UCSF
UC San Francisco Previously Published Works

Title
MicroRNA-mediated regulation of T helper cell differentiation and plasticity

Permalink
https://escholarship.org/uc/item/3fv47698

Journal
Nature Reviews Immunology, 13(9)

ISSN
1474-1733

Authors
Baumjohann, Dirk
Ansel, K Mark

Publication Date
2013-09-01

DOI
10.1038/nri3494

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

NIH Public Access
Author Manuscript
Nat Rev Immunol. Author manuscript; available in PMC 2014 April 09.

Published in final edited form as:
Nat Rev Immunol. 2013 September ; 13(9): 666–678. doi:10.1038/nri3494.

MicroRNA Regulation of T Helper Cell Differentiation and Plasticity

Dirk Baumjohann and **K. Mark Ansel**
Department of Microbiology & Immunology, Sandler Asthma Basic Research Center, University of California San Francisco, San Francisco, CA 94143, USA.

Abstract

T helper (T_H) cells orchestrate appropriate cellular and humoral immune responses to a wide variety of pathogens and are central to the success of vaccines. However, their dysregulation can cause allergies and autoimmune diseases. The T_H cell universe is characterized by a diversity of distinct cell types, such as T_H1, T_H2, T_H17 cells, regulatory T cells, and T follicular helper cells, each featuring specific functions and gene expression programs, but also by plasticity among the different T_H cell subsets. Here, we review recent advances and emerging concepts on how microRNAs, small endogenously expressed oligonucleotides that modulate gene expression, fit into the regulatory networks that govern T helper cell fate decisions and regulate their effector functions.

Introduction

Following their activation by antigen and costimulatory signals, $CD4^+$ T cells can differentiate into several distinct types of effector T helper (T_H) cells. Since the discovery of T_H1 and T_H2 cells approximately 25 years ago[1], an ever-increasing number of T_H cell subsets have been described. Historically, T_H cell subsets were distinguished based on the cytokines they secrete following restimulation with antigen. For instance, T_H1 cells produce interferon-γ (IFNγ which is required for clearance of intracellular pathogens, whereas T_H2 cells produce interleukin-4 (IL-4), IL5 and IL-13, which mediate immune responses against helminths. However, as the diversity of subsets increased and distinct subsets were found to express overlapping sets of cytokines, 'lineage-defining' or 'master' transcription factors have become important classifiers of Th cell subsets. For a long time, T_H1 and T_H2 cells have been widely referred to as stably differentiated lineages. However, the recent emergence of additional subsets, such as peripherally derived regulatory T (T_{Reg}) cells, T follicular helper (T_{FH}) cells, T_H17, T_H9 and T_H22 cells, forced some reconsideration in the field and focused attention on the plasticity of T_H cells[2–5]. It has become clear that a complex network of transcription factors, epigenetic changes, and post-transcriptional regulators is responsible for the development and maintenance of the different T helper cell subsets and their characteristic gene expression programs[6–10].

MicroRNAs (miRNAs) are small (~21 nucleotide) endogenously expressed RNAs that regulate gene expression. They are sequentially processed from longer transcripts by the

Correspondence to K.M.A. mark.ansel@ucsf.edu.

Further information
Web site1 title: "Ansel Lab", Web site1 URL: http://ansel.ucsf.edu/
Web site2 title: "miRBase", Web site2 URL: http://www.mirbase.org
Web site3 title: "TargetScan", Web site3 URL: http://www.targetscan.org
Web site4 title: "miRTarBase", Web site4 URL: http://mirtarbase.mbc.nctu.edu.tw

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

RNase III enzymes DROSHA and DICER and exert their function by guiding the Argonaute (AGO) protein-containing miRNA-induced silencing complex (miRISC) **[G]** to specific target mRNAs by complementary base pairing (Box 1). The miRISC destabilizes target mRNAs and reduces their translation into protein[11, 12]. Whether an mRNA is targeted by miRISC depends on several factors, including alternative splicing and poly-A site usage, and interplay with RNA binding proteins. Moreover, the expression of miRNAs is regulated at several stages during their biogenesis, often involving feedback from their target gene products[13]. Each miRNA has many targets, and several mRNAs are subject to regulation by more than one miRNA (Box 2). Thus, similarly to transcription factors, miRNAs are integral parts of gene expression networks that determine cell identity and function. Conventional methods for the study of coding genes have been complemented by a large number of miRNA-specific technologies that improve our ability to measure miRNA expression, determine their biological functions, and empirically identify their mRNA targets (Box 3).

Because of their evolutionary conserved abundance and wide range of functions, miRNAs are emerging as an integral part of the cellular machinery that confers overall robustness to biological processes[14]. There is also growing evidence that miRNAs are critical modulators of development and function in the immune system and that miRNAs regulate important aspects of T_H cell differentiation[15–21].

In this review, we first discuss how miRNAs are important for modulating naive $CD4^+$ T cell maintenance, activation and expansion and how miRNA expression itself is regulated during these processes. We then examine how miRNAs regulate the differentiation of distinct effector T_H cell subsets and their lineage-defining effector functions by focusing on T_H1, T_H2, T_H17 and T_{FH} cells. Finally, we review the literature that is available on the miRNA-mediated regulation of T_{Reg} cell differentiation and function, before concluding with an outlook on future challenges and the clinical relevance of miRNA biology.

MicroRNA regulation of T cell activation

Effector T_H cell differentiation is highly influenced by the strength of T cell antigen receptor (TCR) stimulation, the nature of the co-stimulatory molecules expressed on antigen presenting cells, and by the cytokine environment [9]. Early systematic miRNA profiling studies in cells of the hematopoietic system identified cell type-specific patterns of miRNA expression that suggested an important role for miRNAs in cell lineage specification and effector functions[22]. These findings were corroborated and extended in subsequent miRNA profiling studies that used small RNA sequencing or high throughput quantitative PCR to profile $CD4^+$ and $CD8^+$ T cells[23], naive and *in vitro* activated $CD4^+$ T cells[24], several human T_H cell subsets[25] and a broad range of hematopoietic cell types[26].

