Stem Cell-Derived Regulatory T Cells for Therapeutic Use in Arthritis

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Abstract

Pluripotent stem cells (PSCs) can be utilized to obtain a renewable source of healthy regulatory T cells (Tregs) to treat autoimmune arthritis as they have the ability to produce almost all cell types in the body, including Tregs. However, the right conditions for the development of antigen (Ag)-specific Tregs from PSCs (i.e., PSC-Tregs) remain unknown. An ongoing project will determine the mechanisms underlying the Ag-specific PSC-Treg treatments that aim to modulate tolerance in autoimmune arthritis. The knowledge gained from these studies will provide new insights into cell-based therapies in autoimmune arthritis, and advance the understanding of fundamental mechanisms underlying Treg differentiation.

Keywords: Pluripotent stem cells; Autoimmune arthritis; Stem cells

Regulatory T cells (Tregs) are an integral component of the normal immune system and contribute to the maintenance of peripheral tolerance. Tregs can down-regulate immune responses and are essential for immune homeostasis. They act as key effectors in preventing and treating rheumatoid arthritis (RA) [1,2].

Hematopoietic stem cell (HSC)-derived hematopoietic progenitors migrate into the thymus and develop into different types of T cells. The transcription factors Aire (largely expressed in thymic medullary epithelial cells - mTECs) and FoxP3 have key functions in clonal deletion and Treg selection [3]. There are links between Aire expression, FoxP3 up-regulation and Treg selection; Aire deficiency affects the negative selection of self-reactive T cells, and FoxP3 controls the development and function of the naturally occurring Tregs (iTregs) [4]. Our laboratory has shown the development of stable Tregs from CD4+ T cells by over-expressing FoxP3 and bcl-xL [5].

Recent advances in the use of large-scale in vitro expansion of Tregs followed by in vivo re-infusion of these cells raises the possibility that this strategy may be successfully utilized for the treatment of rheumatoid arthritis (RA) [6]. Although polyclonally expanded populations of Tregs exhibit suppressive activity, antigen (Ag)-specific Tregs are more efficient at suppressing local autoimmune disorders such as RA, type-1 diabetes (T1D), inflammatory bowel diseases (IBD), allergic reactions and graft-versus-host disease (GVHD) [7-11]. In addition, tissue/organ-associated Treg targeting stabilizes FoxP3 expression and avoids induction of a potentially detrimental systemic immunosuppression [12,13]. For Treg-based immunotherapy, in vitro generation of tissue/organ (e.g., synovium)-associated and non-terminally differentiated effector Tregs for in vivo re-infusion is an optimal approach. However, current methodologies are limited in terms of the capacity to generate, isolate, and expand a sufficient quantity of such Tregs from patients for therapeutic interventions.

A number of challenges exist in Treg-based immunotherapy:

First

Only low numbers of Tregs can be harvested from the peripheral blood mononuclear cells (PBMCs). CD4 and CD25 have been used to isolate Tregs for ex vivo expansion. CD4+CD25+ T cells are not homogenous and contain both Tregs and conventional effector T cells (Tcones). Current expansion protocols activate both Tregs and Tcones, and because it takes a longer time for Tregs to enter the S phase of cell cycle, T cells outgrow Tregs [14]. In addition, Tregs can lose suppressive activity after repetitive stimulation with α-CD3 plus α-CD28 antibodies (Abs) with or without rIL-2 in vitro.

Second

No approach to date has demonstrated the capacity to isolate the entire Treg population with 100% specificity from patients (the current clinical approach). Even FoxP3 or more recently Eos, a transcriptional factor that is considered the gold standard for identification of Tregs, is expressed transiently in some activated non- regulatory human T cells [15], highlighting the difficulty in both identifying and isolating a pure Treg population. The adoptive transfer of non-regulatory T cells with Tregs has a potential to worsen autoimmune diseases.

Third

Gene transduction of CD4+ T cells from PBMCs with Ag-specific T cell receptor (TCR) [16] or chimeric Ag receptor (CAR) [17] and/or TCR with FoxP3 elicits the generation of suppressive T cell populations [8] and overcomes the hurdle of the limited numbers of Ag-specific T cells. However, the engineered Tregs express endogenous and exogenous polyclonal TCRs, which reduce their therapeutic potential (the current experimental approach). Also, TCR mispairing is a concern with regards to the safety of TCR gene-transferred Tregs for clinical use, because the formation of new heterodimers of TCR can induce immunopathology [18]. Therefore, there is a need to improve this strategy and generate monoclonal Tregs.

