Clinical Immunology (2014) 153, 298–307

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Regulatory T cells control diabetes without compromising acute anti-viral defense☆

Carmen Baca Jones[1], Philippe P. Pagni[1], Georgia Fousteri[1], Sowbarnika Sachithanantham, Amy Dave, Teresa Rodriguez-Calvo, Jacqueline Miller, Matthias von Herrath*

Type 1 Diabetes Center, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA

Received 20 February 2014; accepted with revision 14 May 2014
Available online 22 May 2014

KEYWORDS
Regulatory T cells;
Diabetes;
Viral infection;
Stability;
Safety;
Therapy

Abstract While previous reports have demonstrated the efficacy of regulatory T cell therapy in the prevention of diabetes, systemic immunocompromise and Treg instability remain key safety concerns. Here we examined the influence of induced Treg (iTreg) cell therapy on anti-viral host defense and autoimmune T cell responses during acute viral infection in a murine model of autoimmune diabetes. Protective transfers of iTregs maintained IL-10 expression, expanded in vivo and controlled diabetes, despite losing FoxP3 expression. Adoptive transfer of iTregs affected neither the primary anti-viral CD8 T cell response nor viral clearance, although a significant and sustained suppression of CD4 T cell responses was observed. Following acute viral clearance, iTregs transferred early suppressed both CD4 and CD8 T cell responses, which resulted in the reversion of diabetes. These observations indicate that iTregs suppress local autoimmune processes while preserving the immunocompetent host's ability to combat acute viral infection.

1. Introduction

FoxP3$^+$ regulatory T cells (Treg) are a class of suppressive T cells central to the induction and maintenance of immune tolerance [1–6]. In the steady state Tregs function by suppressing T-cell proliferation and cytokine responses directed against autoantigens and commensal bacteria. The ability of Tregs to maintain and restore tolerance makes them attractive candidates for the development of cellular therapies aimed at treating autoimmune disorders, such as type 1 diabetes (T1D). Although the key triggering events are still subject to debate,

☆ This work was supported by NIH grants AI089624-03 and 5T32DK007044.
* Corresponding author. Fax: +1 858 752 6993.
E-mail addresses: carmenbacajones@gmail.org (C. Baca Jones), ppag@novonordisk.com (P.P. Pagni), fousteri.georgia@hsr.it (G. Fousteri), sowie@liai.org (S. Sachithanantham), adave323@gmail.com (A. Dave), trodriguez@liai.org (T. Rodriguez-Calvo), jfmiller@liai.org (J. Miller), matthias@liai.org (M. von Herrath).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.clim.2014.05.006
1521-6616/

T1D is widely recognized as a chronic autoimmune disorder resulting from the destruction of insulin-producing beta cells of the pancreas [7–9]. In addition to the body of evidence generated with murine models, several key disease characteristics in humans implicate an autoimmune etiology in the development of T1D, including the presence of lymphocyte infiltration into the pancreas, antibodies reactive to islet cell proteins and various described genetic susceptibilities [10–13]. Murine models of T1D have indicated a deficiency in either frequency and/or function of Tregs as a parameter in the development of disease [14–18]. Furthermore the provision of functional Tregs (either adoptively transferred or in vivo induced) has been found to be protective [19], thus fueling the interest in Tregs as a promising T1D immunotherapeutic approach [20,21]. Early studies employing the NOD-SCID model found that transfer of total CD4+ CD25+ T cells taken from young pre-diabetic NOD mice conferred protection against diabetic onset [14,22,23]. Similarly, in immunocompetent NOD and rat models of T1D, transfer of Tregs was found to either block or reverse diabetes [20,21].

Clinical application of Treg therapy in the treatment of T1D is currently being investigated in both the U.S. and Poland. A phase I clinical trial (ClinicalTrials.gov Identifier NCT01210664), underway at the University of California San Francisco, is aimed at assessing the safety of autologous in vitro expanded Treg therapy in adults recently diagnosed with T1D. Additionally, a report detailing the effects of autologous Treg transfer in newly diagnosed children found that a single transfer of Tregs reduced insulin dependency and preserved C-peptide levels [24]. Although promising, it remains unclear whether such therapy will prove safe in the face of acute viral infection, and moreover whether the presence of exogenous Tregs would impair the host's ability to clear an acute viral infection. Likewise it remains to be determined whether infection with a viral pathogen would inhibit the ability of Treg therapy to exert its protective effect. This report investigates the stability and function of in vitro induced Tregs (iTregs) under the influence of acute viral infection and how therapeutic iTregs affect host defense against viral pathogens, while maintaining their ability to protect against T1D.