Alterations in miRNA-mediated control following T cell activation

Not surprisingly, T-cell specific deletion of essential factors in the miRNA biogenesis pathway, such as DICER[27–29], DROSHA[30] and DGCR8[31], reduced the survival and proliferation of T cells following activation (Figure 1a). However, miRNA-deficient $CD4^+$ T cells also exhibit a surprising increase in effector T_H cell differentiation and cytokine production[27, 30, 32]. The enhanced differentiation of miRNA-deficient T cells indicates that miRNAs are critically involved in the maintenance of the naïve T cell state[27, 30, 32]. This raises the question of how T cells overcome the miRNA barrier when they receive signals that induce differentiation.

One possibility is that key mRNAs that are held in check by miRNA control in naïve T cells are transcriptionally induced to such a large degree upon activation that their repression by miRNAs is rendered relatively insignificant. However, at least two other contributing

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mechanisms have been discovered. Proliferating T cells utilize alternative polyadenylation sites to produce mRNAs with shortened 3′ UTRs and thus fewer miRNA binding sites[33]. In addition, T cell activation induces the ubiquitylation of AGO2 and its proteasome-dependent turnover, reducing its half-life to less than 2 hours[32]. This results in a global reduction in miRNA abundance, and also rapidly remodels the miRNA repertoire to favor expression of a few miRNAs that are transcriptionally upregulated upon T cell activation.

Among the miRNAs that are substantially downregulated upon T cell activation are some, such as miR-29, that inhibit the differentiation of particular T_H cell subsets[31, 34, 35]. Others, such as miR-125, appear to inhibit effector T cell differentiation in general. miR-125b is highly expressed in human naïve $CD4^+$ T cells as compared with various memory T cell populations, and it inhibits several genes involved in T cell differentiation, including *IFNG*, *IL2RB*, *IL10RA*, and *PRDM1* (encoding BLIMP1)[25] (Figure 1b). Interestingly, miR-125b is not regulated in this way in mouse $CD4^+$ T cells[25]. Instead, its close relative miR-125a is highly expressed in naïve mouse T cells and rapidly downregulated upon activation[32]. In addition, among the miR-125b target genes identified in human T cells, only *Prdm1* has retained the predicted miR-125 binding site during the evolution of its respective murine gene[25]. Attention to species differences like these is important, since the miRNomes of human and murine lymphocytes and their targets are apparently under evolutionary pressure and are not strictly conserved.

miRNA-mediated regulation of TCR signaling

During their development in the thymus, T cells undergo the processes of positive selection **[G]** and negative selection **[G]**. In the periphery, activation of mature naïve T cells that encounter foreign antigen leads to clonal selection and differentiation into effector cells. In all of these processes, the sensitivity of a TCR to recognize cognate peptides has important implications for the fate of the T cell. It is not surprising that the signaling cascade downstream of TCR activation is highly regulated at multiple levels, and several miRNAs have been shown to critically participate in this regulation (Figure 1b).

The miR-181 miRNA family **[G]** is one such regulator of TCR signaling with clear effects on T cell development and homeostasis. Inhibition of miR-181 in thymocytes reduces TCR sensitivity and impairs positive and negative selection in the thymus[36]. Conversely, overexpression of miR-181a in mature T cells augments sensitivity to peptide antigen in the periphery[36]. miR-181 targets multiple phosphatases that dampen TCR signals, including SH2 domain-containing protein tyrosine phosphatase 2 (SHP2), protein tyrosine phosphatase, non-receptor type 22 (PTPN22), dual-specificity protein phosphatase 5 (DUSP5) and DUSP6, thereby reducing the TCR engagement threshold for cellular responses[36] (Figure 1b). As a consequence, an endogenous peptide that positively selects T cells in the thymus but fails to activate specific T cell responses in the periphery can become an autoantigen when T cells develop in the absence of miR-181 activity[37].

The miR-181 family consists of 4 unique mature miRNAs encoded in three miRNA clusters **[G]**, but one of these, *miR-181a1b1*, is particularly important in T cells. Three independent studies identified defects in thymocyte development in *mir181a1b1*-deficient mice[38–40]. Natural killer T (NKT) cell development is particularly dependent on miR-181[38, 40]. Providing an agonistic TCR ligand[40] or eliminating the miR-181 target phosphatase and tensin homologue (PTEN)[38] rescued the defective NKT cell development of *mir181a1b1*-deficient mice, indicating an important role for miR-181 in regulating PI3K signaling. In addition to its effect on TCR signals, miR-181 targets *Nrarp* (Notch-regulated ankyrin repeat protein) and dampens Notch signals that are critical for thymocyte development[39].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

miR-181a1b1-deficiency also compromises peripheral T cell homeostasis and TCR signaling[38, 39]. In addition, naturally occurring changes in miR-181 expression have been implicated in the age-associated decline in $CD4^+$ T cell immunity. Decreased miR-181a expression with age is associated with increased DUSP6 expression and reduced TCR sensitivity[41]. Neonatal $CD4^+$ T cells expressed more miR-181a than did adult peripheral blood $CD4^+$ T cells, concomitant with increased activation-induced calcium flux and extracellular signal-regulated kinase (ERK) phosphorylation. However, neonatal cells display an independent defect in AP1-dependent transcription, ultimately resulting in impaired T cell activation[42]. Taken together, these data suggest that regulation of miR-181a expression may influence age-dependent changes in T cell responsiveness, but additional mechanisms have a dominant influence in special contexts, as in the case of human neonates.

miR-146a is an important feedback regulator of nuclear factor-κB (NF-κB) signaling (Figure 1b). It is abundantly expressed in human memory T cells, and further induced by NF-κB p50 activation in response to TCR engagement[43–45]. In turn, miR-146a targets TRAF6 and IRAK1, forming a negative feedback loop that controls the intensity and duration of NF-κB signaling[46]. Absence of this feedback loop causes spontaneous myeloproliferation and myeloid cell tumorigenesis in miR-146a-deficient mice[45]. In T cells, it causes hyperresponsiveness to TCR signals and failure to resolve T cell-mediated inflammation[46]. Together with its role in T_{Reg} cells (discussed below), these effects make miR-146a a powerful inhibitor of autoimmunity.

miRNA-mediated control of T cell proliferation and survival

The miR-17~92 cluster comprises six miRNAs from four miRNA families that regulate several aspects of T cell activation and activated T cell fate. This cluster and the related miR-106a~363 and miR-106b~25 clusters are all expressed in $CD4^+$ and $CD8^+$ T cells[32, 47]. Overexpression of miR-17~92 in T cells causes lymphoproliferation and an autoimmune-like disease characterized by elevated serum antibody levels and production of autoantibodies[48]. Both miR-17 and miR-92 family miRNAs can enhance T cell proliferation in the absence of other miRNAs[31]. Another miR-17~92 cluster member, miR-19, positively regulates activated T cell survival. These miRNAs mediate their effects through many direct target genes, including PTEN and the pro-apoptotic protein BIM[48, 49] (Figure 1b).

