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

Jianxun Song
Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

Abstract

Pluripotent stem cells (PSCs) can be utilized to obtain a renewable source of healthy regulatory T cells (T\textsubscript{regs}) to treat autoimmune arthritis as they have the ability to produce almost all cell types in the body, including T\textsubscript{regs}. However, the right conditions for the development of antigen (Ag)-specific T\textsubscript{regs} from PSCs (i.e., PSC-T\textsubscript{regs}) remain unknown. An ongoing project will determine the mechanisms underlying the Ag-specific PSC-T\textsubscript{reg} 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 T\textsubscript{reg} differentiation.

Keywords

Pluripotent stem cells; Autoimmune arthritis; Stem cells

Regulatory T cells (T\textsubscript{regs}) are an integral component of the normal immune system and contribute to the maintenance of peripheral tolerance. T\textsubscript{regs} can down-regulate immune responses and are essential for immune homeostasis. They can 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 T\textsubscript{reg} selection [3]. There are links between Aire expression, FoxP3 upregulation and T\textsubscript{reg} selection; Aire deficiency affects the negative selection of self-reactive T cells, and FoxP3 controls the development and function of the naturally occurring T\textsubscript{regs} (nT\textsubscript{regs}) [4]. Our laboratory has shown the development of stable T\textsubscript{regs} from CD4\textsuperscript{+} T cells by over-expressing FoxP3 and bcl-xL [5].

Recent advances in the use of large-scale \textit{in vitro} expansion of T\textsubscript{regs} followed by \textit{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 T\textsubscript{regs} exhibit suppressive activity, antigen (Ag)-specific T\textsubscript{regs} 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 T\textsubscript{reg} targeting stabilizes FoxP3 expression and avoids induction of a potentially detrimental systemic immunosuppression [12,13]. For T\textsubscript{reg}-based immunotherapy, \textit{in vitro} generation of tissue/organ (e.g., synovium)-associated and non-terminally differentiated effector T\textsubscript{regs} for \textit{in vivo} reinfusion is an optimal approach. However, current methodologies are limited in terms of the capacity to generate, isolate, and expand a sufficient quantity of such T\textsubscript{regs} from patients for therapeutic interventions.

A number of challenges exist in T\textsubscript{reg}-based immunotherapy:

\textbf{First}

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

\textbf{Second}

No approach to date has demonstrated the capacity to isolate the entire T\textsubscript{reg} 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 T\textsubscript{regs}, is expressed transiently in some activated non-regulatory human T cells [15], highlighting the difficulty in both identifying and isolating a pure T\textsubscript{reg} population. The adoptive transfer of non-regulatory T\textsubscript{effs} with T\textsubscript{regs} has a potential to worsen autoimmune diseases.

\textbf{Third}

Gene transduction of CD4\textsuperscript{+} 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 T\textsubscript{regs} 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 T\textsubscript{regs} 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 T\textsubscript{regs}. 
Fourth

The differentiation state of T\textsubscript{regs} is inversely related to their capacity to proliferate and persist. The “right” T\textsubscript{regs} resist terminal differentiation, maintain high replicative potential (e.g., expression of common-\gamma chain-\gamma\textsubscript{c}, 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 their survival. In addition, the “right” T\textsubscript{regs} 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” T\textsubscript{regs} persist and provide protective immunity.

Fifth

Because there are too few cells, harvesting sufficient numbers of tissue-associated T\textsubscript{regs} from PBMCs for TCR gene transduction can be problematic.

Taken together, strong arguments support the development of T\textsubscript{reg}\textsuperscript{-}based therapies in autoimmune arthritis using engineered T\textsubscript{regs}. While clinical trials show safety, feasibility, and potential therapeutic activity of T\textsubscript{reg}\textsuperscript{-}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 T\textsubscript{regs} using current approaches are usually intermediate or later effector T\textsubscript{regs} [22], which only have short-term persistence \textit{in vivo}.

To date, pluripotent stem cells (PSCs) are the only source available to generate a high number of the “right” T\textsubscript{regs} [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 (ESCs) [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-T\textsubscript{regs} 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\textsuperscript{+}T\textsubscript{regs}. We have developed a novel system to generate stable Ag-specific iPSC-T\textsubscript{regs}. 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-T\textsubscript{regs}. Once such strategies become available, there is potential to facilitate the generation of tolerance for autoimmune arthritis. Thus, important advances towards T\textsubscript{reg}\textsuperscript{-}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-Tregs remain unknown. PSCs co-cultured on a monolayer of the bone marrow (BM) stromal cell line OP9 cells transfected 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-Tregs.

