Editorial

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Immunotherapy

Current trends with FOXP3$^+$ regulatory T cell immunotherapy to contest autoimmunity and inflammation

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"Although several clinical trials are already performed with *ex vivo* expanded polyclonal Tregs, due to costs and the requirement of advanced and highly sophisticated facilities to expand clinical grade Tregs, *in vivo* modulation of Tregs by targeting IL-2 axis via various approaches is gaining prominence."

First draft submitted: 2 May 2019; Accepted for publication: 3 May 2018; Published online: 16 May 2019

Keywords: autoimmune diseases • chronic inflammatory diseases • therapy • tolerance • Treg • Tregs

Immunity to infection or self-antigen is established by a concerted action of multiple cell types of innate and adaptive immune systems. Of these two disparate immune responses, the later is far more detrimental and may result in autoimmunity. Under ideal conditions, the host immune system eliminates such a potential risk by a myriad of immune tolerance mechanisms. However, failure of these highly coordinated mechanisms poses the threat due to heightened occurrence of self-reactive T cells along with B cells, that launch an immune response against resident tissues, culminating in tissue destruction, inflammation and autoimmune diseases.

Among the many therapeutic approaches against autoimmune disorders, possible utility of regulatory T cells (Tregs) has attracted much attention. Tregs are a subpopulation of CD4$^+$ T cells expressing signature IL-2 receptor α chain (CD25) and the forkhead box P3 (FOXP3) transcription factor. These CD4$^+$CD25$^+$FOXP3$^+$ Tregs are generated in the thymus (t/nTreg cells; n, natural) and are also induced from naïve CD4$^+$ T cells in the periphery (p/iTreg cells; i, induced). Together, they avert the development of uncontrolled inflammation [1]. As evidenced in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, deficiency of Tregs due to *foxp3* mutation leads to severe autoimmune and inflammatory conditions.

Previously, numerous experimental models have demonstrated that adoptive transfer of Tregs could suppress autoimmune diseases. These approaches mostly used polyclonal Treg populations. Based on the success of such approaches in experimental models, adoptive Treg therapy was initially applied in patients to control graft-versus-host disease by using freshly isolated or *ex vivo* expanded CD4$^+$CD25$^+$ Tregs or CD4$^+$CD127^{low}CD25^{high} Tregs [2–5]. These trials incited the application of more advanced technologies for obtaining sufficiently large number of pure *ex vivo* expanded polyclonal Tregs for the therapeutic purposes. CD4$^+$CD127^{low}CD25^{high} Tregs were isolated from the patients, expanded *ex vivo* through anti-CD3/CD28 antibody-coated beads in the presence of IL-2 and adoptively transferred back to the patients [6–8]. While Phase I clinical trial with adult T1D patients provided a proof of concept for the safer utilization of Tregs as adoptive immunotherapy, a further study in a patient with systemic lupus erythematosus has demonstrated accumulation of adoptively transferred Tregs in the inflamed skin and attenuation of IFN-γ and associated inflammatory responses. A similar Treg therapy in pediatric T1D patients has sustained the survival of pancreatic islets and reduced the requirement of exogenous insulin in majority of the patients who received Treg therapy [9]. Several clinical trials are currently ongoing as well as recruiting the patients for other autoimmune and inflammatory conditions including pemphigus and transplantation (NCT03239470, NCT02932826, NCT02428309, NCT02711826, NCT03011021, NCT02526329 to name a few). Data from these studies are expected to further strengthen the field of Treg therapy.

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However, use of polyclonal Tregs might induce systemic immunosuppression and predispose the patients to infections and tumors. This approach is also further complicated by the plasticity and instability of pTregs in the inflamed tissues of patients. Under the influence of chronic inflammation, pTregs could be converted into pathogenic effector T cells [10]. Although tTregs and pTregs could be distinguished by using epigenetics markers, phenotypically they share the surface markers and hence pose difficulties for isolating specifically tTregs for the subsequent therapeutic purposes. The recent report on the ability of FOXP3$^+$ Tregs to induce activation of human basophils, the chief mediators of allergic inflammatory responses adds further dilemma on the choice of Treg-based therapy for allergic inflammatory conditions [11]. Investigators across the field have come up with multiple strategies to tackle these challenges both in patients and experimental models.

As inflammation drives Tregs dysfunctional and also promotes pathogenic conversion of pTregs, therapeutic approaches that neutralize inflammatory mediators and suppress the activation of innate and adaptive immune cells could be used to drive Treg expansion *in vivo*. One such approach is the use of therapeutic normal immunoglobulin or intravenous immunoglobulin to induce Treg expansion by imparting distinct signaling events in antigen presenting cells [12]. An alternative strategy could be targeting the molecules and/or cellular pathways that are found to be dysfunctional among the Tregs of patients.