PI3K signaling and downstream regulators of metabolic activity are subject to intense miRNA control in T cells. PTEN is a common target of several miRNA families that are differentially expressed during T cell development and activation, including miR-17, miR-19, miR-181 and miR-21[38, 48, 50]. Another phosphatidylinositol phosphatase, SHIP-1, is a validated direct target of miR-155[49, 51], the miRNA most highly induced by TCR stimulation[32, 52, 53]. miR-155 deficiency reduces T cell-dependent humoral and cellular immune responses, likely through its activity on many mRNA targets[53–56]. Transcriptome-wide biochemical analysis of Ago2 binding to mRNAs in activated primary mouse T cells identified over 300 sites containing miR-155 seed recognition sequences, and almost 200 miR-155-dependent sites, including a large fraction (~40%) that lack canonical miR-155 seed recognition sequences[49]. Thus, the regulation of T cell behavior by miR-155, and by extension miRNAs in general, involves a large network of co-regulated target mRNAs.

IL-2 is another important regulator of $CD4^+$ T cell metabolism and proliferation[57]. It is produced by activated T cells and can act in an autocrine fashion, and it is also frequently added to *in vitro* T helper cell cultures as a growth factor. miR-146a has been implicated as a negative regulator of IL-2 production in activated human $CD4^+$ T cells[44], and miR-9 has been described as a positive regulator of IL-2 production through a mechanism involving direct targeting of *PRDM1*, which encodes the transcriptional repressor B lymphocyte-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

induced maturation protein 1 (BLIMP1)[58, 59]. In addition, miR-182 enhances clonal selection downstream of IL-2 by inhibiting forkhead box O1 (FOXO1), a transcription factor that is highly expressed in naïve T cells and limits T cell proliferation[60]. At early time points during T cell stimulation, FOXO1 is inactivated by phosphorylation downstream of TCR signals. Later in the response, however, IL-2 produced by activated T cells induces miR-182, which represses Foxo1 post-transcriptionally (Figure 1b).

In summary, miRNAs can have powerful effects on biological processes by regulating genes that control signaling thresholds. Several miRNAs operate in this capacity to regulate T cell development, homeostasis, and activation. These effects have important consequences for T cell immune function, and they are complemented by miRNA regulation of particular T cell differentiation pathways and effector mechanisms.

MicroRNA regulation of effector T helper cells

MicroRNAs are increasingly recognized as important modulators of T_H cell fate decisions and effector functions. Their 'fine-tuning' activity is particularly well suited to act early in the response when small changes in the expression of key genes can have a big effect on cell fate. In addition, miRNAs can regulate the plasticity and effector functions of differentiated T_H cell subsets.

T_H1 cell differentiation and function

T_H1 cells, orchestrate immune responses against viruses, intracellular pathogens and tumors. The first evidence for miRNA regulation of T_H cell differentiation came from studies with DICER-deficient $CD4^+$ T cells, which exhibit increased differentiation into effector cells expressing the T_H1 cell lineage-defining transcription factor T-bet and its transcriptional target, the T_H1 cell hallmark cytokine IFNγ[27]. T cells lacking DROSHA or DGCR8 display a very similar phenotype of aberrant T_H1 cell differentiation[30, 31].

A functional screen in DGCR8-deficient $CD4^+$ T cells revealed that among $CD4^+$ T cell-expressed miRNAs, miR-29a and miR-29b are the most potent inhibitors of T_H1 cell differentiation and IFNγ expression[31]. miR-29 is highly expressed in naïve T cells, and it inhibits T_H1 cell differentiation through multiple direct targets (Figure 2). T-bet is one of the direct miR-29 targets in $CD4^+$ T cells, but miR-29 also targets the closely related transcription factor eomesodermin (EOMES), which is usually not expressed in $CD4^+$ T cells[31]. EOMES regulates IFNγ production in natural killer (NK) cells and $CD8^+$ T cells, and in the absence of miRNAs it is also inappropriately expressed in $CD4^+$ T cells and contributes to the aberrant IFNγ[31]. miR-29 may also directly target the *Ifng* mRNA[35]. Transgenic mice expressing a miRNA sponge **[G]** that reduces miR-29a-mediated control exhibited higher serum IFN-γ levels and more effective clearance of the intracellular bacterium *Listeria monocytogenes*[35]. A recent study confirmed the importance of miR-29 in restraining IFN-γ expression by T cells using *mir-29ab1* knock-out mice[34].

Several other miRNAs regulate T_H1 cell differentiation and function (Figure 2). miR-146a-deficient $CD4^+$ T cells produce more IFNγ *in vitro* and in vivo[46, 51, 61]. As discussed above, miR-146a inhibits T cell responses in general by targeting TRAF6 and IRAK1[46]. However, it may also specifically regulate T_H1 differentiation by targeting signal transducer and activator of transcription 1 (STAT1)[61]. By contrast, miR-155 enhances both T_H1 and T_H17 cell–dependent tissue inflammation[55]. Among its many identified targets in T cells[49], miR-155 inhibits the negative regulators of cytokine signaling SOCS1 and SHIP1[51, 62]. In addition, miR-155 targets *Ifngr1* mRNA and may be responsible for the downregulation of IFNγ receptor in activated $CD4^+$ T cells that differentiate into T_H1 cells[52]. Epistasis experiments showed that miR-155 deficiency is dominant over miR-146 deficiency, as cells

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

lacking both miRNAs are as defective in IFN-γ expression and anti-tumor immunity as those lacking miR-155 only[51].

