in immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Other mutations associated with Treg cell dysfunction are in loci, such as CD25 and cytotoxic T lymphocyte–associated antigen 4, which have been linked to autoimmunity risk in genome-wide association studies.[62] In the NOD mouse model, Treg cells do not control pancreatic islet destruction because of a survival disadvantage secondary to the paucity of IL-2 in chronically inflamed islets.[63] Single infusion *ex vivo* of expanded Treg cells prevents diabetes development in prediabetic mice and stably reverse diabetes in mice with recent disease onset.[13] Islet antigen-specific Treg cells are several orders of magnitude more potent than polyclonal Treg cells and promptly stop the progressive islet destruction by controlling LN priming of effector T cells and subvert effector functions in inflamed islets. These robust preclinical data provide a strong rationale for evaluating this therapeutic strategy in patients with type 1 diabetes mellitus.

Two small clinical trials of Treg cell therapy in patients with type 1 diabetes have been published. A trial conducted at the Medical University of Gdansk enrolled children within 2 months of diabetes onset. The patients were treated with autologous polyclonal $CD4^+CD25^+CD127^{lo/-}$ Treg cells, with doses ranging from 10×10^6/kg to 30 (This should be "20" not "30") $\times 10^6$/kg.[64] There were no serious adverse events caused by the Treg cell therapy after 1 year of follow-up. Treated patients had statistically lower insulin requirements and higher C-peptide levels compared with matched control subjects. Furthermore, 2 of 12 treated patients were insulin independent compared with 0 of 10 control subjects.[65]

A second trial of polyclonal autologous Treg cell therapy enrolled 14 adult patients who were infused with Treg cells in 4 dose-escalating cohorts ranging from 5×10^6 to 2.6×10^9 *ex vivo*–expanded Treg cells. Several patients had stable C-peptide levels and insulin use for up to 2 years after therapy, although the study was not controlled or powered to determine efficacy. Pharmacokinetic monitoring of autologous Treg cells after infusion based on deuterium labeling of the infused cells showed that the ACT Treg cells peaked in circulation in the first 2 weeks and then followed a 2-phase decay, losing 75% of the peak level in the first 3 months and then stabilizing for at least 1 year (the last time point for follow-up). Importantly, deuterium labeling was never found in non-Treg cells, indicating that the infused Treg cells were stable.[66]

In summary, a number of ACT trials with Treg cells have been published. The following section and Fig 2 highlight the large number of active clinical trials of Treg cell therapy for various indications.

Emerging ACT Treg cell therapy for autoinflammatory and autoimmune diseases

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a complex disease involving aberrant innate and adaptive immune responses, with increasing evidence for a key contribution of Treg cells, especially during disease flares.[67-71] ACT of *ex vivo*–expanded Treg cells in autoantibody-positive diseased mice delayed the onset of renal complications and prolonged survival.[67,72] A pilot study of 37 patients with SLE treated with a 5-day course of low-dose IL-2 showed clinical efficacy with increased Treg cell numbers and decreased SLE Disease Activity Index scores.[73] In a recent report a patient with SLE with active skin disease (discoid lupus) received 1×10^8 autologous polyclonally expanded Treg cells. Although the SLE Disease Activity Index score remained unchanged after infusion, the number of immune infiltrates was greatly reduced in postinfusion biopsy specimens.[74]

Inflammatory bowel disease

Inflammatory bowel disease includes both Crohn disease and ulcerative colitis. Inflamed mucosa of patients with inflammatory bowel disease showed only a modest increase in Treg cell numbers compared with other inflammatory conditions, such as diverticulitis.[75] Furthermore, mucosal, but not peripheral blood–derived, T cells are resistant to Treg cell suppression because of overexpression of Smad7, an inhibitor of TGF-β signaling.[76] $CD45RA^+$ Treg cells from the blood of patients with Crohn disease can be expanded. The Levings group recently demonstrated that Treg cells can be tailored for migration into T_H1-inflammed sites by adding IFN-γ and IL-12 during expansion, resulting in epigenetically stable $CXCR3^+$T-$bet^+$$FOXP3^+$ Treg cells.[77]

Numerous preclinical models exist, including dextran sulfate sodium colitis; intrarectal administration of agents, such as 2,4,6-trinitrobenzene sulfonic acid or oxazolone; and IL-10 knockout mice.[78] The most common model used to study Treg cell function in patients with experimental colitis is based on adoptive transfer of CD4 naive or Treg cell–depleted T cells into syngeneic immunodeficient severe combined immunodeficiency or $Rag^{-/-}$ mice.[79] By using this model, type 1 regulatory cells (IL-10–producing Treg cells) were shown to be more potent at preventing colitis than $CD4^+CD25^{hi}$ T cells, suggesting a critical role for IL-10.[80]

Canavan et al[81] developed a humanized mouse model in which they implanted fetal small bowel tissue subcutaneously for 12 to 16 weeks before induction of colitis. Colitis was induced with enteropathogenic *Escherichia coli* 18 hours after injection of 10^6 Treg cells together with rIL-2 (10^4 IU; Proleukin, Prometheus Laboratories, San Diego, Calif). Treg cells homed to the inflamed human lamina propria and suppressed *in vitro* lamina propria–derived effector T cells.