Fourth

The differentiation state of Tregs is inversely related to their capacity to proliferate and persist. The “right” Treg resist terminal differentiation, maintain high replicative potential (e.g., expression of common γ chain-γc, CD132), are less prone to apoptosis (e.g., low expression of PD-1), and have the ability to respond to homeostatic cytokines [19], which facilitates Copyright: © 2016 Song J. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
their survival. In addition, the “right” Treg express high levels of molecules that facilitate their homing to lymph nodes (LNs), such as CD62L and CC-chemokine receptors (e.g., CCR4, CCR7), and maintain stability or plasticity under certain inflammatory conditions. Furthermore, after an effective immune response, the “right” Treg persist and provide protective immunity.

Fifth

Because there are too few cells, harvesting sufficient numbers of tissue-associated Treg from PBMCs for TCR gene transduction can be problematic.

Taken together, strong arguments support the development of Treg-based therapies in autoimmune arthritis using engineered Treg. While clinical trials show safety, feasibility, and potential therapeutic activity of Treg-based therapies using this approach, concerns about autoimmunity due to cross-reactivity with healthy tissues remains a major safety issue [20,21]. In addition, genetically modified Treg using current approaches are usually intermediate or later effectors Treg [22], which only have short-term persistence in vivo.

To date, pluripotent stem cells (PSCs) are the only source available to generate a high number of the “right” Treg [23,24]. Human induced PSCs (iPSCs) can be easily generated from patients' somatic cells by transduction of various transcription factors and exhibit characteristics identical to those of embryonic stem cells (ESC) [25]. Many genetic methods as well as protein-based approaches have been developed to produce iPSCs with potentially reduced risks, including that of immunogenicity and tumorigenicity [26]. Because of the plasticity and the potential for an unlimited capacity for self-renewal, iPSCs have high potential for advancing the field of cell-based therapies.

Our laboratory was the first to show that the development of Ag-specific iPSC-CTLs or iPSC-Treg can be used for cell-based therapies of cancers and autoimmune disorders [23,24,27-30] other groups reported similar results [31-33]. We demonstrated that genetically modified iPSCs with Ag-specific TCR and the transcriptional factor Foxp3, followed by differentiation driven by Notch signaling can enable iPSCs to pass hematopoietic and T lineage differentiation checkpoints, resulting in the development of Ag-specific CD4+Treg. We have developed a novel system to generate stable Ag-specific iPSC-Treg. Our ongoing studies will validate this system and provide new insights into the methodologies and mechanistic requirements for efficient development of inflamed tissue-associated iPSC-Treg. Once such strategies become available, there is potential to facilitate the generation of tolerance for autoimmune arthritis. Thus, important advances towards Treg-based immunotherapy in autoimmune arthritis are anticipated from the proposed studies.

PSCs are exposed to a number of signals responsible for their progression. Although the exact signals are not fully understood, part of the mechanism known to be critical for directing T-cell fate occurs via Notch signaling. The Notch is evolutionarily conserved; regulating cell fate decisions in a number of cell and tissue types. Ligand binding by members of the Jagged or Delta-like (DL) families results in the proteolytic cleavage and release of the intracellular fragment of the Notch heterodimer. Translocation to the nucleus then allows for its regulation of gene expression. Notch-1, specifically, is critical for the establishment of T-cell fate. The loss of function results in the blockade of T cell development and enhanced B cell production, while over-expression results in the blockade of B cell lymphopoiesis and leads to the generation of T cells [34]. However, the intracellular signaling pathways by which Notch signaling regulates the differentiation of Ag-specific PSC-Treg remain unknown. PSCs co-cultured on a monolayer of the bone marrow (BM) stromal cell line OP9 cells transduced with the Notch ligand DL1 or 4 exhibits the ability to differentiate into most hematopoietic lineages and T cells [31]. Our studies will determine the critical regulations of Hes1 [35], Runx1 [36], and surviving [37] by Notch signaling during the development of autoAg-specific PSC-Treg.

Although Ag-specific human iPSC-Treg may have promising therapeutic effects in cell-based therapies, their efficiency is limited by the need to generate a large number of such cells using complex and expensive in vitro differentiation. In addition, the lengthy duration for generating human iPSCs may limit their use in individualized therapies. Alternatively, we will perform cell-based therapies using the TCR/Foxp3 gene-transduced iPSCs, which can differentiate into auto Ag-specific iPSC-Treg in vivo and suppress autoimmunity. We will perform arthritis induction before or after the adoptive transfer of the gene-transduced iPSCs. We will inject Notch agonists or recombinant cytokines (e.g., HIL-7, rFlt3L) to boost in vivo development of auto Ag-specific iPSC-Treg.

In summary, a current roadblock to progress in the field is the lack of an efficient system to generate the “right” auto Ag-specific Treg that could be used for cell-based therapies in autoimmune arthritis. We propose the use of PSC-Treg to address this limitation, allowing derivation of a large number of stable autoAg-specific PSC-Treg for cell-based therapies. Development of such an approach provides an important step toward personalized therapies for autoimmune arthritis.

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References


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