The miR-17~92 cluster also impacts T_H1 cell differentiation. $CD4^+$ T cells freshly isolated from peripheral lymph nodes of 10- to 12-month-old mice that expressed a transgene encoding the human miR-17~92 cluster showed a dramatic increase in the percentage of IFNγ-expressing cells[48]. Similarly, naïve miR-17~92 transgenic $CD4^+$ T cells produced higher amounts of IFNγ under T_H1 polarizing conditions *in vitro*[48]. Conversely, T cell-specific deletion of the miR-17~92 cluster in mice resulted in a gene dose-dependent reduction of IFNγ-secreting $CD4^+$ T cells[63]. Reconstitution of miR-17~92-deficient $CD4^+$ T cells with individual miRNA mimics **[G]** identified miR-19b as sufficient to restore IFNγ production[63].

miRNAs can influence T_H1 cell differentiation indirectly by targeting genes in immune cells other than $CD4^+$ T cells. For example, miR-21 regulates T_H1 cell differentiation by modulating IL-12 production by dendritic cells (DCs). IL-12 is an important cytokine that induces T-bet and IFNγ expression and supports the growth and survival of T_H1 cells[64]. miR-21 is highly expressed in DCs, and directly targets the mRNA that encodes IL-12p35[65]. miR-21 deficiency increases IL-12 expression in DCs, and enhances T_H1 cell development and delayed type hypersensitivity responses *in vivo*[66]. It is possible that other miRNAs that modulate DC activity and cytokine production also have important indirect effects on T_H cell differentiation and immune function.

Studies of T_H1 cell differentiation and function have led the way to insights into the T-cell intrinsic and extrinsic roles for miRNAs in T cell differentiation, and set paradigms for the logic of miRNA-mediated regulation of cell differentiation in general. Some of the miRNAs that regulate T_H1 cells also play identical or related roles in other IFN-γ producing lymphocytes, such as cytotoxic $CD8^+$ T cells and NK cells[67, 68]. Lessons learned through the study of T_H1 cells will likely continue to inform our understanding of miRNA regulation of the immune system.

T_H2 cell differentiation

T_H2 cells orchestrate innate immune responses against helminths[69], and dysregulated T_H2 responses cause allergies and asthma[70]. Over the past decades, much progress has been made in our understanding of the molecular requirements for the generation of T_H2 cells. *In vitro* differentiation systems and more recently elegant *in vivo* approaches have established the powerful influence of a positive feedback loop involving the hallmark T_H2 cytokine IL-4 and the transcription factors STAT6 and GATA-binding protein 3 (GATA3) in amplifying T_H2 responses, though the initial signals leading to T_H2 cell differentiation have been harder to define[71]. In addition to IL-4, T_H2 cells express the genetically linked cytokines IL-5 and IL-13, which drive type 2 inflammation through their effects on myeloid and non-hematopoietic cells[7].

Compared to T_H1 cells, miRNA regulation of T_H2 cell differentiation is poorly understood. Overexpression of miR-21 in T cells increases T_H2 cell differentiation *in vitro*[72], whereas overexpression of miR-27 or miR-128 decreases the secretion of IL-4 and IL-5 by activated $CD4^+$ T cells[73]. Further work is needed to characterize the importance of these miRNAs in T_H2 cell responses. In addition, miR-155-deficient T cells display a mild bias towards T_H2 differentiation *in vitro*. This is accompanied by dysregulation of many target genes, including MAF (also known as c-MAF), a transcription factor that regulates IL-4 production[53, 54]. Approximately 50% of ageing miR-155 knockout mice develop spontaneous lung inflammation, which displays some but not all of the hallmarks of type 2 inflammation that have been associated with asthma[54]. This pathology may be caused by

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

cell-intrinsic defects in T_H2 cell differentiation, but it may also reflect other changes in T cell responses or requirements for miR-155 in other cells.

The lung's accessibility for local drug delivery makes asthma an attractive target for miRNA-directed therapies. MicroRNA expression patterns associated with asthma have been detected in both lung epithelial cells and purified T cells from human asthmatic subjects[74, 75]. In addition, several proof-of-concept studies have shown that miRNA inhibitors can be protective in mouse models of allergic asthma. House dust mite allergen (HDM) challenge increases miR-126 expression in the airway wall, and intranasal administration of a miR-126-specific antagomir **[G]** (cholesterol-linked single stranded RNA complementary to miR-126) reduces airway hyperresponsiveness and eosinophil recruitment to the lung[76]. Treatment with the miR-126-specific antagomir also reduced lung eosinophilia in a mouse model of chronic asthma, but it did not have sustained effects on inflammation or airway remodeling[77]. As miR-126 is strongly expressed in the airway wall[76, 77], but is present in T cells and other hematopoietic cells at only very low abundance[26, 31], it seems likely that miR-126-specific antagomir acts on epithelial cells or other non-hematopoietic cells in the lung rather than directly on infiltrating T_H2 cells in these models.

A miR-145-specific antagomir also inhibited allergic airway disease in the HDM model, possibly through effects on smooth muscle cells, which express a large amount of miR-145[78]. Members of the let-7 family of miRNAs are highly expressed in most cells. Let-7 directly targets *Il13* mRNA and reduces IL-13 production by T cells *in vitro*[79, 80]. However, intravenous administration of locked nucleic acid **[G]** (LNA) inhibitors of let-7 ameliorated lung inflammation and airway hyperresponsiveness in a mouse model of asthma, rather than exacerbating the disease as may have been expected if the dominant effect of let-7 inhibitors was to derepress IL-13 expression in T_H2 cells. Let-7 also targets *Il10* mRNA, and increased IL-10 levels in HIV patients correlate with decreased let-7 expression in $CD4^+$ T cells[81].

Taken together, these studies show that individual miRNAs can have a strong impact on type 2 immunity, but also underscore the complexity of miRNA biology *in vivo*. In particular, they indicate that targeted delivery of miRNAs or miRNA inhibitors specifically to T cells will be important for manipulating miRNA-mediated control of T_H2 cell gene expression, differentiation and effector functions, be it for scientific or therapeutic purposes.

T_H17 cell differentiation

T_H17 cells are defined by expression of the transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and cytokines of the IL-17 family[82] and have an important role in the defense against extracellular bacteria and fungi. In addition, they have been implicated in the etiology and pathology of several autoimmune diseases including psoriasis, multiple sclerosis, colitis, arthritis, and even asthma[83].

MiRNA profiling in T cells from patients with multiple sclerosis, as well as studies in mice and rats with experimental autoimmune encephalomyelitis (EAE) **[G]**, a model system for multiple sclerosis, have revealed distinct contributions for miRNAs in the regulation of T_H cell subsets involved in this disease[84]. Expression of miR-326 in T_H cells correlated with disease severity in mice with EAE and also in patients with multiple sclerosis[85]. miR-326 targets ETS1, a negative regulator of T_H17 cell differentiation and increases T_H17 differentiation *in vitro*[85].

Conversely, expression of miR-10a in $CD4^+$ T cells can limit T_H17 cell differentiation[86]. This effect depends on the presence of retinoic acid, which induces both miR-10a and T-bet.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Suppression of the miR-10a targets *Bcl6* or *Ncor* by RNA interference limited T_H17 cell differentiation in wild-type, but not T-bet-deficient T cells. As BCL-6 and NCOR modulate the expression and transcriptional activity of T-bet[87–89], and T-bet in turn inhibits the expression of RORγt[90], miR-10a and T-bet may cooperate to balance T_H1 and T_H17 differentiation in retinoic acid-rich microenvironments. In line with these observations, miR-10a overexpression in myelin oligodendrocyte glycoprotein (MOG)-specific $CD4^+$ T cells delays neurological disease in EAE[86].

miR-155 knockout mice are strongly resistant to EAE. Detailed analysis revealed both a T cell-intrinsic role for miR-155 in T_H17 cell differentiation, as well as an indirect role through the regulation of production of T_H17 cell-polarizing cytokines by DCs[55, 91]. Impaired T_H17 cell responses of miR-155-deficient T cells were also observed in a mouse model of *Helicobacter pylori* infection and in a mouse model of T_H17-driven chronic colitis[56]. Thus, miR-155 promotes T cell–mediated tissue inflammation through the regulation of both T_H1 and T_H17 cell responses. Finally, miR-301a enhances T_H17 cell differentiation, possibly through targeting PIAS3, which inhibits STAT3 signaling and T_H17 cell differentiation[92].

Although the last few years have seen substantial progress in defining the transcriptional requirements for T_H17 cells[93], studies on miRNA function in T_H17 cells remain sparse. Given the high plasticity associated with this T_H cell subset, it is anticipated that miRNAs will play an important role in defining and maintaining the gene expression program of differentiated T_H17 cells.

T_{FH} cell differentiation

T follicular helper (T_{FH}) cells preferentially localize in close proximity to B cells in the follicles and germinal centers (GCs) of secondary lymphoid organs[94–96] and regulate humoral immunity[97]. T_{FH} cells are necessary for effective T cell-dependent antibody responses such as those induced by common vaccines, and T_{FH} cell dysregulation is involved in several autoimmune diseases[98]. T_{FH} cells express the chemokine receptor CXC chemokine receptor 5 (CXCR5), the co-inhibitory molecule programmed cell death 1 (PD-1) and the co-stimulatory molecule inducible T cell co-stimulator (ICOS), and they shape the humoral immune response by providing cytokines that influence immunoglobulin production by B cells[97]. The transcriptional repressor BCL-6 is necessary and sufficient to induce T_{FH} cell differentiation[88, 89, 99]. An initial $BCL6^+CXCR5^+$ T_{FH} cell population develops directly from naïve $CD4^+$ T cells after priming by DCs[100, 101]. Subsequent interactions with antigen-specific B cells further polarize T_{FH} cells, induce GCs, and contribute to the maintenance of the T_{FH} cell phenotype[99, 102–104].

Several miRNAs are differentially expressed in T_{FH} cells as compared to other T_H cell subsets[26]. miRNAs are essential for an early step in T_{FH} cell differentiation, as activated DGCR8-deficient $CD4^+$ T cells fail to upregulate CXCR5 and downregulate CCR7, thus preventing their migration to the T-B zone border and into B cell follicles[105]. This defect stands in sharp contrast to the role of miRNAs in other T_H cell subsets, such as T_H1 and T_H2, as these can still be generated from naïve miRNA-deficient $CD4^+$ T cells, even with aberrantly high efficiency in some cases[27, 31]. The fact that T_{FH} cell differentiation strictly requires a transcriptional repressor (BCL-6) and post-transcriptional repressors (miRNAs) indicates that silencing of alternative differentiation pathways has a crucial role in specifying or maintaining the T_{FH} cell gene expression program.

To date, only a few studies have provided insight into the roles of individual miRNAs or miRNA families in T_{FH} cells. In *sanroque* mice, a mutation of the RNA-binding protein Roquin-1 leads to excessive T_{FH} cell accumulation, which ultimately causes a lupus-like

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

autoimmune disease[106]. Initially, it was suggested that Roquin limits T_{FH} cell responses by repressing the 3' UTR of *Icos* mRNA through a process involving miR-101 binding within processing bodies (P-bodies) **[G]**[107]. However, later studies clarified that Roquin represses *Icos* by direct binding to its 3' UTR in a miRNA-independent manner[108].

The miR-17~92 cluster promotes T_{FH} cell differentiation and function independent of its effect on T cell proliferation[105, 109]. miR-17~92 targets PTEN in $CD4^+$ T cells[48], and this leads to a modest reduction in the number of developing $BCL6^+CXCR5^+$ T_{FH} cells very early following protein immunization[105, 109]. However, other derepressed miR-17~92 target genes likely contribute to the significantly reduced frequency of T_{FH} cells that accrues later in the response. In this regard, derepression of the miR-17~92 target gene *Phlpp22*, an Akt phosphatase involved in the ICOS signaling pathway, has been linked to the defective migration of activated miR-17~92-deficient T cells into B cell follicles[109]. A feedback mechanism may limit the expression of miR-17~92 in T_{FH} cells, as BCL-6 transduction reduced miR-17~92 expression in primary T cells, and miR-17~92 overexpression reduced CXCR5 expression in B cells[89]. However, the degrees to which the reported miRNA expression differences reflected the T cell activation status versus direct effects of BCL6 remain unclear.

miR-17~92 also prevents the expression of T_{FH} cell-inappropriate genes characteristic of Th17 or Th22 cells, including CC chemokine receptor 6 (*Ccr6)*, *Il22*, and *Rora*, which encodes the transcription factor RORα[105]. All four miRNA families represented in the miR-17~92 cluster target the *Rora* mRNA, and aberrant RORα expression is at least partly responsible for the increased expression of CCR6 and IL-22 in miR-17~92-deficient T_{FH} cells. These findings highlight the ability of miRNAs to ensure the fidelity of T_H subset-specific gene expression programs. Similarly, miR-10a inhibits the conversion of peripherally derived T_{Reg} cells into T_{FH} cells under lymphopenic conditions by directly targeting BCL6 and its co-repressor NCOR2[86]. Transient BCL-6 expression occurs during the development of other T_H cell subsets, including central memory T (T_{CM}) cells and T_H1 cells[101, 110–112]. Although T_{CM} and effector memory T (T_{EM}) cells are important for protective immunity against reinfection with certain pathogens and memory T cells contribute to the pathogenesis of chronic autoimmune diseases[113], not much is known about the involvement of miRNAs in the generation and maintenance of T_{CM} and T_{EM} cells.

Further work is needed to comprehensively profile miRNA expression in developing T_{FH} cells and to dissect how these miRNAs contribute to the balance between T_{FH} versus effector cell differentiation and to the maintenance of the T_{FH} cell identity.

MiRNA-mediated control of T_{Reg} cells

$CD4^+$ regulatory T (T_{Reg}) cells are essential mediators of peripheral tolerance[114]. They counterbalance the actions of effector T cells, preventing inappropriate or exaggerated immune responses that cause autoimmunity and immunopathology. Thymus-derived T_{Reg} cells are formed during positive selection in the thymus, but naïve $CD4^+$ T cells can also differentiate into peripherally derived T_{Reg} cells [115]. The gene expression program and functional identity of both T_{Reg} cell types depend on the lineage-defining transcription factor forkhead box P3 (FOXP3). Research on the requirements for miRNA expression in T_{Reg} cells, as well as on the specific contributions of individual miRNAs in T_{Reg} cell development, function, and plasticity has generally outpaced research on conventional T cells, though much remains to be learned.

MiRNAs support T_{Reg} cell development and homeostasis, and they also are important for the maintenance of their immunosuppressive function. Early studies suggested that miRNAs

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

have a predominantly tolerogenic role in T cells, as $CD4^+$ T cells lacking miRNAs failed to develop into T_{Reg} cells in the thymus and showed reduced differentiation into *in vitro*-induced T_{Reg} cells[28]. Moreover, a substantial percentage of mice with miRNA-deficient $CD4^+$ T cells developed spontaneous autoimmunity as they aged[28]. Notably, it was recently confirmed that miRNA deficiency in conventional $CD4^+$ T cells is in fact protective against the induction of autoimmunity[29]. By contrast, conditional deletion of DICER or DROSHA only in $FOXP3^+$ T_{Reg} cells causes early onset of severe spontaneous autoimmunity[30, 116, 117]. Thus, miRNA expression in T_{Reg} cells is important for the maintenance of self tolerance (Figure 3a).

Several miRNAs have been identified to impact T_{Reg} cell development and function (Figure 3b). Although T_{Reg} cells and conventional (non-T_{Reg}) effector $CD4^+$ T cells express a very similar set of miRNAs, expression profiling has uncovered several miRNAs that are differentially expressed in T_{Reg} cells[25, 26, 28]. miR-155 is highly expressed in T_{Reg} cells, and miR-155-deficient mice exhibit reduced numbers of thymus-derived and peripherally derived T_{Reg} cells[62]. FOXP3 controls this high level of miR-155 expression in T_{Reg} cells, and binds to an intronic region of *Bic*, the gene that encodes the primary transcript of miR-155[62, 118, 119]. miR-155 ensures T_{Reg} cell homeostasis by targeting the suppressor of cytokine signaling 1 (SOCS1)[62], a negative regulator of the IL-2 signaling pathway, which has a crucial role in T_{Reg} cell development. miR-155-deficiency also reduces IL-2 production by conventional $CD4^+$ T cells[54], suggesting that this miRNA may regulate IL-2-directed T_{reg} cell homeostasis through both cell intrinsic and extrinsic mechanisms.

miR-146a also impacts T_{Reg} cell function substantially. miR-146a-deficiency limited mainly to $FOXP3^+$ T_{Reg} cells using a mixed bone marrow chimera approach resulted in an IFNγ- and T_H1 cell-mediated pathology[61], similar to the disease observed in mice with T_{Reg} cell-specific DICER or DROSHA deficiency[30, 116, 117]. This pathology could result from increased expression of the miR-146a target STAT1 in T_{Reg} cells[61]. However, dysregulated miR-146a-deficient myeloid cells[45] may also contribute in this system, so confirmation of a T_{Reg} cell-intrinsic role for miR-146a in the development of autoimmunity remains to be confirmed with conditional miR-146a-deficient mice.

The miR-17~92 cluster represses T_{Reg} cell formation *in vitro*[63]. miR-17 directly targets TGF-β receptor II (*Tgfbr2*) and cAMP-responsive element binding protein 1 (*Creb1*), both of which are implicated in T_{Reg} cell differentiation *in vitro*[63]. In thymus-derived T_{Reg} cells, miR-17~92 seems to be particularly important during antigenic responses.miR-17~92 is dispensable for the development of thymus-derived T_{Reg} cells *in vivo*[63, 120], but miR-17~92-deficiency does reduce the number of MOG-specific T_{Reg} cells that are peripherally induced during EAE[120]. Similarly, transgenic overexpression of miR-17~92 does not affect total $FOXP3^+$ T_{Reg} cell numbers in a protein immunization model, but does increase the frequency of follicular T_{Reg} cells that emerge from thymus-derived T_{Reg} cells[105]. T_{Reg} cell-specific miR-17~92-deficiency also reduces the frequency of IL-10 producing T_{Reg} cells, which are particularly important for dampening immune responses[120]. Further studies are needed to identify the molecular targets and mechanisms of miR-17~92 in the regulation of T_{Reg} cell activity.

miR-10a is selectively expressed in T_{Reg} cells[26], and it regulates the stability of peripherally derived and *in vitro*-induced T_{Reg} cells. Retinoic acid synergizes with TGFβ to induce miR-10a expression in conventional $CD4^+$ T cells, and these conditions also promote the differentiation of peripherally derived T_{Reg} cells[86, 121]. miR-10a is not required for FOXP3 induction in *in vitro*-induced T_{Reg} cells, but it contributes to T_{Reg} cell stability by maintaining high FOXP3 expression[121]. miR-10a also inhibited the conversion of peripherally derived T_{Reg} cells into T_{FH} cells under lymphopenic conditions[86], a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

phenomenon that was originally described for FOXP3$^+$ T_{Reg} cells that had been adoptively transferred in the Peyer's patches of T cell-deficient mice[122]. miR-10a expression in T_{Reg} cells correlates inversely with susceptibility to autoimmune disease: T_{Reg} cells from the autoimmunity-resistant C57Bl/6 mouse strain have high miR-10a expression, whereas T_{Reg} cells from the autoimmunity-prone NOD mouse strain have low miR-10a expression levels[121]. miR-10a may prevent inappropriate activation of a T_{FH} cell gene expression program in T_{Reg} cells by directly targeting the mRNAs encoding the T_{FH} cell lineage-defining transcriptional repressor BCL-6 and its co-repressor NCOR2[86]. As discussed above, miR-10a also limits T_H17 cell differentiation[86], adding another layer to the complex regulatory network that constrains T_{Reg} cell plasticity.

The availability of genetic tools that facilitate the study of T_{Reg} cells *in vivo* has hastened the pace of research on miRNA function in T_{Reg} cells. miRNAs clearly perform critical functions in regulating the development and function of T_{Reg} cells in maintaining immune tolerance. Still, further studies are needed to dissect the impact of individual miRNAs and their targets in T_{Reg} cells, and to elucidate their role in the plasticity that is associated with this T_H cell subset.

Concluding remarks and future perspectives

MiRNAs are now generally recognized as important regulators of gene expression in most, if not all, vertebrate cells. The last few years have seen much progress in the field of miRNA regulation of lymphocyte development and immune function[123], and particularly in T helper cells[67, 124, 125]. T helper cell differentiation is particularly sensitive to small changes in transcriptional regulation, which is also reflected in the variety of known T_H cell subsets and the observed plasticity among some of these apparently differentiated cell types. Since miRNAs have evolved as a mechanism to fine tune biological processes[14], it is very likely that miRNAs represent an equally plastic and adjustable system for the regulation of T_H cell differentiation and plasticity.

The complexity and redundancy of miRNA networks underscores the importance of precise gene regulation in the programming of the great variety of cells that comprise a human body and their appropriate responses to developmental and environmental cues. This is also reflected in the observation that defects in single miRNAs within the same cell type can have diverse effects in different experimental settings, as discussed in this review for the different T_H cell subsets. Most of the progress on miRNA function in T_H cells has so far been based on mouse *in vitro* and *in vivo* models, and relatively little is known about miRNA function in human T_H cells. As one of the means to compensate for this lack of knowledge, it will be interesting to use systems biology approaches such as exome sequencing to discover mutations in patients that might correlate with altered miRNA expression. Such tools might also allow screening for alterations in miRNA target sequences that might contribute to the etiology of immune diseases.

Elucidating miRNA function in T_H cells can be leveraged to promote our understanding of the complex molecular pathways that govern the behavior and cell fate decisions of these cells. Future studies will not only provide insights into the role of miRNAs themselves, but most likely will also reveal novel mechanisms of immune regulation through the identification and functional analysis of miRNA target genes. Ideally, these studies will identify novel targets for the treatment of conditions in which T helper cell functions are impaired or exaggerated. Recent years have seen considerable progress in the field of miRNA-based therapies[126]. The first miRNA-targeted therapy, miraversen, blocks miR-122 in hepatocytes and reduced hepatitis C virus RNA in patients enrolled in a phase 2a trial[127]. Serious challenges remain though before miRNA-targeted therapies can be used in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

immunological diseases. It will be particularly important to develop enhanced methods for the delivery of these modalities specifically to T_H cells in patients[128]. One advantage of the immune system is that many of its effector cells recirculate between sites of inflammation and secondary lymphoid organs via the blood and lymph. Thus, systemic delivery of agents that target certain immune cell types might be easier accomplished than delivery to solid organs. Local administration at sites of inflammation may also be sufficient to safely alter immune activation while averting the need for cell type specific targeting. In any case, our expanding knowledge of these powerful but tiny regulators of T_H cell function promises new avenues for harnessing and shaping immune responses.

Acknowledgments

This work was supported by the NIH (HL109102, HL107202), and a Scholar Award from The Leukemia & Lymphoma Society (K.M.A.), and by the Swiss Foundation for Grants in Biology and Medicine (D.B.).

References

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986; 136:2348–2357. [PubMed: 2419430]
2. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009; 30:646–655. [PubMed: 19464987]
3. Locksley RM. Nine lives: plasticity among T helper cell subsets. J Exp Med. 2009; 206:1643–1646. [PubMed: 19635860]
4. Murphy KM, Stockinger B. Effector T cell plasticity: flexibility in the face of changing circumstances. Nat Immunol. 2010; 11:674–680. [PubMed: 20644573]
5. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science. 2010; 327:1098–1102. [PubMed: 20185720]
6. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol. 2009; 9:91–105. [PubMed: 19151746]
7. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. Annu Rev Immunol. 2006; 24:607–656. [PubMed: 16551261]
8. Kanno Y, Vahedi G, Hirahara K, Singleton K, O'Shea JJ. Transcriptional and epigenetic control of T helper cell specification: molecular mechanisms underlying commitment and plasticity. Annu Rev Immunol. 2012; 30:707–731. [PubMed: 22224760]
9. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol. 2010; 28:445–489. [PubMed: 20192806]
10. Ansel KM, Lee DU, Rao A. An epigenetic view of helper T cell differentiation. Nat Immunol. 2003; 4:616–623. [PubMed: 12830136]
11. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–233. [PubMed: 19167326]
12. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010; 79:351–379. [PubMed: 20533884]
13. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010; 11:597–610. [PubMed: 20661255]
14. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012; 148:1172–1187. [PubMed: 22424228]
15. Hoefig KP, Heissmeyer V. MicroRNAs grow up in the immune system. Curr Opin Immunol. 2008; 20:281–287. [PubMed: 18554884]
16. Ceribelli A, Satoh M, Chan EK. MicroRNAs and autoimmunity. Curr Opin Immunol. 2012; 24:686–691. [PubMed: 22902047]
17. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010; 10:111–122. [PubMed: 20098459]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

18. Nakayamada S, Takahashi H, Kanno Y, O'Shea JJ. Helper T cell diversity and plasticity. Curr Opin Immunol. 2012; 24:297–302. [PubMed: 22341735]
19. Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. Cell. 2009; 136:26–36. [PubMed: 19135886]
20. Belver L, Papavasiliou FN, Ramiro AR. MicroRNA control of lymphocyte differentiation and function. Curr Opin Immunol. 2011; 23:368–373. [PubMed: 21353514]
21. Jeker LT, Bluestone JA. Small RNA regulators of T cell-mediated autoimmunity. J Clin Immunol. 2010; 30:347–357. [PubMed: 20393792]
22. Monticelli S, et al. MicroRNA profiling of the murine hematopoietic system. Genome Biol. 2005; 6:R71. [PubMed: 16086853] This study was the first to systematically profile miRNA expression in the murine hematopoietic system.
23. Landgraf P, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell. 2007; 129:1401–1414. [PubMed: 17604727]
24. Barski A, et al. Chromatin poises miRNA- and protein-coding genes for expression. Genome Res. 2009; 19:1742–1751. [PubMed: 19713549]
25. Rossi RL, et al. Distinct microRNA signatures in human lymphocyte subsets and enforcement of the naive state in CD4+ T cells by the microRNA miR-125b. Nat Immunol. 2011; 12:796–803. [PubMed: 21706005] Elegant study that used high-throughput miRNA profiling to establish miRNA expression in human lymphocyte subsets.
26. Kuchen S, et al. Regulation of microRNA expression and abundance during lymphopoiesis. Immunity. 2010; 32:828–839. [PubMed: 20605486] High-throughput sequencing technology was used to comprehensively characterize the microRNome in many different immune cell types.
27. Muljo SA, et al. Aberrant T cell differentiation in the absence of Dicer. J Exp Med. 2005; 202:261–269. [PubMed: 16009718] This study provided the first evidence that miRNAs are important regulators of T helper cell differentiation.
28. Cobb BS, et al. A role for Dicer in immune regulation. J Exp Med. 2006; 203:2519–2527. [PubMed: 17060477]
29. Tian L, et al. Loss of T cell microRNA provides systemic protection against autoimmune pathology in mice. J Autoimmun. 2012; 38:39–48. [PubMed: 22225602]
30. Chong MM, Rasmussen JP, Rudensky AY, Littman DR. The RNAseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. J Exp Med. 2008; 205:2005–2017. [PubMed: 18725527] These three papers described that miRNA expression in Treg cells is required to prevent autoimmunity.
31. Steiner DF, et al. MicroRNA-29 Regulates T-Box Transcription Factors and Interferon-gamma Production in Helper T Cells. Immunity. 2011; 35:169–181. [PubMed: 21820330] These papers showed that miR-29 is an important regulator of IFN-γ production in Th1 cells.
32. Bronevetsky Y, et al. T cell activation induces proteasomal degradation of Argonaute and rapid remodeling of the microRNA repertoire. J Exp Med. 2013; 210:417–432. [PubMed: 23382546]
33. Sandberg R, Neilson JR, Sarma A, Sharp PA, Burge CB. Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. Science. 2008; 320:1643–1647. [PubMed: 18566288]
34. Ma F, et al. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-gamma. Nat Immunol. 2011; 12:861–869. [PubMed: 21785411] These papers showed that miR-29 is an important regulator of IFN-γ production in Th1 cells.
35. Smith KM, et al. miR-29ab1 Deficiency Identifies a Negative Feedback Loop Controlling Th1 Bias That Is Dysregulated in Multiple Sclerosis. J Immunol. 2012 These papers showed that miR-29 is an important regulator of IFN-γ production in Th1 cells.
36. Li QJ, et al. miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell. 2007; 129:147–161. [PubMed: 17382377]
37. Ebert PJ, Jiang S, Xie J, Li QJ, Davis MM. An endogenous positively selecting peptide enhances mature T cell responses and becomes an autoantigen in the absence of microRNA miR-181a. Nat Immunol. 2009; 10:1162–1169. [PubMed: 19801983]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

38. Henao-Mejia J, et al. The MicroRNA miR-181 Is a Critical Cellular Metabolic Rheostat Essential for NKT Cell Ontogenesis and Lymphocyte Development and Homeostasis. Immunity. 2013; 38:984–997. [PubMed: 23623381]
39. Fragoso R, et al. Modulating the strength and threshold of NOTCH oncogenic signals by mir-181a-1/b-1. PLoS Genet. 2012; 8:e1002855. [PubMed: 22916024]
40. Zietara N, et al. Critical role for miR-181a/b-1 in agonist selection of invariant natural killer T cells. Proc Natl Acad Sci U S A. 2013; 110:7407–7412. [PubMed: 23589855]
41. Li G, et al. Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. Nat Med. 2012; 18:1518–1524. [PubMed: 23023500]
42. Palin AC, Ramachandran V, