



miRNA Regulation of T Cells in Islet Autoimmunity and Type 1 Diabetes

Martin G. Scherm^{1,2} · Carolin Daniel^{1,2,3}

Published online: 28 July 2020

© The Author(s) 2020

Abstract

Purpose of Review Regulatory T cells (Tregs) are critical contributors to immune homeostasis and their dysregulation can lead to the loss of immune tolerance and autoimmune diseases like type 1 diabetes (T1D). Recent studies have highlighted microRNAs (miRNAs) as important regulators of the immune system, by fine-tuning relevant genes in various immune cell types. In this review article, we discuss recent insights into miRNA regulation of immune tolerance and activation. Specifically, we discuss how the dysregulation of miRNAs in T cells contributes to their aberrant function and the onset of islet autoimmunity, as well as their potential as targets of novel intervention strategies to interfere with autoimmune activation.

Recent Findings Several studies have shown that the dysregulation of individual miRNAs in T cells can contribute to impaired immune tolerance, contributing to onset and progression of islet autoimmunity. Importantly, the targeting of these miRNAs, including miR-92a, miR-142-3p and miR-181a, resulted in relevant effects on downstream pathways, improved Treg function and reduced islet autoimmunity in murine models.

Summary miRNAs are critical regulators of immune homeostasis and the dysregulation of individual miRNAs in T cells contributes to aberrant T cell function and autoimmunity. The specific targeting of individual miRNAs could improve Treg homeostasis and therefore limit overshooting T cell activation and islet autoimmunity.

Keywords Immune regulation · Islet autoimmunity · Type 1 diabetes · Regulatory T cell · miRNA · Biomarker

Introduction

Type 1 diabetes (T1D) is an organ-specific autoimmune disease. Its high prevalence early in life and increasing incidence

worldwide make it a considerable burden for the healthcare systems. The disease is characterized by the T cell-mediated destruction of the insulin producing beta cells in the pancreas, leading to impaired insulin secretion, hyperglycemia, and increased risk of both acute and chronic complications associated with significant morbidity and mortality [1]. FOXP3⁺ regulatory T cells (Tregs) are key players for the maintenance of immune homeostasis. Impaired Treg induction, stability and function are critically involved in the loss of immune tolerance and the activation of autoimmunity [2]. microRNAs (miRNAs) have been recently shown to contribute to immune function and homeostasis by controlling gene expression in various immune cell types, including Tregs. Targeting of miRNAs represents a promising approach to interfere with overshooting immune activation. In this review, we discuss the role of T cells for the onset and progression of islet autoimmunity and the complex interplay with miRNA regulatory networks. Furthermore, we address the therapeutic potential of miRNA targeting for future immunomodulatory strategies with the vision to interfere with autoimmunity.

This article is part of the Topical Collection on *Pathogenesis of Type 1 Diabetes*

✉ Carolin Daniel
carolin.daniel@helmholtz-muenchen.de

Martin G. Scherm
martin.scherm@helmholtz-muenchen.de

¹ Institute of Diabetes Research, Group Immune Tolerance in Type 1 Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, Heidemannstrasse 1, 80939 Munich, Germany

² Deutsches Zentrum für Diabetesforschung (DZD), Ingolstaedter Landstrasse 1, 85764 Munich-, Neuherberg, Germany

³ Division of Clinical Pharmacology, Department of Medicine IV, Ludwig-Maximilians-Universität München, 80337 Munich, Germany

miRNA Basics and Biogenesis

Small non-coding RNAs (ncRNAs) have emerged as important contributors to the regulation of various biological processes including immune regulation, function and homeostasis. They are defined by their Argonaute (AGO) family protein-mediated mode of action and their length of 20–30 nucleotides. There are three distinct families of small ncRNAs with regulatory function: miRNA (microRNA), siRNA (small interfering RNA) and piRNA (PIWI-interacting RNA). The miRNA class is the most abundant in most tissues. miRNAs are ~22 nucleotides long, single-stranded and transcribed from the genome, alone or in polycistronic clusters. They control gene expression by complementary binding of their target mRNA, recruiting AGO family proteins and inducing mRNA cleavage, translational repression or mRNA deadenylation.

The recognition of the respective mRNA targets is facilitated by the miRNA seed sequence, a sequence which is specific for each miRNA, spanning from nucleotide position 2 to 7. While the seed sequence is crucial for target recognition and thus for the regulatory function of miRNAs, the binding of the mRNA target is additionally supported by nucleotides 8 and 13–16 of the miRNA [3]. There are groups of miRNAs, called miRNA clusters or families, which have highly similar seed sequences and substantially overlapping target genes [4]. Previous studies suggested that miRNA binding preferentially occurs in the 3' untranslated region (UTR) [5], but more recent studies showed that miRNA binding sites are located at comparable levels in the 3' UTR and the coding region of the mRNA targets, with only a slight preference for the 3' UTR [6, 7].

The biogenesis of miRNAs is a complex multi-stage process which takes place in the nucleus as well as the cytoplasm. It comprises the transcription of miRNA genes into primary miRNA (pri-miRNA) transcripts, processing into pre-miRNAs and subsequently into mature miRNAs. Pri-miRNAs are mainly transcribed by RNA polymerase II [8], and the primary transcript can contain a single miRNA or a cluster of multiple miRNAs [9]. The pri-miRNA is processed by Drosha [10] and DGCR8 [11, 12] and the resulting pre-miRNA by Dicer [13] to produce the mature miRNA duplex. One strand of the mature miRNA is loaded into AGO to form the miRNA-induced silencing complex (miRISC) and to guide the complex to its target mRNAs, while the other strand is discarded [14].

Regulatory Role of miRNAs

The important role of miRNAs for various biological processes is illustrated by their complex regulatory networks. Each individual miRNA targets a multitude of genes and in

addition most of these genes are targeted by more than one miRNA. Most human protein-coding genes have been shown to be miRNA targets with more than 60% containing multiple conserved miRNA binding sites and in addition even larger numbers of non-conserved sites [3–5]. So far, about 2600 mature human miRNA sequences are defined in the miRNA database MirBase [15]; the actual number of human miRNAs is thought to be much higher, as illustrated by the constant increase of newly defined miRNA sequences [16]. It has been shown that miRNAs harbor clear tissue-specific expression patterns, which are regulated at the transcription level and illustrate their important contribution to the regulation of tissue homeostasis and function [3, 17]. Given these complex regulatory networks and the broad role of miRNAs for the regulation of various biological processes, the dysregulation of miRNA expression can contribute to various diseases, including autoimmunity, cancer [18] and neurological diseases [19]. The first evidence for an important role of miRNAs in the regulation of the immune system dates back to 2004, when differential expression of several miRNAs was reported in specific immune cell subsets [20]. Since then, multiple studies clearly indicated the implication of miRNAs in various processes of immune regulation.

Immune Regulation and T1D Autoimmunity

The clear discrimination between self and non-self is a critical requirement for the proper functioning of the immune system, which is detecting and eliminating harmful pathogens and distinguishing them from the organism's own tissues. This delicate balance between immune activation and immune tolerance is a complex and tightly regulated process that requires precise control of lymphocyte development and function, with constant adjustments as required. The importance of proper immune regulation becomes evident when these control mechanisms are dysregulated, which leads to the development and activation of autoreactive lymphocytes and thereby is a critical contributor to autoimmunity. There are more than 80 diseases with an autoimmune etiology, which can be classified into systemic or organ-specific diseases, such as Multiple Sclerosis, Rheumatoid Arthritis and T1D, which is the most common autoimmune disease in young children [21]. The disease is characterized by impaired immune tolerance mechanisms, resulting in the immune-mediated destruction of pancreatic beta cells, diabetes symptoms and complications [22].

