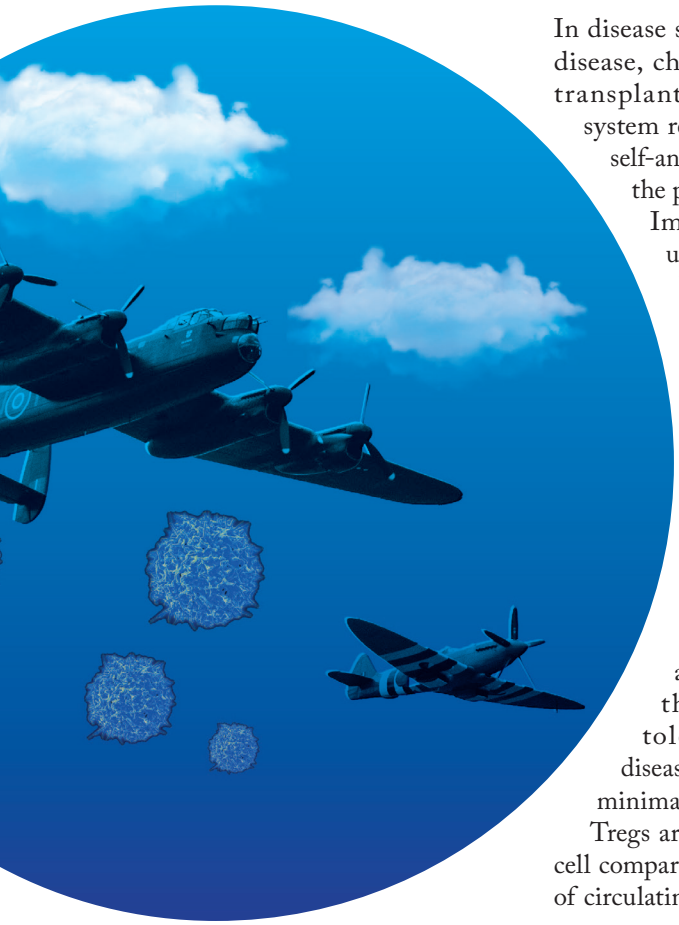
Cell Therapy for Autoimmune Disease: Armored Tregs to the Rescue

How Treg-cell therapy could transform the way we treat autoimmune disease and transplant rejection

By Raul Elgueta and Cristina del Carmen Rosello



In disease states such as autoimmune disease, chronic viral infection, and transplant rejection, the immune system responds inappropriately to self-antigens or doesn't resolve once the pathogen has been removed. Immunosuppressants may be used to reduce inflammation, but current biologic and small-molecule therapies must be administered over the long-term and can only alleviate symptoms. Regulatory T cells (Tregs) maintain a healthy immune response by suppressing inappropriate activation. And, in recent years, researchers have turned to Tregs to develop adoptive cellular therapies that can restore immune tolerance in autoimmune disease and transplantation – with minimal side effects.

Tregs are a subcomponent of the T cell compartment. Around five percent of circulating CD4+ T cells are Tregs, which can be identified by expression of the transcription factors FOXP3 and Helios, together with high expression of cell surface marker IL-2 receptor (CD25). In addition, the subunit α of the IL-7 receptor (CD127) is downregulated, which is inversely correlated to the suppression function of human Tregs (1). Lastly, the demethylation of the Treg-specific demethylated region (TSDR), an evolutionary conserved noncoding region of the FOXP3 locus, is the best marker for the stability of Tregs (2). Clinically stable Tregs are defined as a CD4+CD25+CD127low/- with over 80 percent of demethylation in the TSDR.

Once Tregs are activated via their cognate antigen, they suppress immune response by i) releasing inhibitory cytokines; ii) expressing suppressor cell surface molecules, such as CTLA-4, PD-1, Vista; and iii) depriving nutrients needed for T cell activation. These mechanisms block dendritic cell maturation and abrogate effector T cell proliferation and function. And that's why this subset of CD4+ T cells are showing promise in the development of

cellular therapies for autoimmune disease and transplantation.

Current state of play

Currently, three main Treg-cell products are being developed for adoptive cell therapy: polyclonal Tregs, antigen-specific Tregs and chimeric antigen receptor (CAR) Tregs (see Table 1). In clinical trials, polyclonal Treg cells isolated from peripheral blood tend to be used, as these cells can be readily expanded in vitro. Polyclonal Treg-cell therapy has been found feasible and safe in different clinical settings, including kidney transplant and autoimmune type 1 diabetes (3,4). However, these studies have failed to demonstrate efficacy – and this failure has been attributed to the low Treg specificity of the therapy.