A 12-week, open-label, uncontrolled, multicenter, single-infusion phase I/IIa clinical study of type 1 regulatory ovalbumin-specific Treg cells was performed in 20 patients with refractory CD. The infusion was well tolerated and showed dose-related efficacy.[82] A multicenter phase II trial has been completed, but results are still not available (NCT02327221). Finally, a double-blind, placebo-controlled, phase I-II clinical trial using tTreg cells has not started recruiting (NCT03185000).

Pemphigus vulgaris

Pemphigus vulgaris (PV) is an autoimmune bullous disease caused by IgG autoantibodies targeting the desmosomal adhesion proteins desmoglein (Dsg) 1 and Dsg3. The antibodies target the desmosome, resulting in acantholysis. Treg cell numbers seem to be reduced in patients with PV.[83] In the preclinical model of transferring splenocytes from Dsg3-immunized $Dsg3^{-/-}$ and $Rag2^{-/-}Dsg3^{+/+}$ mice, polyclonal expanded Treg cells, including those from $Dsg3^{-/-}$ animals, reduced disease activity and anti-Dsg3 antibody production.[84] Schmidt et al[85] used a HLA-DRB1 04:02 transgenic PV mouse model immunized with human Dsg3 and showed a critical role for Treg cells in inhibiting the Dsg3-driven T-cell response, as well as Dsg3-specific antibodies. A phase I multicenter clinical trial of polyclonal autologous Treg cells therapy in patients with PV started in 2017 and is currently actively recruiting patients (NCT03239470).

Autoimmune hepatitis

Autoimmune hepatitis (AIH) is diagnosed based on an increase in liver enzyme levels in the presence of autoantibodies and immune cell infiltration in the liver.[86] The number and role of Treg cells in AIH pathogenesis has been controversial. Longhi et al[87] showed decreased Treg cell numbers in peripheral blood of 41 patients compared with control subjects, whereas others have not seen differences in Treg cell frequency in peripheral blood.[88,89] It has been reported that liver tissue normally has very low concentrations of IL-2, and thus generally, it is not supportive of Treg cell survival and function.[90] Higher frequencies of intrahepatic Treg cells correlate with better response to therapy.[88] The development of a preclinical model for AIH has been challenging.[91-93] Autoimmune regulator(This should be "AIRE". No one will understand "Autoimmune regulator")–deficient mice have an AIH-like disease with autoantibodies and lymphoplasmatic and hepatic infiltrates. Treg cell numbers were decreased, and ACT of 8×10^5 polyclonal nonexpanded Treg cells reversed the AIH histological lesions.[93] A phase I clinical trial for treating AIH with Treg cells has been registered (NCT02704338).

Allergy and asthma

Mouse models of allergy and asthma have implicated Treg cells as an important component of disease pathogenesis. For example, a mutation in *IL4RA* destabilized pTreg cells and drives T_H17-skewed airway inflammation.[94] Treg cells play a role in oral tolerance in a peanut allergy model in mice.[95] In patients (Delete the "In patients")Treg cell numbers can be decreased in patients with asthma, and there is also some evidence for reduced FOXP3 expression and Treg cell dysfunction.[96-98] In addition, asthmatic patients have an increased frequency of IL-17^+ Treg cells.[99] This population might represent destabilized Treg cells becoming pathogenic T_H17 effector cells. Because antigen triggers are often known in patients with allergy and asthma, these diseases could potentially be treated with antigen-specific Treg cells.

Current challenges and future prospects of Treg cell therapy

Although Treg cell therapy has a clear immunologic rationale, there are many challenges at this early stage of its implementation and testing. The magnitude of *ex vivo* expansion of Treg cells can be highly variable depending on patient population and underlying diseases. There are also limited clinical-grade (Good Manufacturing Practice) reagents and instruments specifically designed for Treg cell manufacturing. In many disease models, tissue antigen-specific Treg cells are more potent than polyclonal Treg cells in controlling disease progression. However, the specificities of these cells are largely unknown. Because of the very low frequency of tissue antigen-specific Treg cells and the propensity of Treg cells to destabilize with repeated *in vitro* stimulation, there is no established approach to manufacture tissue antigen-specific Treg cells. Clinically, the types and stages of diseases that are likely to be responsive to Treg cell therapy are yet to be defined. Moreover, patients with autoimmune disease are often treated with a wide variety of immunosuppressive drugs that can interact with Treg cells, making it challenging to evaluate the effect of Treg cell therapy.

Synthetic biology approaches, such as chimeric antigen receptors, have brought transformative advances to cancer therapy. Such approaches have the potential to improve the efficacy of Treg cell therapy. The efficacy of redirecting Treg cell specificity by using chimeric antigen receptors has been demonstrated in multiple mouse models.[100-106] Alternately, tissue antigen-specific TCRs could be identified by using single-cell TCR sequencing and used to redirect Treg cell specificity.[62,107] Several groups have inserted TCRs specific for insulin and glutamate decarboxylase into human Treg cells, which enables antigen-specific suppression.[108,109] At this stage, it remains unclear whether repurposed TCRs derived from effector T cells have the same biological function as TCRs from Treg cells. In addition to redirecting specificity, synthetic biology can be used to promote Treg cell fitness. For example, mutated IL-2 receptors could be introduced into Treg cells to allow for orthogonal signaling with an engineered mutant IL-2 to selectively expand infused Treg cells.[110]

Concluding remarks

In summary, Treg cell therapy is now emerging as a potential therapy for a wide variety of autoimmune and inflammatory diseases. There are a number of clinical trials underway. Results emerging from these trials over the coming years will be critical to charting the future of Treg cell therapies. Based on Treg cell biology, we can expect 2 distinct goals of Treg cell therapy: (1) to restore peripheral self-tolerance in an antigen-specific fashion and (2) to suppress inflammation and promote tissue repair (Fig 3). In the settings of transplantation, type 1 diabetes, and celiac disease, restoration of tolerance is of primary consideration, although in many diseases, such as IgE-mediated allergy[111] and amytrophic lateral sclerosis,[112] suppressing chronic inflammation and inducing tissue repair will be needed to restore tissue homeostasis. With better understanding of the immunopathophysiology of these diseases, future Treg cell therapy can be tailored by using tools of synthetic biology to achieve durable disease remission.

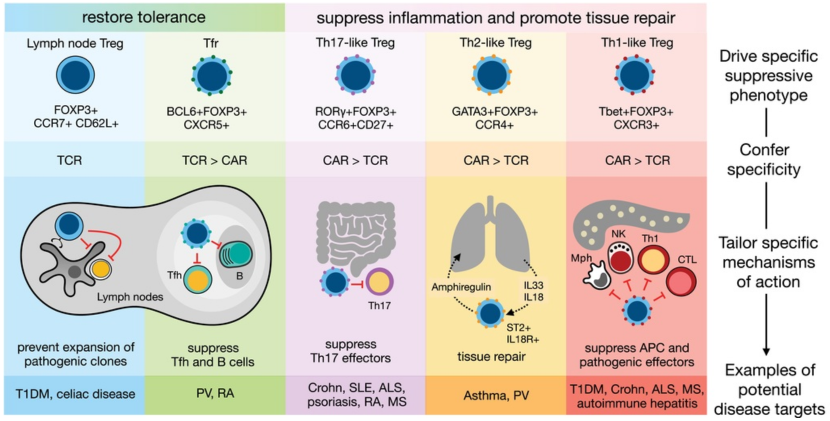

Fig 3 Potential future applications of Treg cells to target specific tissues and diseases. Therapeutic Treg cell products could be differentiated to suppress specific inflammatory T-cell subsets. Treg cells can be engineered further to traffic to diseased tissue through expression of specific antigen receptors. In conferring antigen specificity, we note that the suppressive activity of Treg cells in lymph nodes would more likely involve TCRs targeting specific antigens, whereas CARs might be more effective in tissues. Taken together, these approaches might be sufficient to target specific tissues or organs and treat a variety of autoimmune and autoinflammatory diseases. *ALS*, Amytrophic lateral sclerosis; *APC*, antigen-presenting cell; *CAR*, chimeric antigen receptor; *CTL*, cytotoxic T cells; *Mph*, macrophage; *MS*, multiple sclerosis; *NK*, natural killer cells; *RA*, rheumatoid arthritis; *T1D*, type 1 diabetes mellitus; *Tfh*, follicular helper T cells; *Tfr*, follicular regulatory T cells.

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Abbreviations: HSCT/GVHD, hematopoietic stem cell transplant/graft versus host disease; SOT, solid organ transplant; AD, autoimmune disease

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