T1D progresses through distinct identifiable stages prior to the onset of the clinically overt disease [23, 24]. The appearance of autoantibodies against islet autoantigens defines the pre-symptomatic phase of T1D, which is therefore termed islet autoimmunity. During the last decades, several

autoantigens have been identified, including insulin [25], glutamic acid decarboxylase (GAD) [26], insulinoma-antigen 2 (IA2) [27, 28] and zinc transporter 8 (ZNT8) [29]. Importantly, autoantibodies can be present in the blood many years before the onset of hyperglycemia [24, 30]. Despite ongoing research efforts and although recent studies significantly contributed to our understanding of T1D pathogenesis, the molecular mechanisms underlying the heterogeneity, the onset of islet autoimmunity and the progression to symptomatic diabetes remain poorly understood.

Role of T Cells in T1D

The immune-mediated destruction of the insulin producing beta cells in the pancreatic islets of Langerhans is initiated by insulinitis, a process of immune cell infiltration into the islets. The infiltrating immune cells consist of CD8⁺ T cells, CD4⁺ T cells, B cells and macrophages, with CD8⁺ T cells being the most abundant cell type [31]. Despite the high abundance of CD8⁺ T cells in the pancreatic infiltrates, various subsets of CD4⁺ T cells have been identified as important contributors to autoimmune diseases, including T1D, multiple sclerosis [32], rheumatoid arthritis [33] and Crohn's disease [34]. In T1D, multiple studies have identified and further characterized infiltrating CD4⁺ T cells autoreactive against islet antigens. These identified subsets include Th1, Th2 [35], Th17 [36, 37], Th9, Th22 [38] and TFH cells [39, 40]. Although T cells clearly play a major role in the destruction of pancreatic beta cells, the underlying mechanisms promoting aberrant T cell activation in autoimmunity remain poorly understood.

Recent studies have focused on the contribution of specific T cell subsets to immune activation and autoimmunity and their findings point towards an important role of impaired function of Tregs [7, 41–44]. Tregs are crucial contributors to the maintenance of immune homeostasis and their impaired induction, stability and function can critically contribute to the loss of immune tolerance and the activation of autoimmunity. They are characterized by the expression of CD4, CD25, and the transcription factor FOXP3, which is crucial for their development, maintenance and function [45, 46]. This is clearly illustrated by the deleterious consequences of mutation in the FOXP3 gene, resulting in fatal autoimmune phenotypes in both mice (scurfy mice) and humans (IPEX—immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) [47, 48]. In addition to their generation in the thymus, Tregs can be induced from naive CD4⁺ T cells in the periphery, most efficiently by the application of a strong-agonistic T cell receptor (TCR) ligand under subimmunogenic conditions [49–51].

miRNAs as Biomarkers in T1D

Biomarkers provide an objectively quantifiable manner to characterize a disease. This enables both diagnosis and treatment as early and precisely as possible, and facilitates highly targeted and personalized interventions aimed at the maximum benefit for the patient. Consequently, the interest in biomarkers for studying diseases and developing novel treatment strategies continues to grow in many areas of clinical practice.

Levels of circulating miRNAs can be analyzed in small volumes of peripheral blood. Given this minimal invasiveness and the importance of miRNAs for immune homeostasis, several studies have investigated the impact of circulating miRNAs on disease development and their suitability to predict diseases progression [52–55]. miRNAs involved in the function of lymphocytes and beta cells were found to be differentially expressed in whole blood and serum obtained from newly diagnosed T1D patients compared to healthy controls. The abundance of miR-21a and miR-93, which are involved in NF- κ B signaling and negatively regulate apoptotic and inflammatory genes, was significantly reduced in peripheral blood mononuclear cells (PBMCs) of T1D patients [53]. In contrast, residual beta cell function and glycemic control in individuals with recent onset of T1D were associated with high levels of miR-25 [52]. miR-146a was significantly down-regulated in PBMCs of patients with newly diagnosed T1D and the decreased miRNA expression was associated with high GAD autoantibody titers in the serum [56], while an upregulation of miR-326 correlated with the presence of autoantibodies against GAD and IA2 [54]. A meta-analysis of studies investigating circulating miRNA profiles in T1D patients revealed eleven differentially expressed miRNAs: miR-21-5p, miR-24-3p, miR-100-5p, miR-146a-5p, miR-148a-3p, miR-150-5p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375 and miR-1275. These miRNAs are involved in immune regulation, cell proliferation and insulin processing, making them potential biomarkers for T1D [57].

Despite the importance of the pre-symptomatic phase of T1D only limited studies have investigated miRNA levels in individuals with islet autoimmunity before the onset of clinical T1D. One of these rare studies found similar miRNA levels in the serum of high-risk individuals positive for multiple islet autoantibodies and healthy controls and no specific miRNA signature could be identified in the high-risk group. Interestingly, several miRNAs were associated with glucose homeostasis and autoantibody titers and these miRNAs correlated with glycemic status and ongoing islet autoimmunity in high-risk individuals [58]. Another study investigated miRNAs in the serum of 150 autoantibody-positive and 150 autoantibody-negative family-matched siblings and found several miRNAs in the serum reflecting islet autoimmunity and progression to T1D with miR-21-3p, miR-29a-3p and miR-424-5p showing the strongest correlation [55]. Despite

these important insights, the full potential of circulating miRNAs for the assessment of T1D pathogenesis and progression is still limited due to: (1) In organ-specific diseases like T1D, profiles from whole blood or serum might not reflect the relevant changes in the affected organ or cell type. (2) Relevant changes in miRNA profiles might result from changes in the miRNA profile or the abundance of specific immune cell populations, rather than global changes in the miRNA expression. (3) Changes in miRNA abundance might result from the loss of glucose homeostasis and therefore not be related to disease pathogenesis. To overcome these limitations and to increase the explanatory power of circulating miRNAs identified in the abovementioned studies, the analysis of specific miRNAs in cell types which are directly involved in the activation and progression of autoimmunity gets into the focus.

miRNA Regulation of Regulatory T Cells

In line with their regulatory role in various biological settings, including the immune system, miRNAs have also been highlighted as direct modulators of Tregs. The Treg-specific interference with the miRNA processing machinery in mice had no effect on thymic development of Tregs, but it resulted in reduced suppressive function of Tregs and deleterious systemic autoimmunity [59••, 60••, 61, 62]. Based on these findings, multiple studies investigated the underlying mechanisms of these defects and specifically the contribution of individual miRNAs. These studies revealed miRNA regulation of thymic Treg development, Treg induction, FOXP3 expression, Treg stability and suppressive function (Table 1).

The expression of miR-10a is restricted to Tregs following the exposure to retinoic acid and the abundance of this miRNA correlates with a low susceptibility to autoimmune diseases in mice [85, 86]. miR-10a stabilizes the Treg-specific gene signature by targeting several effector T cell genes including *Bcl6* and *Ncor2*. However, the functional targets of miR-10a may be targeted by other miRNAs as well, because miR-10a deficiency alone does not result in impaired Treg function or autoimmunity. The analysis of miRNA expression in Tregs revealed several differentially expressed miRNAs with both up- and downregulation compared to CD4⁺CD25⁻ T cells. Interestingly, miR-31 which was downregulated in Tregs, directly targets the 3'UTR of *Foxp3* mRNA [68]. This predicted binding was experimentally confirmed using lentiviral transduction of T cells, which resulted in reduced FOXP3 levels. In addition, miR-21, which was highly expressed in Tregs, is a positive, though indirect, regulator of FOXP3 expression. miR-155 is highly abundant in Tregs and directly regulated by FOXP3. In mouse models miR-155 deficiency resulted in impaired Treg development and homeostasis and consequently reduced levels of Tregs

in the thymus and the spleen [87, 88]. In miR-155-deficient Tregs the expression of FOXP3 is reduced and instable while in vitro Treg induction is unaffected. miR-155 targets suppressor of cytokine signaling 1 (SOCS1), a negative regulator of STAT5 signaling which determines the responsiveness to IL-2, a critical regulator of Treg homeostasis [89]. However, miR-155-deficient Tregs can prevent autoimmune diseases in mice, indicating that miR-155 is crucial for FOXP3 expression and Treg stability but does not directly affect their suppressive function [87].

The efficient in vitro Treg induction is also subject to regulation by miRNAs. In vitro Treg induction experiments using both Dicer and Drosha deficient naive CD4⁺ T cells resulted in a significantly reduced expression of FOXP3 in induced Tregs compared to wild type naive CD4⁺ T cells [59••, 60••]. Interestingly, miRNAs with both positive and negative regulatory effects on in vitro Treg induction have been identified in a miRNA screen [90] and several miRNAs form networks to cooperatively regulate Treg induction. For example, miR-150 induced reduction of mTOR occurs only in presence of miR-99a and a similar cooperation has been shown for miR-15a-16 and 15b-16. Another miRNA targeting the PI3K/Akt/mTOR pathway is miR-126. It targets p85 β , which is a regulatory subunit of PI3K, reducing PI3K/Akt/mTOR pathway activity and favoring Treg induction. By contrast, miR-126 inhibition increases the activity of the PI3K/Akt/mTOR pathway, inhibiting FOXP3 expression and impairing Treg induction [75]. miR-155 also contributes to proper Treg induction in vitro by targeting *Socs1* which is in line with its role for thymic Treg generation [91]. As mentioned above, the miRNA screen also revealed miRNAs with a negative effect on Treg induction in vitro [90]. Two members of the miR-17~92 cluster, miR-17 and miR-19, negatively regulate Treg induction while they have no impact on thymic Treg development [92]. miR-17 has been shown to directly target the cAMP-responsive element binding protein 1 (*Creb1*) and the TGF β -receptor II, both involved in proper Treg induction. The TGF β signaling pathway is also a target of the miR-23-miR-27-miR-24 cluster, and consequently a high abundance of this cluster impairs Treg generation [93].

Mechanistic Dissection of miRNAs in T Cells during Autoimmunity

As illustrated above, several recent studies illustrated the importance of miRNA mediated regulation for immune homeostasis by controlling the accurate function of various immune cell subsets, including Tregs. While this makes them of considerable importance for the field, the underlying, relevant pathways ultimately triggering insufficient immune tolerance and the onset of T1D autoimmunity, remain insufficiently understood. Given the importance of proper Treg function,

Table 1 miRNAs involved in Treg induction, maintenance and function

miRNA	miRNA regulation	miRNA target	effect on Tregs	organism	target validation		miRNA effect validation		reference
					indirect	direct	in vitro	in vivo	
let7i	up	IGF1R	decreased Treg induction	human	X		X		[63]
miR10a	down	IFN γ	increased Foxp3 abundance	mouse		X	X	X	[64]
miR15a/16	up	Foxp3	decreased Foxp3 abundance	human		X	X	–	[65]
miR15b/16	up	Rictor, mTor	increased Treg induction	mouse	X		X	X	[66]
miR17	up	IKZF4	decreased Treg frequencies	mouse	X		X	X	[67]
miR19b	up	PTEN	decreased Treg frequencies	mouse	X		X	X	[67]
miR21	up	unknown	increased Foxp3 abundance	human	–	–	X	–	[68]
miR21	down	STAT3	decreased Treg frequencies	human	X		–	–	[69]
miR24	down	Foxp3	increased Foxp3 abundance	human		X	–	–	[70]
miR25	up	TGF β signaling	decreased suppression	human		X*	–	–	[71]
miR31	down	Foxp3	increased Foxp3 abundance	human		X	X	–	[68]
miR92a	up	KLF2 (...)	decreased Treg induction	human/mouse		X	X	X	[42]
miR95	up	unknown	increased Foxp3 abundance	human	–	–	–	–	[70]
miR99a	up	mTor	increased Treg induction	mouse		X	X	X	[72]
miR100	up	SMAD2/3	decreased Treg induction	human	X		X		[73]
miR125a-5p	down	CXCL13	decreased Treg frequencies	human		X*	–	–	[74]
miR126	down	p85B	decreased Treg induction	human/mouse	X		X	X	[75]
miR142-3p	up	Tet2	decreased Treg induction and stability	human/mouse		X	X	X	[7]
miR146a	down	STAT1	decreased suppression	human		X*	–	–	[76]
miR146a	down	STAT1	decreased suppression	mouse		X	X	X	[77]
miR146b	up	TRAF6	decreased suppression	human		X	X	X	[78]
miR150	up	mTor	increased Treg induction	mouse		X	X	X	[72]
miR181a	up	PI3K signaling	decreased Treg induction	human/mouse		X	X	X	[44]
miR182	up	Foxo1	decreased Treg frequencies	mouse	X		X	X	[79]
miR200a	up	unknown	decreased Treg frequencies	human	–	–	–	–	[80]
miR210	down	Foxp3	increased Foxp3 abundance	human		X	–	–	[70]
miR210	up	Foxp3	decreased Treg frequencies	human		X	X	–	[81]
miR214	up	PTEN	increased Treg frequencies	mouse	X		X	X	[82]
miR326	up	Ets-1	decreased Treg frequencies	human	X		–	–	[83]
miR663	up	TGF β 1	decreased Treg frequencies	human/mouse		X	X	X	[84]

*shown previously

induction and stability, three recent studies focused on the direct interplay between the dysregulated expression of individual miRNAs in T cells during the onset of autoimmunity and impaired Treg induction from naive CD4⁺ T cells.

miR-142-3p

The first study showed that during islet autoimmunity increased miR-142-3p expression leads to impaired epigenetic remodeling and consequently interferes with the efficient induction of Tregs and Treg stability (Fig. 1). Specifically, the upregulation of miR-142-3p during the onset of islet autoimmunity resulted in decreased expression of its direct target, the

methylcytosine dioxygenase Tet2, which is a mediator of DNA demethylation. By catalyzing DNA demethylation of regulatory regions and consequently altering the accessibility of the DNA to transcription factors, Tet enzymes are involved in the regulation of various cellular processes. They're involved in the differentiation of CD4⁺ T cells in humans and mice, as well as Treg homeostasis and function by active demethylation of the *FOXP3* conserved noncoding sequence 2 (CNS2), which ensures long-term stability of FOXP3 expression in Tregs [94–97]. The miR-142-3p mediated reduced abundance of the epigenetic modifier Tet2 was directly linked to impaired DNA demethylation at the *FOXP3* CNS2 locus during islet autoimmunity and led to reduced frequencies of FOXP3⁺ Tregs in the pancreas of mice with ongoing islet