In the context of transplantation, the second approach for adoptive Treg therapy is the use of antigen-presenting cells from donors to stimulate in vitro Tregs from recipients (5). This method provides greater specificity than polyclonal Tregs, but the yield of cells is very low in comparison, and it cannot be applied to expand Tregs from patients with autoimmune diseases. Therefore, this Treg product has not successfully moved forward into the clinic.

The third approach is the expansion of polyclonal Tregs genetically engineered to contain a chimeric antigen receptor (CAR) or a transgenic T cell receptor (TCR) expressed on the cell surface to increase the specificity of the therapy. In recent years, CAR T-cell therapy has been successful in the oncology field, but has seen significant cytotoxic side effects associated with cytokine release syndrome and neurotoxicity. In contrast, CAR Treg-cell therapy would be expected to have the opposite effect and dampen down inflammation in autoimmune disease and promote transplant tolerance. Transgenic TCRs and CARs should play an important role in the future adoptive Treg-cell therapy clinical landscape, given the antigen specificity they are able to introduce.

Adoptive cell therapy	*Strengths*	*Weaknesses*
Polyclonal Treg therapy	• Easy to isolate and get a good cell yield after expansion	• No specificity • Low suppression capacity
Antigen-specific Tregs	• High specificity and suppression capacity	• Cell yield is low after expansion
Genetically engineered Tregs (TCR or CAR)	• High specificity and suppression capacity • High number of antigen-specific cells is obtained after expansion	• Cost of the therapy is elevated • Long waiting time
Allogeneic engineered Tregs	• Reduced cost and waiting time • Reduced variability across the process	• Risk of rejection
hiPSC-Tregs	• Limitless capacity for gene engineering • Rejuvenated Tregs • Universal cell line (GMP grade characterized cell line, edition of HLA)	• Lack of protocols to generate Tregs from hiPSCs

Table 1. Different adoptive regulatory T cell therapy strategies

What about allogeneic approaches?

Autologous adoptive cellular therapy is currently the most promising model of Treg-cell treatment, but there are challenges. First, the starting material required must be of high quality. In many cases, patients' T cells are exhausted and unable to be expanded or their numbers are too low for the manufacturing process. Second, engineering and expansion protocols are long and there is a risk of the patient deteriorating rapidly – shrinking the window of time where the therapy could be efficacious. Finally, the price per treatment tends to be high; for example, the cost of the CD19 CAR T-cell therapy for B cell lymphoma is currently around $475,000 (6). Thus, an alternative therapeutic approach is needed to reduce both the cost and time of the manufacturing process.

Allogeneic or "off-the-shelf" Treg-cellular therapy could be the answer. This approach involves generating CAR Tregs expanded from a bank of healthy donors with the best possible human leukocyte antigen (HLA)-match. In the short term, this may be sufficient to establish the suppressive environment in both autoimmune disease and in transplant tolerance. The isolation and preparation of Tregs from healthy donors is advantageous

