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Chemokine axis as a therapeutic target to enhance the recruitment of Tregs and treat organ-specific autoimmune and inflammatory diseases

“CD4⁺CD25⁺Foxp3⁺ Tregs are one of the major players implicated in the maintenance of immune tolerance.”

KEYWORDS: autoimmune diseases ■ CCL17 ■ CCL22 ■ CCR4 ■ CD4⁺CD25⁺FoxP3⁺ T cells ■ chemokine ■ immunotherapy ■ Tregs

Central tolerance is not foolproof and hence requires additional mechanisms in the periphery to control immune aggression and to prevent autoimmunity and inflammation. CD4⁺CD25⁺FoxP3⁺ Tregs are one of the major players implicated in the maintenance of immune tolerance [1,2]. Several lines of evidence both from experimental models (e.g., scurfy mice) and from patients (e.g., immunodysregulation polyendocrinopathy enteropathy X-linked syndrome [IPEX]) clearly demonstrate that deficiency of Tregs or defects in their functions consequent to mutations in key Treg-associated molecules lead to inflammatory conditions and autoimmunity [1,2].

“...the idea of Treg therapy is heavily dependent on the isolation of polyclonal Tregs.”

CD4⁺ T cells in the periphery can be differentiated and polarized into various effector subsets including Th1, Th2 and Th17 cells [3–5]. Like other CD4⁺ T-cell subsets, Tregs are heterogeneous. In addition to natural Tregs (nTregs) that are thymic in origin and self-antigen specific, Tregs can also be induced (iTregs) in the periphery from naive T cells under the influence of cytokines and low antigenic stimulation. Both nTregs and iTregs share several common features. In contrast to iTregs, nTregs are stable in phenotype and are potent immune suppressors. The transcription factor FoxP3 that governs Treg functions is stably expressed in nTregs, and the *FoxP3* gene is more widely dimethylated. In addition, nTregs specifically express Helios, an Ikaros transcription factor family member [6]. The relative importance of nTregs and iTregs in the prevention of autoimmunity is still not clear. Since the thymus does not have access to all self-antigens of the periphery, it is expected that

nTregs against all self-antigens are not generated in the thymus. In this context, iTregs might act as an additional shield of protection to prevent autoimmunity. Also, Tregs generated against foreign antigens, including those from viruses, bacteria, parasites and fungi are induced in nature.

How do Tregs exert immune tolerance? Various reports indicate that Tregs have multifaceted mechanisms with diverse targets. Thus, Tregs can regulate the functions of cells of both innate and adaptive immune compartments, including dendritic cells, macrophages, NK cells, T and B lymphocytes, by several mutually non-exclusive mechanisms both via soluble factors (i.e., TGF-β, IL-10 and IL-35) and cell-associated molecules (such as CTLA-4, LFA-1, CD39 and LAG-3) [1,2,7,8].

Given that Tregs are potent immune suppressors despite representing a tiny population among CD4⁺ T cells, several approaches have been attempted to explore their utility for the immunotherapy of autoimmune and inflammatory diseases, and in transplantation to prevent graft-versus-host disease [9–11]. These approaches are broadly classified into two: first, *in vivo* modulation of Treg functions by immunomodulation; and second, isolation and *ex vivo* expansion of Tregs followed by their adoptive transfer.

Treg cellular immunotherapy by adoptive transfer has shown promise in experimental models. Since the genetic background of mice can be manipulated by transgenic, knock-in or knock-out methods, the results from experimental models provide only a proof-of-concept. There are several drawbacks to transferring this technology to the clinics. Foremost is the requirement of a GMP facility for the isolation and expansion of Tregs. However, the technique of isolation of antigen-specific human Tregs required for immunotherapy is still in the



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infancy stage. For the moment, the idea of Treg therapy is heavily dependent on the isolation of polyclonal Tregs. This poses an additional problem: the surface phenotype of Tregs is not fully elucidated and hence any contaminating effector T cells in the cellular preparation can revert to pathogenic T cells upon adoptive transfer. What is the stability of these *ex vivo*-expanded Tregs upon adoptive transfer? Several reports have also demonstrated that Tregs are defective in several autoimmune diseases (e.g., rheumatoid arthritis) [12] and hence we cannot use such defective Tregs for cellular therapy. In addition, the cost associated with such a technology is enormously high.

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 “...CCR4–CCL22–CCL17 represents a potential target to manipulate Tregs for therapeutic purposes...”