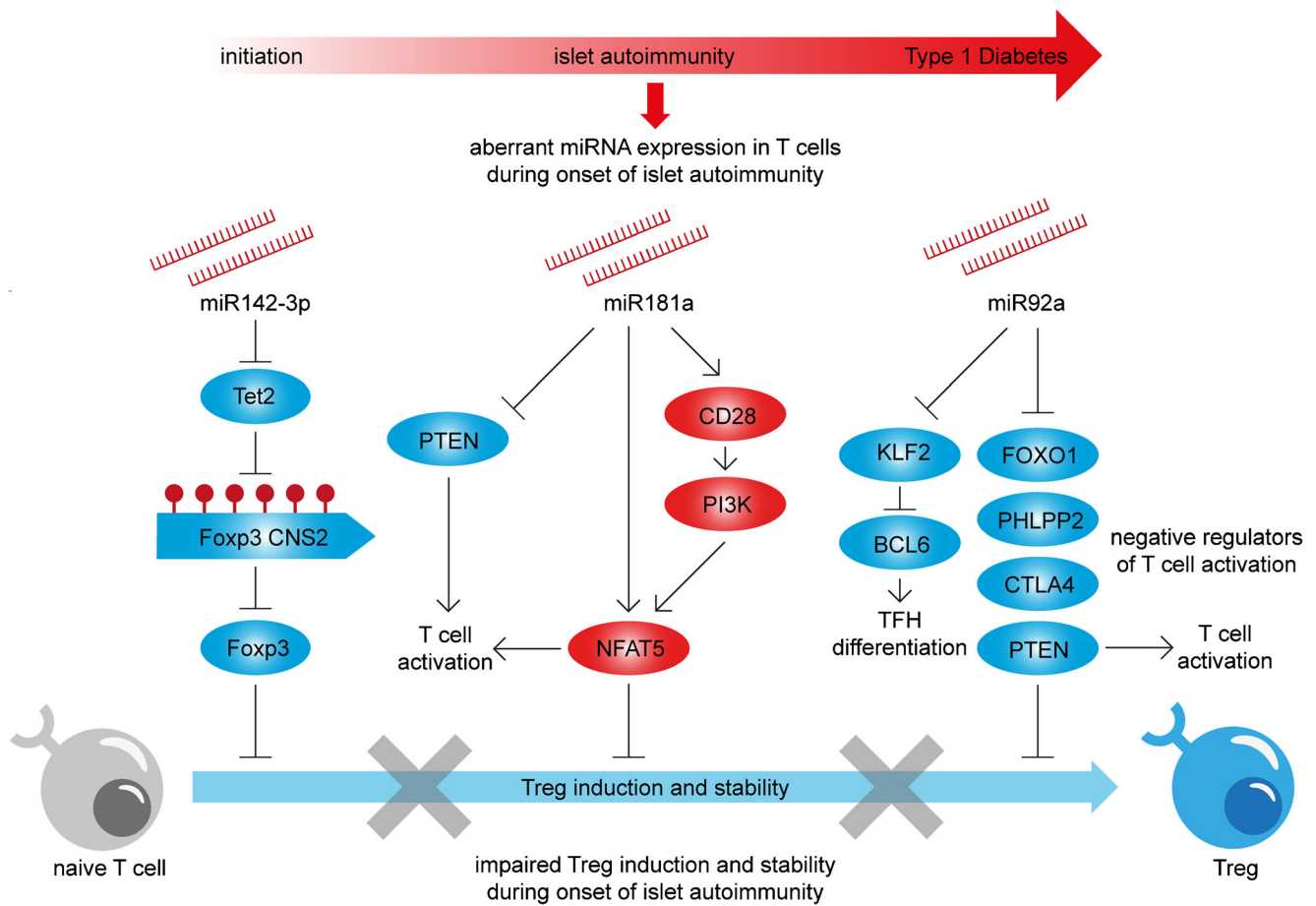


Fig. 1 Role of T cell specific miRNAs in autoimmune activation. During the onset of islet autoimmunity high levels of miR-142-3p, miR-181a and miR-92a and their downstream pathways contribute to enhanced T cell activation and impairments in Treg induction from naive CD4⁺ T cells

autoimmunity. The inhibition of miR-142-3p restored Tet2 levels, improved Treg induction and stability in vitro and reduce islet autoimmunity in mouse models of islet autoimmunity in vivo. As a next step, the relevance of these findings for established human T1D and their translatability was shown in humanized mouse models. Furthermore, and in line with the complex regulatory networks targeted by individual miRNAs, the study revealed multiple miR-142-3p targets with important roles in Treg development and function. Specifically, *Stat5*, *Smad3* and Tgfb receptors (*Tgfr1*, *Tgfr2* and *Tgfr3*) were identified as potential targets. *Stat5* is a downstream target of IL-2 involved in Treg development [98]; TGF β exhibits potent immunoregulatory properties, including the expression of FOXP3 in Tregs via phosphorylation of Smad proteins [99]. Both signaling pathways add to the identified mechanism of Tet2-mediated DNA demethylation to ensure FOXP3 expression and maintenance and consequently proper Treg function and stability. These findings suggest that miR-142-3p targets a regulatory network of genes involved in the regulation of Treg function and stability; such a broad impact helps explain how the targeting of a single miRNA has such a considerable effect on Treg homeostasis in a highly

complex setting like autoimmunity. In addition, Tet2 catalyzes the demethylation of regulatory regions of various genes. This study showed the importance of Tet2-mediated demethylation of the *FOXP3* CNS2 for Treg function and stability in the context of autoimmunity. However, additional regulatory regions might be controlled by Tet2 and contribute to the regulation of FOXP3. These results offer a new mechanistic model where during islet autoimmunity miR-142-3p/Tet2-mediated impairments in Treg induction and stability contribute to the activation and progression of islet autoimmunity. Moreover, specific targeting of miR-142-3p or Tet2 might contribute to the development of novel intervention strategies, aiming at improved Treg induction and stability to interfere with the onset of islet autoimmunity.

miR-181a

In a second study, excessive T cell activation was linked to differential miRNA expression during the onset of islet autoimmunity (Fig. 1). The excessive T cell activation was found in children with recent onset of islet autoimmunity

and resulted in enhanced frequencies and proliferation of FOXP3^{int}CD4⁺ T cells, which interferes with efficient Treg induction [43, 44]. Specifically, this increased T cell activation could be directly linked to higher levels of miR-181a, which reportedly alters signaling thresholds in T cells by modulating the sensitivity to antigenic stimulation [100]. The phosphatase PTEN, which is a negative regulator of T cell activation, was identified as a direct target of miR-181a and PTEN inhibition resulted in increased PI3K signaling and activation of NFAT5. NFAT5 plays an important role for T cell activation and its abundance was increased as a result of high miR-181a levels. Furthermore, elevated miR-181a levels increased the expression of the costimulatory molecule CD28 which is also involved in the activation of the PI3K pathway [101] and consequently T cell activation. In line with these findings, the inhibition of miR-181a improved Treg induction from naive CD4⁺ T cells, while a miR-181a mimic resembled the impaired Treg induction observed during recent onset of islet autoimmunity. These findings were confirmed in mouse models, including those of T1D [44]. Specifically, high levels of miR-181a and NFAT5, decreased abundance PTEN and reduced Treg induction capacity from naive CD4⁺ T cells were found in NOD mice with islet autoimmunity. The in vivo inhibition of miR-181a in those mice reduced islet autoimmunity, decreased CD28 and NFAT5 expression and enhanced PTEN levels. Treg induction was also improved by pharmacological inhibition of NFAT5, and in *Nfat5* deficient mice where increased levels of PTEN were also observed.

miR-92a

The third study provided evidence that high levels of miR-92a during the onset of islet autoimmunity mediate the induction of T follicular helper (TFH) cell precursors as well as impaired Treg induction from naive CD4⁺ T cells, two mechanisms that are likely involved in the onset and progression of islet autoimmunity [42] (Fig. 1). TFH cells, which are a subset of CD4⁺ T cells, provide help to B cells to produce high-affinity antibodies, making them crucial contributors to humoral immunity [102–104]. This important role for antibody production suggests TFH precursors as an important effector T cell subset in autoimmunity. Both insulin-specific and polyclonal TFH precursors were increased in children with recent onset of islet autoimmunity [42]. Analysis of differential miRNA expression in these children identified miR-92a as upregulated and there was a positive correlation between miR-92a expression and the frequencies of TFH precursor cells in the blood. In experimental in vitro TFH induction assays, the inhibition of miR-92a reduced TFH induction, while a miR-92a mimic had the opposite effect [42]. This was in line with the identification of several negative

regulators of T cell activation including *Ctla4*, *Foxo1* and *Pten* as targets of miR-92a. In addition, *Klf2* was identified as a novel target of miR-92a and the miR-92a mediated down-regulation of *Klf2* was essential for the induction of TFH precursor cells. The decreased levels of PTEN resulted in the activation of the PI3K pathway which limits efficient Treg induction. Consequently, in vitro Treg induction was impaired in the presence of a miR-92a mimic, and high miR-92a levels in children with recent onset of islet autoimmunity were accompanied by reduced frequencies of insulin-specific Tregs. Conversely, PI3K signaling promoted TFH cell induction, since the effect of a miR-92a mimic is reduced in presence of a PI3K inhibitor and increased when PTEN signaling is inhibited.

In addition to the studies described above, miR-92a and miR-181a are also involved in the pathogenesis of other autoimmune conditions, including lymphoproliferation, Th17-mediated inflammation and autoimmune neuroinflammation [67, 105, 106].

Targeting of T Cell miRNAs and Clinical Relevance

The studies described above illustrate mechanisms by which increased miRNA expression directly contributes to autoimmune activation and progression. These findings, as well as the general importance of miRNAs for the precise regulation of the immune system, suggest that targeting miRNAs could represent an innovative intervention strategy to improve Treg induction and stability to interfere with islet autoimmunity. The relatively simple and well-known structure of miRNAs makes them attractive drug targets. This enables the efficient targeting of miRNAs using highly specific inhibitors, while mimics can be used to increase their activity, facilitating the fine-tuning of the regulation of their target genes. The complex regulatory networks of miRNAs, with each miRNA targeting a multitude of genes, hinder the direct and precise control of individual genes. However, these complex regulatory networks provide a potential explanation of the considerable effect of individual miRNAs in various settings, including their involvement in disease pathogenesis.

In autoimmunity and T1D miRNA-targeting agents were successfully used in vitro, in mouse models of autoimmune disease, and in humanized mice, resulting in upregulation of the respective miRNA targets, improvements in Treg function and reduced islet autoimmunity [7, 42, 44]. Furthermore, several clinical trials are currently investigating miRNA-based therapeutics for the treatment of hepatitis C virus infection, Alport syndrome, fibrosis, lymphoma, leukemia, inflammatory bowel disease, liver cancer and wound healing (summarized in: [107, 108]). To ensure both efficacy and safety of miRNA-targeting drugs, these approaches require the selective and

targeted delivery of miRNA inhibitors to the desired cell population, which remains challenging [109]. This is particularly important for the efficient treatment of organ-specific autoimmune diseases such as T1D but also for other disease settings which require a precise and local intervention in affected cell types. Several recent approaches resulted in considerable advances regarding the uptake of miRNA targeting agents by immune cells, as well as their targeted delivery. The use of oligonucleotide encapsulation techniques and nanoparticles led to improved uptake by immune cells and combinations of antibodies and nanoparticles are an important step towards the precise targeting of T cells or even T cell subsets [110, 111]. However, the design and validation of future treatment approaches will require the development of additional targeted strategies for T cell-specific targeting of miRNAs as well as optimized humanized mouse models to mimic the mechanisms underlying human autoimmune diseases in a preclinical model.

Conclusion

In order to better understand the molecular mechanisms promoting the onset of autoimmunity and the progression to T1D, the contribution of individual T cell subsets and how these subsets are regulated are of great importance. miRNAs are emerging as critical contributors to immune function and homeostasis, and they fine-tune the expression of relevant genes in various immune cell types, including Tregs. Therefore, recent studies focused on the contribution of individual miRNAs to aberrant T cell function in the setting of autoimmunity and described mechanisms by which miRNA levels impact autoimmune activation and progression. These insights suggest that the targeted and specific modulation of miRNA levels has the potential to reestablish Treg function and stability and in turn interfere with autoimmunity in future intervention strategies.

Acknowledgments C.D. holds a professorship grant from the Excellence Program for Outstanding Female Scientists from the Helmholtz Association, is supported by a Research Group at Helmholtz Zentrum München, the German Center for Diabetes Research (DZD) and through a membership in the CRC1054 of the Deutsche Forschungsgemeinschaft (B11).

Funding Information Open Access funding provided by Projekt DEAL.

Compliance with Ethical Standards

Conflict of Interest Martin G. Scherm and Carolin Daniel each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

- 1 Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*. 2010;464:1293–300. <https://doi.org/10.1038/nature08933>.
- 2 Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell*. 2008;133:775–87. <https://doi.org/10.1016/j.cell.2008.05.009>.
- 3 Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15:509–24. <https://doi.org/10.1038/nrm3838>.
- 4 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–97. [https://doi.org/10.1016/S0092-8674\(04\)00045-5](https://doi.org/10.1016/S0092-8674(04)00045-5).
- 5 Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19:92–105. <https://doi.org/10.1101/gr.082701.108>.
- 6 Kameswaran V, Bramswig NC, McKenna LB, Penn M, Schug J, Hand NJ, et al. Epigenetic regulation of the DLK1-MEG3 MicroRNA cluster in human type 2 diabetic islets. *Cell Metab*. 2014;19:135–45. <https://doi.org/10.1016/j.cmet.2013.11.016>.
- [7•] Scherm MG, Serr I, Zahm AM, Schug J, Bellusci S, Manfredini R, et al. miRNA142-3p targets Tet2 and impairs Treg differentiation and stability in models of type 1 diabetes. *Nat Commun*. 2019. <https://doi.org/10.1038/s41467-019-13587-3> **During islet autoimmunity a miR-142-3p/Tet2/Foxp3 axis in murine and human CD4+ T cells leads to impaired epigenetic remodeling and consequently interferes with the efficient induction of Tregs and impaired Treg stability.**
- [8] Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J*. 2004;23:4051–60. <https://doi.org/10.1038/sj.emboj.7600385>.
- [9] Berezikov E. Evolution of microRNA diversity and regulation in animals. *Nat Rev Genet*. 2011;12:846–60. <https://doi.org/10.1038/nrg3079>.
- [10] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003;425:415–9. <https://doi.org/10.1038/nature01957>.
- [11] Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. *Nature*. 2004;432:231–5. <https://doi.org/10.1038/nature03049>.

- [12] Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, et al. The microprocessor complex mediates the genesis of microRNAs. *Nature*. 2004;432:235–40. <https://doi.org/10.1038/nature03120>.
- [13] Hutvagner G, McLachlan J, Pasquinelli AE, Bálint É, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme dicer in the maturation of the let-7 small temporal RNA. *Science* (80-). 2001;293:834–8. <https://doi.org/10.1126/science.1062961>.
- [14] Rand TA, Petersen S, Du F, Wang X. Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell*. 2005;123:621–9. <https://doi.org/10.1016/j.cell.2005.10.020>.
- [15] Kozomara A, Birgaonu M, Griffiths-Jones S. MiRBase: from microRNA sequences to function. *Nucleic Acids Res*. 2019;47:D155–62. <https://doi.org/10.1093/nar/gky1141>.
- [16] Rodríguez-Galán A, Fernández-Messina L, Sánchez-Madrid F. Control of immunoregulatory molecules by miRNAs in T cell activation. *Front Immunol*. 2018;9:1–10. <https://doi.org/10.3389/fimmu.2018.02148>.
- [17] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell*. 2007;129:1401–14. <https://doi.org/10.1016/j.cell.2007.04.040>.
- [18] Lujambio A, Lowe SW. The microcosmos of cancer. *Nature*. 2012;482:347–55. <https://doi.org/10.1038/nature10888>.
- [19] Im HI, Kenny PJ. MicroRNAs in neuronal function and dysfunction. *Trends Neurosci*. 2012;35:325–34. <https://doi.org/10.1016/j.tins.2012.01.004>.
- [20] Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs Modulate Hematopoietic Lineage Differentiation. *Science* (80-). 2004. <https://doi.org/10.1126/science.1091903>.
- [21] Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet*. 2009;373:2027–33. [https://doi.org/10.1016/S0140-6736\(09\)60568-7](https://doi.org/10.1016/S0140-6736(09)60568-7).
- [22] Steffes MW, Sibley S, Jackson M, Thomas W. β -Cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care*. 2003;26:832–6. <https://doi.org/10.2337/diacare.26.3.832>.
- [23] Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of jdrf, the endocrine society, and the American diabetes association. *Diabetes Care*. 2015;38:1964–74. <https://doi.org/10.2337/dc15-1419>.
- [24] Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *Jama*. 2013;309:2473–9. <https://doi.org/10.1001/jama.2013.6285>.
- [25] Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* (80). 1983;222:1337–9. <https://doi.org/10.1126/science.6362005>.
- [26] Baekkeskov S, Aanstoot HJ, Christgai S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature*. 1990;347:151–6. <https://doi.org/10.1038/347151a0>.
- [27] Lan MS, Lu J, Goto Y, Notkins AL. Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human Insulinoma. *DNA Cell Biol*. 2009;13:505–14. <https://doi.org/10.1089/dna.1994.13.505>.
- [28] Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, et al. Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol*. 1994;152:3183–8.
- [29] Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci*. 2007;104:17040–5.
- [30] Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. *Immunity*. 2010;32:468–78. <https://doi.org/10.1016/j.immuni.2010.03.018>.
- [31] Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, et al. Differential insulinitic profiles determine the extent of β -cell destruction and the age at onset of type 1 diabetes. *Diabetes*. 2016;65:1362–9. <https://doi.org/10.2337/db15-1615>.
- [32] Dittel BN. CD4 T cells: balancing the coming and going of autoimmune-mediated inflammation in the CNS. *Brain Behav Immun*. 2008;22:421–30. <https://doi.org/10.1016/j.bbi.2007.11.010>.
- [33] Cope AP, Schulze-Koops H, Aringer M. The central role of T cells in rheumatoid arthritis. *Clin Exp Rheumatol*. 2007.
- [34] Chao K, Zhang S, Yao J, He Y, Chen B, Zeng Z, et al. Imbalances of CD4+ T-cell subgroups in Crohn's disease and their relationship with disease activity and prognosis. *J Gastroenterol Hepatol*. 2014;29:1808–14. <https://doi.org/10.1111/jgh.12592>.
- [35] Szebeni A, Schloot N, Kecskeméti V, Hosszúfalusi N, Pánczél P, Prohászka Z, et al. Th1 and Th2 cell responses of type 1 diabetes patients and healthy controls to human heat-shock protein 60 peptides AA437-460 and AA394-408. *Inflamm Res*. 2005;54:415–9. <https://doi.org/10.1007/s00011-005-1362-9>.
- [36] Arif S, Moore F, Marks K, Bouckennooghe T, Dayan CM, Planas R, et al. Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated β -cell death. *Diabetes*. 2011;60:2112–9. <https://doi.org/10.2337/db10-1643>.
- [37] Ferraro A, Soggi C, Stabilini A, Valle A, Monti P, Piemonti L, et al. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. *Diabetes*. 2011;60:2903–13. <https://doi.org/10.2337/db11-0090>.
- [38] Ryba-Stanisławowska M, Werner P, Brandt A, Myśliwiec M, Myśliwska J. Th9 and Th22 immune response in young patients with type 1 diabetes. *Immunol Res*. 2016;64:730–5. <https://doi.org/10.1007/s12026-015-8765-7>.
- [39] Ferreira RC, Simons HZ, Thompson WS, Cutler AJ, Dopico XC, Smyth DJ, et al. IL-21 production by CD4+ effector T cells and frequency of circulating follicular helper T cells are increased in type 1 diabetes patients. *Diabetologia*. 2015;58:781–90. <https://doi.org/10.1007/s00125-015-3509-8>.
- [40] Kenefick R, Wang CJ, Kapadi T, Wardzinski L, Attridge K, Clough LE, et al. Follicular helper T cell signature in type 1 diabetes. *J Clin Invest*. 2015;125:292–303. <https://doi.org/10.1172/JCI6238>.
- [41] Heninger AK, Eugster A, Kuehn D, Buettner F, Kuhn M, Lindner A, et al. A divergent population of autoantigen-responsive CD4+ T cells in infants prior to b cell autoimmunity. *Sci Transl Med*. 2017;9:eaa8848. <https://doi.org/10.1126/scitranslmed.aaf8848>.
- [42] J Serr I, Fürst RW, Ott VB, Scherm MG, Nikolaev A, Gökmen F, et al. miRNA92a targets KLF2 and the phosphatase PTEN signaling to promote human T follicular helper precursors in T1D islet autoimmunity. *Proc Natl Acad Sci*. 2016;201606646. <https://doi.org/10.1073/pnas.1606646113> **High levels of miR-92a during onset of islet autoimmunity mediate the induction of TFH precursors as well as impaired Treg induction from naive CD4+ T cells, two mechanisms that are likely involved in the onset and progression of islet autoimmunity.**