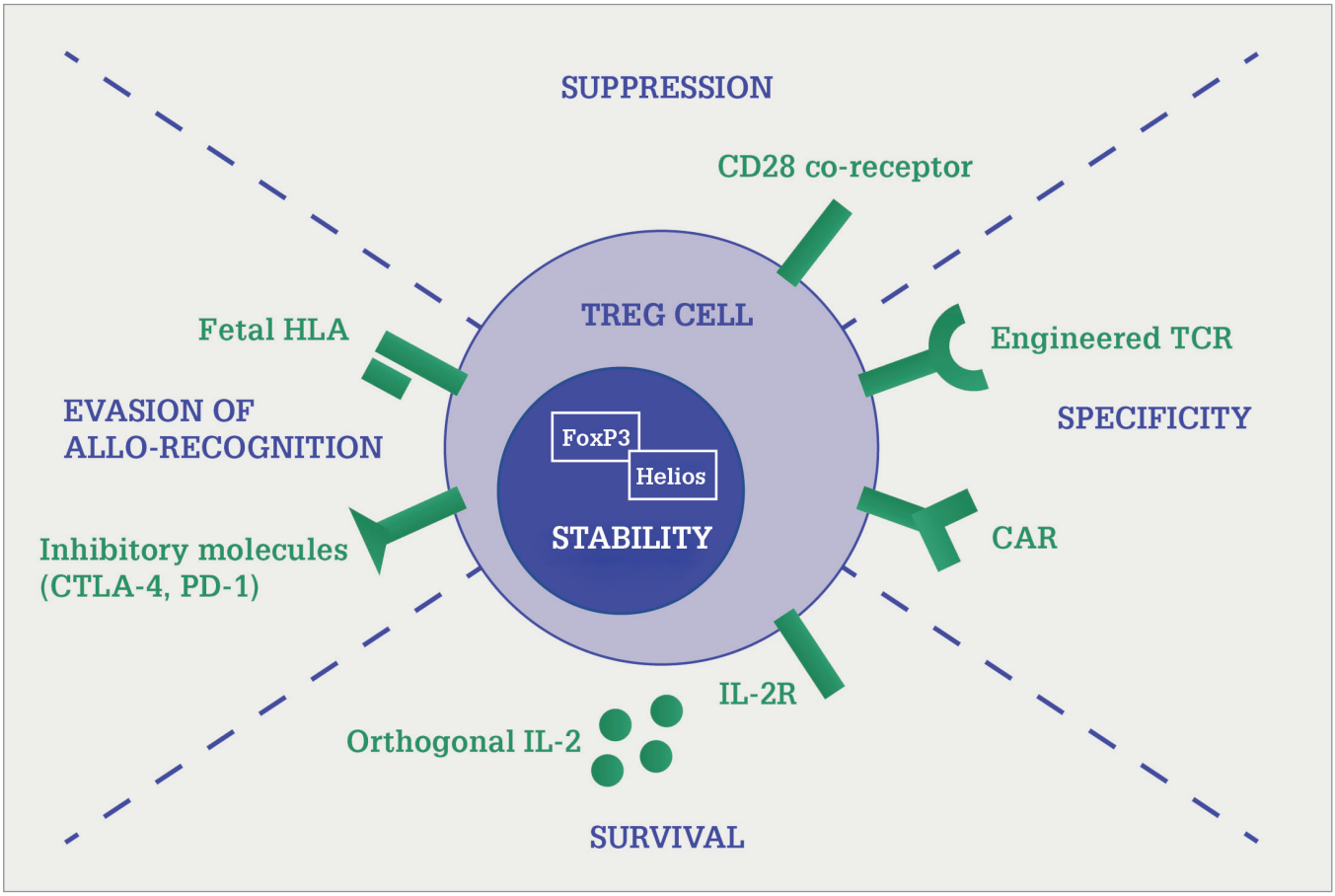
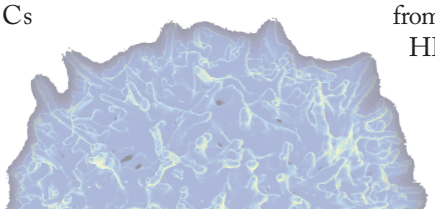


Figure 1. Summary of accessories that can be included in adoptive Treg therapy. These accessories will tailor the survival, stability, specificity, and evasion of allo-recognition of the therapy.

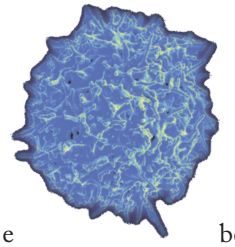
"As we've seen, there is room for improvement regarding the potency and efficacy of Treg-cellular therapies."

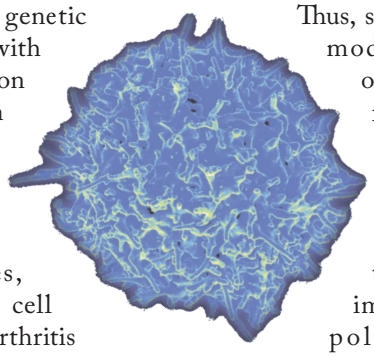
as it helps reduce variability in expansion, increases the quality of the starting material and reduces the treatment time. Nevertheless, this method is susceptible to host-mediated allo-rejection of the transferred cells, which will likely limit repeat dosing and long-term efficacy. Therefore, developing Treg cells that can evade host-mediated immune recognition will present exceptional opportunities in the creation of off-the-shelf therapies.

At this point, the use of human induced pluripotent stem cell-derived Tregs (hiPSC-Tregs) for allogeneic therapy appears an attractive alternative. hiPSCs can be expanded easily and could be an endless source of Tregs given that they are amenable to biotherapeutic manufacturing processes. Computational approaches to cell reprogramming are well placed to identify new genes needed to accelerate and improve the process of generating both consistent and well-characterized batches of hiPSC-Tregs (14). Importantly, hiPSCs would generate "rejuvenated" Tregs with longer telomeres which will improve expansion and prevent cell cycle exhaustion (7). Finally, the genome of hiPSCs can be routinely modified in the lab, bringing a wide range of possibilities: from adding CARs to editing HLA identity.