Because of shortcomings of Treg cellular immunotherapy, the strategy of *in vivo* modulation of Treg functions to impose immune tolerance and to treat autoimmune and graft-versus-host disease has drawn a lot of interest. *In vivo* modulation of Treg functions can be attained either by targeting Tregs directly or modulating the local inflammatory environment. The approaches include: generalized immunosuppressive agents such as rapamycin that are known to boost Treg number and/or functions; immunomodulators such as intravenous immunoglobulin; monoclonal antibodies such as anti-CD3; neutralizing monoclonal antibodies to inflammatory cytokines such as TNF- α ; inhibitors of inflammatory signaling pathways; use of Treg-derived molecules such as CTLA-4; or targeting Treg migratory properties [8,10,12–21].

Tregs express various homing receptors such as integrin CD62L and CCR7 that direct Tregs from the thymus to lymphoid organs, CXCR4 for trafficking of Tregs to bone marrow and CCR2 for homing of Tregs to inflamed joints. In addition, Tregs also express CCR4 that binds to two ligands with an order of affinity CCL22 > CCL17. Although, none of these receptors are *per se* specific for Tregs, CCR4 has drawn great attention in recent years since CCR4 is expressed by the majority of Tregs in humans and CCR4 expression defines *bona fide* Tregs with potent suppressive functions [22–25]. Since the ligands for CCR4 are expressed by a wide range of cells including dendritic cells, B cells, macrophages, endothelial cells in dermal post-capillary venules and bronchial and intestinal

epithelial cells, CCR4 directs trafficking of Tregs both towards lymphoid and nonlymphoid tissues [23–25]. In addition, CCL22 and CCL17 induced by the tumor microenvironment and by pathogens can direct Tregs to the site of tumors and infection and consequently can suppress effective immune responses [26–28]. Therefore, CCR4–CCL22–CCL17 represents a potential target to manipulate Tregs for therapeutic purposes: either to boost immune responses as in the case of tumor, infection and vaccination, or to suppress the immune responses by enhancing the Treg recruitment as in the case of autoimmune and inflammatory conditions. In fact, blocking the CCR4–CCL22–CCL17 axis, either by monoclonal antibodies to CCL22, CCR4 antagonists or siRNA, has been shown to boost antitumor and vaccine immunity [26,29–32].

Since blockade of the CCR4–CCL22 axis can limit the negative influence of Tregs on the immune system and can be used to boost the immune response to tumors and vaccines, why not exploit the same axis to enhance the recruitment of Tregs to a specific organ for the therapy of organ-specific autoimmune and inflammatory diseases? This is what Montane *et al* have shown in a recent report by using diabetes-prone nonobese diabetic (NOD) mice model [33]. They created a murine CCL22-encoding double-stranded adeno-associated virus serotype 8 and injected the viral vector into the NOD mice via the pancreatic duct. The viral vector induced the expression of CCL22 and recruited endogenous Tregs that express high levels of TGF- β to islets. Furthermore, these Tregs prevented the development of spontaneous autoimmune diabetes in these mice while preserving insulin-positive cells. This protection was associated with a reduction in the number of circulating autoreactive CD8⁺ T cells and IFN- γ -producing CD8⁺ T cells. Not only that, grafting of syngeneic islets that express adenoviral-mediated CCL22 was also enough to recruit Tregs to islets and to delay recurrence of diabetes in recipient NOD mice. The approach in this model, however, did not reverse the disease process as such.

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 “...Tregs prevented the development of spontaneous autoimmune diabetes in these mice while preserving insulin-positive cells.”

Several questions need to be addressed in the future to translate these results to clinics. How superior is this strategy to therapeutics that are already in clinics or under evaluation for Type 1 diabetes? Can it be adjunctive to

current therapy [34]? Although adenoviruses are the most commonly used vectors, they are intrinsically immunogenic and therefore might require an alternative nonimmunogenic expression system for the patients. How long are these vectors capable of overexpressing CCL22 and how much expression is required for the therapy? Alternatively, once Tregs are recruited to the site of autoimmunity, are they enough to suppress autoimmune responses? Since, Tregs are known to suppress the immune response to infectious agents and their clearance, the possible adverse effects of organ-specific recruitment of Tregs towards predisposition to specific infectious diseases also needs to be carefully looked upon. Nevertheless, the report of Montane *et al.* provides a pointer that the chemokine axis can be exploited to enhance the recruitment of Tregs for the therapy of organ-specific autoimmune diseases such as Type 1 diabetes.

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