Treg homeostasis and their survival in the periphery are governed mainly by IL-2 signaling. The IL-2 could bind and signal via two types of IL-2 receptors (IL-2R), termed di- and trimeric IL-2Rs. CD122 (IL-2Rβ) and the common γ chain (γ_c) form the dimeric IL-2R and is expressed mainly by memory CD8$^+$ T cells and NK cells, whereas trimeric IL-2R, constitutively expressed on Tregs is made of dimeric IL-2Rs in association with CD25 (IL-2Rα). Defective IL-2 signaling in Tregs caused by deficiency of IL-2 or IL-2R subunits CD25 and CD122, negatively impacts Tregs survival and leads to autoimmunity. In fact, low dose-IL-2 therapy has been explored in clinic trials for T1D, systemic lupus erythematosus, graft-versus-host disease and other disorders [13]. Due to high affinity of trimeric IL-2Rs toward IL-2, this low dose IL-2 expands Tregs and hence alleviates inflammation. As an alternative approach, IL-2 complexed to a monoclonal antibody that recognizes CD122/γ_c epitope of IL-2 was constructed in order to selectively activate Tregs. This monoclonal antibody–IL-2 complex displayed promising results in experimental models of several autoimmune and inflammatory diseases including T1D, experimental autoimmune encephalomyelitis and transplantation [14]. Inspiring from these reports, yet another novel approach to expand Tregs was achieved by designing a human anti-IL-2 antibody F5111.2 that stabilizes IL-2 in a conformation that results in the preferential STAT5 phosphorylation in Tregs [15]. When injected to mice, hIL-2-complexed to F5111.2 promoted expansion of Tregs and protected the animals from autoimmune diseases.

A potential generalized immunosuppression mediated by polyclonal Treg immunotherapy could be overcome by using autoantigen-specific Tregs. Studies performed in TCR transgenic mice confirmed the therapeutic utility of antigen-specific Tregs in the therapy of autoimmune diabetes [16]. However, development of such antigen-specific Tregs and their utility in clinical settings still remains in infancy due to the lack of a complete understanding of the mechanisms of immune suppression by Tregs, insufficient information about the target antigen against which Tregs have to be induced or expanded, and the difficulties in the isolation and expansion of antigen-specific Tregs *ex vivo*. Recent advances in retroviral therapy have been extended to generate naive CD4$^+$ T cells stably expressing FOXP3 and TCR transgenes specific to autoantigens. Such a population of cells has been demonstrated to be instrumental in suppressing the development of arthritis [17]. However, these studies remain marred by the biosafety concerns on the utility of integrative viral vectors in large-scale immunotherapy and thus are currently limited only to the experimental models of inflammation.

An alternative approach includes feeding antigen-presenting cells like macrophages and dendritic cells with apoptotic cells. This strategy has been shown to promote immune suppressive phenotype due to increased production of TGF-β and IL-10. Supplementation of antigens derived from the inflamed tissue in a similar setting generated macrophages or dendritic cells (antigen-pulsed or self-peptide loaded) with tolerogenic phenotype that skewed differentiation of CD4$^+$ T cells toward Tregs termed as 'apoptosis-antigen therapy' [18]. This approach has an added advantage of generating not only antigen-specific Tregs but also impedes the number of infiltrating effector T cells and reduces the risk of relapse. More recently, TCR gene transfer technologies and engineered Tregs with chimeric antigen receptor have also been tested in experimental models [19,20]. These approaches have given an auxiliary benefit of stabilized FOXP3 expression in Tregs and hence might resist conversion of Tregs into effector T cells. Although the identity of autoantigen(s) in several autoimmune diseases is (are) still not completely understood, such evolving methodologies might eventually lead to the development of novel autoantigen-specific Treg therapies.

Conclusion & future perspective

The last two decades have been marked by an unprecedented interest in the field of Tregs, where these cells have been explored for their prominent role in immunological tolerance that prevents the onset and progression of inflammatory and autoimmune diseases. Several fundamental aspects of Treg ontogeny, development process, and transcriptional, metabolic and epigenetic regulatory processes have been discovered. Preclinical studies have implicated the therapeutic potential of Tregs in mitigating autoimmune responses and in imposing immune tolerance. Treg therapy has been considered as superior therapy over immunosuppressive drugs to treat inflammation. Although several clinical trials are already performed with *ex vivo* expanded polyclonal Tregs, due to costs and the requirement of advanced and highly sophisticated facilities to expand clinical grade Tregs, *in vivo* modulation of Tregs by targeting IL-2 axis via various approaches is gaining prominence. The preclinical models have shown the importance and feasibility of antigen-specific Treg therapy. However, translation of these approaches to clinic is not straightforward due to many obstacles to overcome. For example, identification of tissue-specific antigens to conceive targeted Treg therapy without compromising the host immunity to infections and tumors is one among them. Thus, fundamental advances in Treg biology, pathogenesis of autoimmune diseases and technologies will eventually lead to the development of novel Treg-based adaptive immunotherapies to surmount autoimmune and chronic inflammatory disorders.

Acknowledgments

The authors tried their best to cover the research publications most relevant to the topic discussed above and apologize to those whose publications could not be cited here due to space constraints.

Financial and competing interest disclosure

J Trinath is supported by Research Initiation Grant (grant number BITS/GAU/RIG/2017/77) and OPERA award (grant number FR/SCM/160317/BIO). J Bayry is supported by Institut National de la Santé et de la Recherche Médicale, Sorbonne Université and Université Paris Descartes. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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