However, there is a clear need to establish robust protocols for the generation of Tregs from hiPSCs. Mohammad Haque and colleagues have developed a method based on the genetic modification of iPSCs with the FOXP3 transcription factor followed by in vitro stimulation with Notch ligand (8). The resulting Treg cells were able to produce suppressive cytokines, inhibit other immune cell activities and suppress arthritis development in an adoptive transfer context (8). Notably, this study was only carried out in a murine model, and efforts are now focused on unraveling how Tregs are developed in the human thymus and in defining protocols to generate phenotypically stable Tregs from hiPSCs. Here, the deployment of next-generation sequencing and gene regulator/epigenetic network data could play a key role. Through the systematic identification of gene regulators and soluble factors, we can expect to enhance the generation, maintenance and stability of hiPSC-Tregs for cellular therapies (14, 15).





Armoring allogeneic Tregs

As we've seen, there is room for improvement regarding the potency and efficacy of Treg-cellular therapies. And four promising avenues are emerging; namely, improving survival, stability, specificity, and evasion of allo-recognition (see Figure 1).

A major concern is the stability of Tregs, which means that Tregs are not in a terminally differentiated state. Due to their plasticity, Tregs can adopt an effector T cell phenotype depending on the environmental signals. Recently, several transcription factors (FOXP3, Helios, BACH2, NRP-1) have been identified as key to maintaining Treg stability. Thus, strategies to genetically modify the expression of these transcription factors and obtain phenotypically stable Tregs are being investigated. For instance, gene transfer of FOXP3 in immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) patient-derived CD4 T cells that generate potent suppressor T cells, which in turn sustain a regulatory phenotype in inflammatory conditions (9).

To promote Treg survival, some researchers have focused on precisely targeting IL-2 to guide cells to their target. Low dose IL-2 treatment has shown to increase Treg numbers in patients, but it is also able to induce proliferation of other proinflammatory immune cell sets. To address this problem, researchers are developing a human anti-IL2/IL-2 complex that preferentially stimulates Treg expansion over effector T cells or the combination of an orthogonal IL-2R engineered Tregs paired with an orthogonal IL-2 (10).

To improve the specificity of Treg therapies, the use of engineered TCRs and CARs are being widely explored. The most intuitive strategy to generate antigen-specific Tregs is to engineer them with a specific TCR recognizing a peptide of interest. Several approaches have been designed to promote preferential pairing of exogenous TCR and avoid pairing with endogenous TCR (extra-disulfide bridges between TCR subunits, "murinization" of TCR constant chain, and so on). Promisingly, Theodore Roth and colleagues engineered primary T cells by replacing the endogenous TCR locus with a tumor antigen-specific TCR using a non-viral CRISPR-Cas9 genome-targeting system (11). This strategy should avoid off-target effects led by exogenous-endogenous TCR mispairing.

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On the other hand, CAR constructs have the advantage of being independent of the HLA complex and do not require a co-receptor. Following CAR construct development in the immune-oncology field, CAR Treg-cell therapy has been evolving from simpler first generation to more complex third-generation CAR designs. Similar to CAR T cells, the inclusion of costimulatory domains in CAR constructs can enhance the suppression ability of Tregs. For instance, Nicholas Dawson and colleagues compared 10 different costimulatory domain CAR variants in gene-edited Tregs and demonstrated that the CD28 co-receptor intracellular domain was fundamental for a potent and stable immunosuppressive response (12). However, different studies have shown

discrepancies in their results, which highlights the need to standardize experimental settings and success criteria.

There have also been attempts to engineer cells to avoid allo-recognition; for example, deleting HLA class I or II molecules avoids allo-rejection of the transferred cells by CD8+ and CD4+ T cells, respectively. However, cells that do not express major histocompatibility complex molecules can be recognized and killed by natural killer (NK) cells. Here, inducing the expression of fetal HLA-E or HLA-G (molecules expressed during maternal-fetal tolerance) can lead to tolerance in NK cells (13). In addition, the expression of PD-1 and CTLA-4 could inhibit allo-activation of T cells, and the induction of CD47 expression – a "do not eat me signal" – will inhibit phagocytosis by macrophages. Thus, the combination of accessory proteins with a universal cell that can evade allo-rejection may provide off-the-shelf Treg cell therapy with the "armor" they need.

What does the future hold?

The successful validation of CAR T cell therapy in oncology has paved the way for Treg-cellular therapies for both transplant tolerance and autoimmune disease. So far, clinical trials and research studies have shown that adoptive Treg therapy is a safe and feasible approach, with plenty of room for improved efficacy via an evolution from polyclonal to antigen-specific approaches. The price and waiting times of these therapies present additional challenges, but off-the-shelf Tregs therapies may provide the solution. And if researchers can successfully tailor the survival, suppression, specificity, and stability of Tregs to each patient, while generating them from a universal and self-renewing source, the current shortcoming of the field should dissolve – a truly exciting prospect for patients.

"Clinical trials and research studies have shown that adoptive Treg therapy is a safe and feasible approach."

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