Although Ag-specific human iPSC-Tregs 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-Tregs in vivo and suppress autoimmune arthritis. 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., rIL-7, rFlt3L) to boost in vivo development of auto Ag-specific iPSC-Tregs.

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

Acknowledgments

This project is funded, in part, under grants with the National Institute of Health Grant R01AI121180, R21AI109239 and K18CA151798, American Diabetes Association 1-16-IBS-281 and the Pennsylvania Department of Health using Tobacco Settlement Funds.

References

1. Peres RS, Liew FY, Talbot J, Carregaro V, Oliveira RD, et al. Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. Proc Natl Acad Sci U S A. 2015; 112:2509–2514. [PubMed: 25675517]
2. Chen M, Su W, Lin X, Guo Z, Wang J, et al. Adoptive transfer of human gingiva-derived mesenchymal stem cells ameliorates collagen-induced arthritis via suppression of Th1 and Th17 cells and enhancement of regulatory T cell differentiation. Arthritis Rheum. 2013; 65:1181–1193. [PubMed: 23400582]
3. Hossain DM, Panda AK, Manna A, Mohanty S, Bhattacharjee P, et al. FoxP3 acts as a cotranscription factor with STAT3 in tumor-induced regulatory T cells. Immunity. 2013; 39:1057–1069. [PubMed: 24315995]

4. Aschenbrenner K, D’Cruz LM, Vollmann EH, Hinterberger M, Emmerich J, et al. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. Nat Immunol. 2007; 8:351–358. [PubMed: 17322887]

5. Haque R, Lei F, Xiong X, Wu Y, Song J. FoxP3 and Bcl-xl cooperatively promote regulatory T cell persistence and prevention of arthritis development. Arthritis Res Ther. 2010; 12:R66. [PubMed: 20384988]

6. Hippen KL, Merkel SC, Schirm DK, Nelson C, Tennis NC, et al. Generation and large-scale expansion of human inducible regulatory T cells that suppress graft-versus-host disease. Am J Transplant. 2011; 11:1148–1157. [PubMed: 21564534]

7. van Herwijnen MJ, Wieten L, van der Zee R, van Kooten PJ, Wagenaar-Hilbers JP, et al. Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. Proc Natl Acad Sci USA. 2012; 109:14134–14139. [PubMed: 22891339]

8. Wright GP, Notley CA, Xue SA, Bendle GM, Holler A, et al. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. Proc Natl Acad Sci U S A. 2009; 106:19078–19083. [PubMed: 19884493]

9. Sela U, Olds P, Park A, Schlesinger SJ, Steinman RM. Dendritic cells induce antigen-specific regulatory T cells that prevent graft versus host disease and persist in mice. J Exp Med. 2011; 208:2489–2496. [PubMed: 22084006]

10. Bacher P, Kniemeyer O, Schönbrunn A, Sawitzki B, Assenmacher M, et al. Antigen-specific expansion of human regulatory T cells as a major tolerance mechanism against mucosal fungi. Mucosal Immunol. 2013; 7:916–928. [PubMed: 24301658]

11. Nguyen TL, Sullivan NL, Ebel M, Teague RM, DiPaolo RJ. Antigen-specific TGF-beta-induced regulatory T cells secrete chemokines, regulate T cell trafficking, and suppress ongoing autoimmunity. J Immunol. 2011; 187:1745–1753. [PubMed: 21746962]

12. Van Belle TL, Ling E, Haase C, Bresson D, Urso B, et al. NKG2D blockade facilitates diabetes prevention by antigen-specific Tregs in a virus-induced model of diabetes. J Autoimmun. 2013; 40:66–73. [PubMed: 22944096]

13. Takiishi T, Korf H, Van Belle TL, Robert S, Grieco FA, et al. Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified Lactococcus lactis in mice. J Clin Invest. 2012; 122:1717–1725. [PubMed: 22484814]

14. Vogtenhuber C, O’Shaughnessy MJ, Vignali DA, Blazar BR. Outgrowth of CD4low/negCD25+ T cells with suppressor function in CD4+CD25+ T cell cultures upon polyclonal stimulation ex vivo. J Immunol. 2008; 181:8767–8777. [PubMed: 19050298]

15. Sharma MD, Huang L, Choi JH, Lee EJ, Wilson JM, et al. An inherently bifunctional subset of Foxp3+ T helper cells is controlled by the transcription factor eos. Immunity. 2013; 38:998–1012. [PubMed: 23684987]

16. Perro M, Tsang J, Xue SA, Escors D, Cesco-Gaspere M, et al. Generation of multi-functional antigen-specific human T-cells by lentiviral TCR gene transfer. Gene Ther. 2010; 17:721–732. [PubMed: 20164855]

17. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011; 365:725–733. [PubMed: 21830940]

18. Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med. 2010; 16:565–570. [PubMed: 20400962]

19. Gratzi IK, Truong HA, Yang SH, Maurano MM, Lee K, et al. Cutting Edge: Memory Regulatory T Cells Require IL-7 and Not IL-2 for Their Maintenance in Peripheral Tissues. J Immunol. 2013; 190:4483–4487. [PubMed: 23543753]

20. Kuball J, Dossett ML, Wolff M, Ho WY, Voss RH, et al. Facilitating matched pairing and expression of TCR chains introduced into human T cells. Blood. 2007; 109:2331–2338. [PubMed: 17082316]

Autoimmune Infect Dis. Author manuscript; available in PMC 2016 December 28.
21. van Loenen MM, de Boer R, Amir AL, Hagedoorn RS, Volbeda GL, et al. Mixed T cell receptor dimers harbor potentially harmful neoreactivity. Proc Natl Acad Sci U S A. 2010; 107:10972–10977. [PubMed: 20534461]

22. Kim YC, Zhang AH, Su Y, Rieder SA, Rossi RJ, et al. Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses. Blood. 2015; 125:1107–1115. [PubMed: 25498909]

23. Haque R, Lei F, Xiong X, Bian Y, Zhao B, et al. Programming of regulatory T cells from pluripotent stem cells and prevention of autoimmunity. J Immunol. 2012; 189:1228–1236. [PubMed: 22732595]

24. Lei F, Haque R, Xiong X, Song J. Directed differentiation of induced pluripotent stem cells towards T lymphocytes. J Vis Exp. 2012:e3986. [PubMed: 22617911]

25. Kim JB, Sebastiano V, Wu G, Arauzo-Bravo MJ, Sasse P, et al. Oct4-induced pluripotency in adult neural stem cells. Cell. 2009; 136:411–419. [PubMed: 19203577]

26. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011; 474:212–215. [PubMed: 21572395]

27. Lei F, Haque R, Weiler L, Vrana KE, Song J. T lineage differentiation from induced pluripotent stem cells. Cell Immunol. 2009; 260:1–5. [PubMed: 19811778]

28. Lei F, Zhao B, Haque R, Xiong X, Budgeon L, et al. In vivo programming of tumor antigen-specific T lymphocytes from pluripotent stem cells to promote cancer immunosurveillance. Cancer Res. 2011; 71:4742–4747. [PubMed: 21628492]

29. Haque M, Song J, Fino K, Sandhu P, Wang Y, et al. Melanoma Immunotherapy in Mice Using Genetically Engineered Pluripotent Stem Cells. Cell Transplant. 2016; 25:811–827. [PubMed: 26777320]

30. Haque M, Song J, Fino K, Sandhu P, Song X, et al. Stem cell-derived tissue-associated regulatory T cells ameliorate the development of autoimmunity. Sci Rep. 2016; 6:20588. [PubMed: 26846186]

31. Themeli M, Kloss CC, Ciriello G, Fedorov VD, Perna F, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. Nat Biotechnol. 2013; 31:928–933. [PubMed: 23934177]

32. Vizzarco R, Masuda K, Yamada D, Ikawa T, Shimizu K, et al. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells. Cell Stem Cell. 2013; 12:31–36. [PubMed: 23290135]

33. Saito H, Okita K, Chang AE, Ito F. Adoptive transfer of CD8+ T cells generated from induced pluripotent stem cells triggers regressions of large tumors along with immunological memory. Cancer Res. 2106; 76:3473–3483.

34. Dervovic DD, Liang HC, Cannons JL, Elford AR, Mohtashami M, et al. Cellular and molecular requirements for the selection of in vitro-generated CD8 T cells reveal a role for notch. J Immunol. 2013; 191:1704–1715. [PubMed: 23851691]

35. Wendorff AA, Koch U, Wunderlich FT, Wirth S, Dubey C, et al. Hes1 is a critical but context-dependent mediator of canonical Notch signaling in lymphocyte development and transformation. Immunity. 2010; 33:671–684. [PubMed: 21093323]

36. Guo Y, Maillard I, Chakraborti S, Rothenberg EV, Speck NA. Core binding factors are necessary for natural killer cell development and cooperate with Notch signaling during T-cell specification. Blood. 2008; 112:480–492. [PubMed: 18390836]

37. Lei F, Song J, Haque R, Xiong X, Fang D, et al. Transgenic expression of survivin compensates for OX40-deficiency in driving Th2 development and allergic inflammation. Eur J Immunol. 2013; 43:1914–1924. [PubMed: 23616302]