- [43] Serr I, Fürst RW, Achenbach P, Scherm MG, Gökmen F, Haupt F, et al. Type 1 diabetes vaccine candidates promote human Foxp3(+)Treg induction in humanized mice. *Nat Commun*. 2016;7:10991. <https://doi.org/10.1038/ncomms10991>.
- [44•] Serr I, Scherm MG, Zahm AM, Schug J, Flynn VK, Hippich M, et al. A miRNA181a/NFAT5 axis links impaired T cell tolerance induction with autoimmune type 1 diabetes. *Sci Transl Med*. 2018;10. <https://doi.org/10.1126/scitranslmed.aag1782> **The dysregulation of miR-181a and the downstream signaling pathway resulting in NFAT5 upregulation critically contribute to an impairment of Treg induction during islet autoimmunity.**
- [45] Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol*. 2003;4:330–6. <https://doi.org/10.1038/ni904>.
- [46] Roncador G, Brown PJ, Maestre L, Hue S, Martínez-Torrecuadrada JL, Ling K-L, et al. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *Eur J Immunol*. 2005;35:1681–91. <https://doi.org/10.1002/eji.200526189>.
- [47] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet*. 2001;27:20–1. <https://doi.org/10.1038/83713>.
- [48] Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol*. 2003;4:337–42. <https://doi.org/10.1038/ni909>.
- [49] von Boehmer H, Daniel C. Therapeutic opportunities for manipulating T(Reg) cells in autoimmunity and cancer. *Nat Rev Drug Discov*. 2013;12:51–63. <https://doi.org/10.1038/nrd3683>.
- [50] Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol*. 2005;6:1219–27. <https://doi.org/10.1038/ni1265>.
- [51] Daniel C, Weigmann B, Bronson R, von Boehmer H. Prevention of type 1 diabetes in mice by tolerogenic vaccination with a strong agonist insulin mimotope. *J Exp Med*. 2011;208:1501–10. <https://doi.org/10.1084/jem.20110574>.
- [52] Nielsen LB, Wang C, Sørensen K, Bang-Berthelsen CH, Hansen L, Andersen MLM, et al. Circulating levels of MicroRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression. *Exp Diabetes Res*. 2012;2012:1–7. <https://doi.org/10.1155/2012/896362>.
- [53] Salas-Pérez F, Codner E, Valencia E, Pizarro C, Carrasco E, Pérez-Bravo F. MicroRNAs miR-21a and miR-93 are down regulated in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes. *Immunobiology*. 2013;218:733–7. <https://doi.org/10.1016/j.imbio.2012.08.276>.
- [54] Sebastiani G, Spagnuolo I, Patti A, Grieco FA, Cataldo D, Ferretti E, et al. MicroRNA expression fingerprint in serum of type 1 diabetic patients. *Diabetologia*. 2012;55:S48.
- [55] Snowwhite IV, Allende G, Sosenko J, Pastori RL, Messinger Cayetano S, Pugliese A. Association of serum microRNAs with islet autoimmunity, disease progression and metabolic impairment in relatives at risk of type 1 diabetes. *Diabetologia*. 2017;60:1409–22. <https://doi.org/10.1007/s00125-017-4294-3>.
- [56] Yang M, Ye L, Wang B, Gao J, Liu R, Hong J, et al. Decreased miR-146 expression in peripheral blood mononuclear cells is correlated with ongoing islet autoimmunity in type 1 diabetes patients. *J Diabetes*. 2015;7:158–65. <https://doi.org/10.1111/1753-0407.12163>.
- [57] Assmann TS, Recamonde-Mendoza M, De Souza BM, Crispim D. MicroRNA expression profiles and type 1 diabetes mellitus: systematic review and bioinformatic analysis. *Endocr Connect*. 2017;6:773–90. <https://doi.org/10.1530/ec-17-0248>.
- [58] Åkerman L, Casas R, Ludvigsson J, Tavira B, Skoglund C. Serum miRNA levels are related to glucose homeostasis and islet autoantibodies in children with high risk for type 1 diabetes. *PLoS One*. 2018;13:e0191067. <https://doi.org/10.1371/journal.pone.0191067>.
- [59••] Chong MMW, Rasmussen JP, Rudensky AY, Littman DR. The RNaseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. *J Exp Med*. 2008;205:2449–9. <https://doi.org/10.1084/jem.20071219090508c> **This study shows that miRNA-dependent regulation is critical for preventing spontaneous inflammation and autoimmunity.**
- [60••] Cobb B, Hertweck A, Smith J, O'Connor E, Graf D, Cook T, et al. A role for dicer in immune regulation. *J Exp Med*. 2006;203:2519–27. <https://doi.org/10.1084/jem.20061692> **This study shows that T reg cell development involves Dicer-generated miRNAs.**
- [61] Liston A, Rudensky AY, Lu L-F, O'Carroll D, Tarakhovskiy A. Dicer-dependent microRNA pathway safeguards regulatory T cell function. *J Exp Med*. 2008;205:1993–2004. <https://doi.org/10.1084/jem.20081062>.
- [62] Zhou X, Jeker LT, Fife BT, Zhu S, Anderson MS, McManus MT, et al. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. *J Exp Med*. 2008;205:1983–91. <https://doi.org/10.1084/jem.20080707>.
- [63] Kimura K, Hohjoh H, Fukuoka M, Sato W, Oki S, Tomi C, et al. Circulating exosomes suppress the induction of regulatory T cells via let-7i in multiple sclerosis. *Nat Commun*. 2018;9:17. <https://doi.org/10.1038/s41467-017-02406-2>.
- [64] Kelada S, Sethupathy P, Okoye IS, Kistasis E, Czieso S, White SD, et al. miR-182 and miR-10a Are Key Regulators of Treg Specialisation and Stability during Schistosoma and Leishmania-associated Inflammation. *PLoS Pathog*. 2013. <https://doi.org/10.1371/journal.ppat.1003451>.
- [65] Liu X, Robinson SN, Setoyama T, Tung SS, D'Abundo L, Shah MY, et al. FOXP3 is a direct target of miR15a/16 in umbilical cord blood regulatory T cells. *Bone Marrow Transplant*. 2014;49:793–9. <https://doi.org/10.1038/bmt.2014.57>.
- [66] Singh Y, Garden OA, Lang F, Cobb BS. MicroRNA-15b/16 enhances the induction of regulatory T cells by regulating the expression of Rictor and mTOR. *J Immunol*. 2015;195:5667–77. <https://doi.org/10.4049/jimmunol.1401875>.
- [67] Liu SQ, Jiang S, Li C, Zhang B, Li QJ. Mir-17-92 cluster targets phosphatase and tensin homology and ikaros family zinc finger 4 to promote th17-mediated inflammation. *J Biol Chem*. 2014;289:12446–56. <https://doi.org/10.1074/jbc.M114.550723>.
- [68] Rouas R, Fayyad-Kazan H, El Zien N, Lewalle P, Rothé F, Simion A, et al. Human natural Treg microRNA signature: role of microRNA-31 and microRNA-21 in FOXP3 expression. *Eur J Immunol*. 2009;39:1608–18. <https://doi.org/10.1002/eji.200838509>.
- [69] Dong L, Wang X, Tan J, Li H, Qian W, Chen J, et al. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. *J Cell Mol Med*. 2014;18:2213–24. <https://doi.org/10.1111/jcmm.12353>.
- [70] Fayyad-Kazan H, Rouas R, Fayyad-Kazan M, Badran R, El Zein N, Lewalle P, et al. MicroRNA profile of circulating CD4-positive regulatory T cells in human adults and impact of differentially expressed microRNAs on expression of two genes essential to their function. *J Biol Chem*. 2012;287:9910–22. <https://doi.org/10.1074/jbc.M111.337154>.
- [71] De Santis G, Ferracin M, Biondani A, Caniatti L, Rosaria Tola M, Castellazzi M, et al. Altered miRNA expression in T regulatory cells in course of multiple sclerosis. *J Neuroimmunol*.

