

Th17 cells in Type 1 diabetes: a future perspective



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Type 1 diabetes (T1D) is classically diagnosed during childhood and adolescence but can occur at any age. T1D is a chronic autoimmune disease in which CD4⁺ and CD8⁺ T cells are thought to mediate destruction of the insulin-producing cells in the pancreatic islets [1]. Loss of β -cell function results in overt hyperglycemia rendering patients absolutely reliant on exogenous insulin therapy for the remainder of their lives to manage their hyperglycemia [2]. Currently, insulin therapy is the only therapeutic option to treat T1D and although it is effective it does not reproduce the intricate, fine level of regulation of plasma glucose levels provided by insulin production by pancreatic β cells [2]. Thus, individuals with T1D on insulin therapy still develop diabetic complications due to the lack of normal physiological control of glucose levels. Over the past few decades, there has been significant progress in treatment regimens for several diseases where the immune system plays an important role; however, similar treatment regimens designed specifically for T1D are slow to

follow. Given the autoimmune component of this disease, it is clear that novel therapeutic approaches aimed at modulating the immune response is warranted for the treatment of T1D.

Immune-mediated pathogenesis of disease

Pathogenesis of T1D is initially characterized by mononuclear cell infiltration into the pancreatic islets followed by T-cell-mediated destruction of the insulin-producing β cells [1]. Autoreactive IFN- γ -producing Th1 cells are believed to mediate disease caused, in part, by the failure of Foxp3⁺CD4⁺ Tregs to control the onset and severity of autoimmunity, also referred to as a loss of tolerance [1,3]. However, studies have demonstrated that loss of IFN- γ or its receptor failed to prevent the spontaneous development of diabetes in nonobese diabetic (NOD) mice, a commonly used mouse model mimicking human T1D [3]. Moreover, a separate study demonstrated that induction of IFN- γ -restored normoglycemia in NOD mice [3]. Collectively, these studies have

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called into question the absolute dependence of T1D progression on Th1 cells alone.

Th17 cells & disease

Recent advances in immunology have identified another population of CD4⁺ T cells, Th17 cells, that have been demonstrated be pathogenic mediators of several autoimmune diseases once attributed to Th1 cells, including multiple sclerosis, rheumatoid arthritis and psoriasis [3]. Th17 cells can be differentiated from naïve CD4⁺ T cells in the presence of TGFβ, IL-6, IL-1β and IL-21 and produce several proinflammatory cytokines, including IL-17A, IL-17F, IL-21 and IL-22 [3]. The transcription factors STAT3, and the nuclear receptors (NR) RORγt and RORα, are required for full Th17 cell development and function [1,3,4]. Th17 cells have pleiotropic effects on various cell types, including epithelial and endothelial cells, and under 'normal' immune homeostatic conditions, provide protection from several bacterial and fungal pathogens. However, Th17 cells garnered significant interest due to their pathogenic responses *in vivo*.

The discovery of Th17 cells spurred a flurry of research aimed at understanding their developmental program and to provide insight into the pathogenesis of autoimmunity. However, as research progressed, an increasingly complex picture of Th17 cell development and function emerged. TGFβ was proven to be required for the development of Th17 and inducible T-regulatory cells based on its ability to upregulate the expression Foxp3, RORα and RORγt, suggesting antagonism between and plasticity within Th17 and T-regulatory cell populations [3-5]. To complicate matters even further, Th17 cells have been demonstrated to convert into IFN-γ-producing Th1-like cells both *in vitro* and *in vivo*, with the cells that co-express IL-17A and IFN-γ considered the most pathogenic [6]. Thus, the inherent plasticity of Th17 cells has confounded efforts to describe and identify clear-cut roles for this cell type in several autoimmune diseases, including T1D.

Th17 cells & Type 1 diabetes

The exact role of Th17 cells in T1D remains somewhat controversial. Studies using IL-25 or antibodies to IL-17A support a pathogenic role for Th17 cells in T1D since their administration to NOD mice reduced or inhibited disease [7]. Other studies have demonstrated

that Th17 cells are located in areas surrounding the pancreatic islets and transfer of *in vitro* differentiated Th17 cells into NOD. SCID-recipient mice induced diabetes [8,9]. However, analysis of transferred cells revealed that the Th17 cells had converted into IFN-γ⁺IL-17⁺ cells *in vivo*, suggesting that this conversion event was indispensable for diabetes development [6]. Finally, we recently demonstrated that administration of SR1001, a dual RORα/γ inverse agonist, to NOD mice inhibited Th17 cells, Th17-mediated cytokine expression and the development of hyperglycemia in NOD mice [10].