Global suppression of T cell responses has led to delayed disease progression in T1D trials; however, such approaches may render the patient sensitive to opportunistic infection, and as such current efforts are focused on more targeted therapeutic approaches [25–28]. Treg therapy is one such strategy currently under investigation in both murine models and clinical trials. Immunotherapeutic transfer of Tregs is aimed at restoring peripheral tolerance to beta cell antigens without ablating protective effector responses to foreign antigens. While classical immunosuppressive therapies have widely recognized off target effects, Treg therapy must also be evaluated for safety. Three scenarios in particular pose potential hazards including: outgrowth of contaminating effector cells, conversion of Tregs in vivo to T effectors (Teff), and global suppression through systemic cytokine release or antigen independent suppression of APC function.

Here the RIP-GP murine model of T1D was utilized to address questions of efficacy and safety of iTreg therapy in T1D and acute viral infection. RIP-GP transgenic mice express the lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP) under the control of the rat insulin promoter (RIP) resulting in the expression of the viral GP on the beta cells [29–31]. Infection of RIP-GP mice with LCMV breaks tolerance and induces T-cell mediated destruction of insulin producing beta cells [32]. The RIP-GP T1D model provides a unique opportunity to test the safety, stability and efficacy of Treg therapy in a worst-case scenario, where the transferred Tregs recognize both the beta cell autoantigen and viral antigen in an acute inflammatory model. We report that a single iTreg transfer leads to the reversion of diabetes despite the loss of FoxP3 expression in transferred iTregs during acute viral infection. While iTreg transfer leads to the early suppression of CD4 T effector responses, suppression of CD8 Teff responses was delayed and did not impact viral titers or clearance. These data suggest that despite the loss of FoxP3 expression, iTregs transferred into immunocompetent RIP-GP mice did not convert to Teff, provided protection from diabetes and did not interfere with host anti-viral defense.

2. Materials and methods

2.1. Mice

C57BL/6 mice were purchased from the Jackson Laboratory and housed for two weeks prior to infection or spleen harvest. RIP-GP mice, which express the viral GP under the control of the rat insulin promoter, were bred in house [30]. Smarta mice, TCR transgenic for the dominant I-A^b-restricted LCMV $GP_{61–80}$ peptide, and FoxP3 GFP reporter mice were bred in house and previously described [33,34]. Smarta mice were backcrossed onto FoxP3-GFP reporter background and screened for both reporter and TCR transgene expression prior to use. Blood glucose was measured weekly using the OneTouch Ultra system (LifeScan). Mice with blood glucose values >300 mg/dL were scored diabetic.

2.2. Ethics statement

The La Jolla Institute for Allergy and Immunology Institutional Animal Care and Use Committee (PHS assurance # A3779-01) approved all animal experimental protocols, adhering to the *Guide for The Care and Use of Animals*. The LIAI Department of Laboratory Animal Care is an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) International accredited unit.

2.3. Treg generation and adoptive transfer

CD4 T cells were isolated from the spleens of naive adult Smarta mice. Following mechanical disruption and RBC lysis, splenocytes were stained with rat monoclonal antibodies to B220, CD11c, CD11b, CD16/32, IA/IE and CD8 (BD Pharmingen). Labeled splenocytes were magnetically depleted (anti-rat IgG Dynal beads, Invitrogen) and CD4 T cells ($B220^-CD11c^-CD11b^-CD16/32^-IA/IE^-CD8^-$) negatively selected. Isolated Smarta CD4 T cells were cultured for 7 days in the presence of plate bound anti-CD3, 0.5 μg/ml soluble anti-CD28, 5 ng/mL recombinant TGFβ (Roche) and 100 U/mL IL-2 (Roche). At the conclusion of the culture cells were live sorted based on FoxP3 reporter expression and 1×10^6 $FoxP3^+$ iTregs were transferred by tail vein injection into either RIP-GP or C57BL/6 mice as indicated.