- 2010;226:165–71. <https://doi.org/10.1016/j.jneuroim.2010.06.009>.
- [72] Warth SC, Hoefig KP, Hiekel A, Schallenberg S, Jovanovic K, Klein L, et al. Induced miR-99a expression represses Mtor cooperatively with miR-150 to promote regulatory T-cell differentiation. *EMBO J*. 2015. <https://doi.org/10.15252/embj.201489589>.
- [73] Negi V, Paul D, Das S, Bajpai P, Singh S, Mukhopadhyay A, et al. Altered expression and editing of miRNA-100 regulates iTreg differentiation. *Nucleic Acids Res*. 2015;43:8057–65. <https://doi.org/10.1093/nar/gkv752>.
- [74] Li JQ, Hu SY, Wang ZY, Lin J, Jian S, Dong YC, et al. Long non-coding RNA MEG3 inhibits microRNA-125a-5p expression and induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura. *Biomed Pharmacother*. 2016;83:905–11. <https://doi.org/10.1016/j.biopha.2016.07.057>.
- [75] Qin A, Wen Z, Zhou Y, Li Y, Li Y, Luo J, et al. MicroRNA-126 regulates the induction and function of CD4+ Foxp3+ regulatory T cells through PI3K/AKT pathway. *J Cell Mol Med*. 2013;17:252–64. <https://doi.org/10.1111/jcmm.12003>.
- [76] Zhou Q, Haupt S, Kreuzer JT, Hammitzsch A, Proft F, Neumann C, et al. Decreased expression of miR-146a and miR-155 contributes to an abnormal Treg phenotype in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2015;74:1265–74. <https://doi.org/10.1136/annrheumdis-2013-204377>.
- [77] Lu LF, Boldin MP, Chaudhry A, Lin LL, Taganov KD, Hanada T, et al. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. *Cell*. 2010;142:914–29. <https://doi.org/10.1016/j.cell.2010.08.012>.
- [78] Lu Y, Hippen KL, Lemire AL, Gu J, Wang W, Ni X, et al. MiR-146b antagomir-treated human Tregs acquire increased GVHD inhibitory potency. *Blood*. 2016;128:1424–35. <https://doi.org/10.1182/blood-2016-05-714535>.
- [79] Wan C, Ping CY, Shang XY, Tian JT, Zhao SH, Li L, et al. MicroRNA 182 inhibits CD4+ CD25+ Foxp3+ Treg differentiation in experimental autoimmune encephalomyelitis. *Clin Immunol*. 2016;173:109–16. <https://doi.org/10.1016/j.clim.2016.09.008>.
- [80] Wang XY, Chen XY, Li J, Zhang HY, Liu J, Sun LD. MiR-200a expression in CD4+ T cells correlates with the expression of Th17/Treg cells and relevant cytokines in psoriasis vulgaris: a case control study. *Biomed Pharmacother*. 2017;93:1158–64. <https://doi.org/10.1016/j.biopha.2017.06.055>.
- [81] Zhao M, Tao WL, Ping LG, Zhang P, Jie DX, Tang Q, et al. Up-regulation of microRNA-210 induces immune dysfunction via targeting FOXP3 in CD4+ T cells of psoriasis vulgaris. *Clin Immunol*. 2014. <https://doi.org/10.1016/j.clim.2013.10.009>.
- [82] Yin Y, Cai X, Chen X, Liang H, Zhang Y, Li J, et al. Tumor-secreted miR-214 induces regulatory T cells: a major link between immune evasion and tumor growth. *Cell Res*. 2014;24:1164–80. <https://doi.org/10.1038/cr.2014.121>.
- [83] Sun XG, Tao JH, Xiang N, Li XM, Wang GS, Fang X, et al. Negative correlation between miR-326 and Ets-1 in regulatory T cells from new-onset SLE patients. *Inflammation*. 2016;39:822–9. <https://doi.org/10.1007/s10753-016-0312-8>.
- [84] Geng L, Tang X, Zhou K, Wang D, Wang S, Yao G, et al. MicroRNA-663 induces immune dysregulation by inhibiting TGF- β 1 production in bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. *Cell Mol Immunol*. 2019;16:260–74. <https://doi.org/10.1038/cmi.2018.1>.
- [85] Jeker LT, Zhou X, Gershberg K, de Kouchkovsky D, Morar MM, Stadthagen G, et al. MicroRNA 10a marks regulatory T cells. *PLoS One*. 2012;7:1–8. <https://doi.org/10.1371/journal.pone.0036684>.
- [86] Takahashi H, Kanno T, Nakayamada S, Hirahara K, Sciumè G, Muljo SA, et al. TGF- β and retinoic acid induce the microRNA miR-10a, which targets Bcl-6 and constrains the plasticity of helper T cells. *Nat Immunol*. 2012;13:587–95. <https://doi.org/10.1038/ni.2286>.
- [87] Kohlhaas S, Garden OA, Okkenhaug K, Vigorito E, Turner M, Scudamore C. Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells. *J Immunol*. 2009;182:2578–82. <https://doi.org/10.4049/jimmunol.0803162>.
- [88] Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, et al. Foxp3-dependent MicroRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity*. 2009;30:80–91. <https://doi.org/10.1016/j.immuni.2008.11.010>.
- [89] Yao R, Ma YL, Liang W, Li HH, Ma ZJ, Yu X, et al. MicroRNA-155 modulates Treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLoS One*. 2012;7:e46082. <https://doi.org/10.1371/journal.pone.0046082>.
- [90] Warth SC, Hoefig KP, Ansel KM, Schallenberg S, Klein L, Heissmeyer V, et al. Induced miR-99a expression represses Mtor cooperatively with miR-150 to promote regulatory T-cell differentiation. *EMBO J*. 2015;34:1195–213. <https://doi.org/10.15252/embj.201489589>.
- [91] Murugaiyan G, Weiner HL, Beynon V, Mittal A, Joller N. Silencing MicroRNA-155 ameliorates experimental autoimmune encephalomyelitis. *J Immunol*. 2011;187:2213–21. <https://doi.org/10.4049/jimmunol.1003952>.
- [92] Jiang S, Li C, Olive V, Lykken E, Feng F, Sevilla J, et al. Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. *Blood*. 2011;118:5487–97. <https://doi.org/10.1182/blood-2011-05-355644>.
- [93] Cho S, Wu C-J, Yasuda T, Cruz LO, Khan AA, Lin L-L, et al. miR-23 approximately 27 approximately 24 clusters control effector T cell differentiation and function. *J Exp Med*. 2016;213:235–49. <https://doi.org/10.1084/jem.20150990>.
- [94] Nair VS, Oh KI. Down-regulation of Tet2 prevents TSDR demethylation in IL2 deficient regulatory T cells. *Biochem Biophys Res Commun*. 2014;450:918–24. <https://doi.org/10.1016/j.bbrc.2014.06.110>.
- [95] Wang L, Liu Y, Han R, Beier UH, Thomas RM, Wells AD, et al. Mbd2 promotes Foxp3 Demethylation and T-regulatory-cell function. *Mol Cell Biol*. 2013;33:4106–15. <https://doi.org/10.1128/MCB.00144-13>.
- [96] Yang R, Qu C, Zhou Y, Konkel JE, Shi S, Liu Y, et al. Hydrogen sulfide promotes Tet1- and Tet2-mediated Foxp3 Demethylation to drive regulatory T cell differentiation and maintain immune homeostasis. *Immunity*. 2015;43:251–63. <https://doi.org/10.1016/j.immuni.2015.07.017>.
- [97] Yue X, Trifari S, Äijö T, Tsagaratou A, Pastor WA, Zepeda-Martínez JA, et al. Control of Foxp3 stability through modulation of TET activity. *J Exp Med*. 2016;213:377–97. <https://doi.org/10.1084/jem.20151438>.
- [98] Mahmud SA, Manlove LS, Farrar MA. Interleukin-2 and STAT5 in regulatory T cell development and function. *JAK-STAT*. 2013;2:e23154. <https://doi.org/10.4161/jkst.23154>.
- [99] Konkel JE, Zhang D, Zanvit P, Chia C, Zangarale-Murray T, Jin W, et al. Transforming growth factor- β signaling in regulatory T cells controls T Helper-17 cells and tissue-specific immune responses. *Immunity*. 2017;46:660–74. <https://doi.org/10.1016/j.immuni.2017.03.015>.
- [100] Li QJ, Chau J, Ebert PJR, Sylvester G, Min H, Liu G, et al. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell*. 2007;129:147–61. <https://doi.org/10.1016/j.cell.2007.03.008>.
- [101] Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. *Proc Natl Acad Sci U S A*. 2008;105:7797–802. <https://doi.org/10.1073/pnas.0800928105>.

- [102] Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med*. 2000;192:1545–52.
- [103] Serr I, Daniel C. Regulation of T follicular helper cells in islet autoimmunity. *Front Immunol*. 2018;9. <https://doi.org/10.3389/fimmu.2018.01729>.
- [104] Schemm MG, Ott VB, Daniel C. Follicular Helper T Cells in Autoimmunity. *Curr Diab Rep* 2016;16. <https://doi.org/10.1007/s11892-016-0770-2>, 75.
- [105] Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol*. 2008;9:405–14. <https://doi.org/10.1038/ni1575>.
- [106] Ghorbani S, Talebi F, Chan WF, Masoumi F, Vojgani M, Power C, et al. MicroRNA-181 variants regulate T cell phenotype in the context of autoimmune neuroinflammation. *Front Immunol*. 2017;8. <https://doi.org/10.3389/fimmu.2017.00758>.
- [107] Bonneau E, Neveu B, Kostantin E, Tsongalis GJ, De Guire V. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. *Electron J Int Fed Clin Chem Lab Med*. 2019.
- [108] Hanna J, Hossain GS, Kocerha J. The potential for microRNA therapeutics and clinical research. *Front Genet*. 2019;10. <https://doi.org/10.3389/fgene.2019.00478>.
- [109] Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. *Nat Rev Drug Discov*. 2014;13:622–38. <https://doi.org/10.1038/nrd4359>.
- [110] Kumar P, Ban HS, Kim SS, Wu H, Pearson T, Greiner DL, et al. T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. *Cell*. 2008;134:577–86. <https://doi.org/10.1016/j.cell.2008.06.034>.
- [111] Peer D, Eun JP, Morishita Y, Carman CV, Shimaoka M. Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. *Science* (80-). 2008;319:627–30. <https://doi.org/10.1126/science.1149859>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.