Conversely, several lines of evidence suggest that Th17 cells may be protective against T1D development. One group demonstrated that immunization of NOD mice with mycobacterial preparations prevented the onset of T1D [11]. Immunization induced IL-17-producing T cells which, when transferred into recipient mice, did not induce diabetes [11]. The authors concluded that the rise in IL-17 after immunization, and thus Th17 cells, exerted a protective effect on T1D development [11]. Using RNA interference, another group demonstrated that silencing of IL-17A *in vivo* did not protect NOD mice from developing T1D [12]. Finally, several groups have described cases in which the presence of specific gut microflora induces Th17 cells *in vivo* and as a result, NOD mice are protected from the development of T1D [3].

Interestingly, clinical data supports a pathogenic role for Th17 cells in T1D. Patients with T1D present with elevated plasma levels of IL-17, increased circulating IL-17-producing cells and islet antigen-specific Th17 cells [13,14]. Pancreatic lymph nodes from patients with T1D have an increase in Th17 cells and analysis of islets from recently deceased T1D patients expressed the transcription factor RORγt and IL-17A [13]. In combination with IL-1β and IFN-γ, IL-17 mediates increased β-cell apoptosis in human pancreatic islet cells *in vitro* [14]. Thus, clinical data suggest that IL-17 producing cells may be pathogenic in the early stages of disease development and therapeutic strategies targeting Th17 cells may be a viable option for the treatment of T1D.

Therapeutic strategies

While treatment methods for T1D have improved over time, insulin replacement

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therapy is still the main treatment option for T1D. However, insulin therapy does not affect the autoimmune process nor has it removed the burden of disease on society. This suggests that therapies designed to inhibit the autoimmune destruction may be a valuable strategy. The use of monoclonal antibodies against CD3, which restores immune self-tolerance through targeting of effector T cells while amplifying Treg cells in patients with recent onset diabetes has yielded promising results [15]. However, not all subjects respond and the duration of repose is variable. Similar results have been obtained with other immunotherapies that were tested individually [16]. Collectively, these studies suggest that immunotherapies hold promise for the treatment of T1D, but perhaps combination therapies may have the greatest efficacy.

Because of the growing evidence supporting the role for Th17 cells in T1D and their dichotomous role with Treg cells, targeting this cell type for the treatment of T1D is a logical choice. Approaches that have yielded promising results in mouse models of T1D include use of IL-25 or neutralizing antibodies to IL-17A [7,9]. An alternative approach would be to specifically target the factors that drive Th17-cell development and function, including ROR α and ROR γ t, in order to target the cell type as a whole, rather than individual cytokines. The RORs are members of the NR superfamily of ligand-regulated transcription factors. Small lipophilic molecules can robustly modulate the transcriptional activity of NRs making them attractive therapeutic targets. In fact, approximately 10–15% of drugs currently approved by the US FDA target NRs, emphasizing their therapeutic value [17]. Based on these criteria, we designed and characterized a ROR α/γ dual inverse agonist, SR1001, that inhibited Th17 cell development and function *in vitro* and had efficacy *in vivo* in several mouse models of autoimmunity, including T1D [10,18]. We demonstrated that efficacy of SR1001 was a consequence of globally inhibiting Th17-cell development and effector function, including expression of all Th17-mediated cytokines [10]. However, SR1001's efficacy may have also been attributed to several other factors, including the decreased IFN- γ expression in T cells or the increased Treg populations we observed *in vivo* [10]. These data suggest that modulating ROR activity may affect Th17-cell plasticity.

The efficacy of SR1001 may also have been a consequence of changes in metabolic processes *in vivo*. Both ROR α and ROR γ have been extensively studied outside of the immune system due to their key roles in the regulation of hepatic glucose and lipid metabolism, processes often dysregulated in T1D [19]. Collectively, the data suggest that modulation of ROR activity and Th17-cell function may prove to be a valid therapeutic strategy for the treatment of T1D.

Conclusions & future perspective

T1D is characterized as an autoimmune disease whereby CD4⁺ T cells are thought to mediate disease pathology. While the effector function of Th1 cells is well established in T1D pathogenesis, emerging evidence suggests that Th17 cells also play an important role. Their exact role and how they relate to Th1 cells is still unclear and more work needs to be done to elucidate the mechanism(s) inducing disease. Regardless, targeting Th17 cells appears to be a viable therapeutic strategy for the treatment of T1D. Considering the number of beneficial effects observed with the use of ROR modulators in a mouse model of T1D, further studies should be performed to evaluate their therapeutic potential, including whether SR1001 may be efficacious for the treatment of recent onset diabetes. Since SR1001 modulates the activity of ROR α and ROR γ , future studies identifying the individual roles for each ROR in the development of T1D are necessary in order to make more focused therapeutics. Finally, given the less than robust outcomes of past clinical trials assessing immune modulators, including anti CD3, we should begin to consider evaluating effects of drugs in combinations. Doing so may yield the greatest results and we may finally generate efficacious immunotherapies for the treatment of T1D.

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References

- 1 Shao S, He F, Yang Y, Yuan G, Zhang M, Yu X. Th17 cells in Type 1 diabetes. *Cell Immunol.* 280(1), 16–21 (2012).
- 2 Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 383(9911), 69–82 (2014).
- 3 Bedoya SK, Lam B, Lau K, Larkin J 3rd . Th17 cells in immunity and autoimmunity. *Clin. Dev. Immunol.* 2013 986789 (2013).
- 4 Yang XO, Pappu BP, Nurieva R *et al.* T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* 28(1), 29–39 (2008).
- 5 Yang XO, Nurieva R, Martinez GJ *et al.* Molecular antagonism and plasticity of regulatory and inflammatory t cell programs. *Immunity* 29(1), 44–56 (2008).
- 6 Haskins K, Cooke A. CD4 T cells and their antigens in the pathogenesis of autoimmune diabetes. *Curr. Opin. Immunol.* 23(6), 739–745 (2011).
- 7 Emamaullee JA, Davis J, Merani S *et al.* Inhibition of Th17 cells regulates autoimmune diabetes in nod mice. *Diabetes* 58(6), 1302–1311 (2009).
- 8 Martin-Orozco N, Chung Y, Chang SH, Wang YH, Dong C. Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into th1 cells. *Eur. J. Immunol.* 39(1), 216–224 (2009).
- 9 Bending D, De La Pena H, Veldhoen M *et al.* Highly purified th17 cells from bdc2.5nod mice convert into th1-like cells in NOD/SCID recipient mice. *J. Clin. Invest.* 119(3), 565–572 (2009).
- 10 Solt LA, Banerjee S, Campbell S, Kamenecka TM, Burris TP. ROR inverse agonist suppresses insulinitis and prevents hyperglycemia in a mouse model of Type 1 diabetes. *Endocrinology* 156(3), 869–881 (2015).
- 11 Nikoopour E, Schwartz JA, Huszarik K *et al.* Th17 polarized cells from nonobese diabetic mice following mycobacterial adjuvant immunotherapy delay Type 1 diabetes. *J. Immunol.* 184(9), 4779–4788 (2010).
- 12 Joseph J, Bittner S, Kaiser FM, Wiendl H, Kissler S. Il-17 silencing does not protect nonobese diabetic mice from autoimmune diabetes. *J. Immunol.* 188(1), 216–221 (2012).
- 13 Marwaha AK, Leung NJ, Mcmurphy AN, Levings MK. Th17 cells in autoimmunity and immunodeficiency: protective or pathogenic? *Front. Immunol.* 3, 129 (2012).
- 14 Reinert-Hartwall L, Honkanen J, Salo HM *et al.* Th1/th17 plasticity is a marker of advanced beta cell autoimmunity and impaired glucose tolerance in humans. *J. Immunol.* 194(1), 68–75 (2015).
- 15 Bach JF. Anti-cd3 antibodies for Type 1 diabetes: beyond expectations. *Lancet* 378(9790), 459–460 (2011).
- 16 Ludvigsson J. Combination therapy for preservation of beta cell function in Type 1 diabetes: new attitudes and strategies are needed! *Immunol. Lett.* 159(1–2), 30–35 (2014).
- 17 Marciano DP, Chang MR, Corzo CA *et al.* The therapeutic potential of nuclear receptor modulators for treatment of metabolic disorders: PPARgamma, RORs, and REV-ERBS. *Cell Metab.* (2014).
- 18 Solt LA, Kumar N, Nuhant P *et al.* Suppression of th17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature* 472(7344), 491–494 (2011).
- 19 Jetten AM, Kang HS, Takeda Y. Retinoic acid-related orphan receptors alpha and gamma: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity. *Front. Endocrinol.* 4, 1 (2013).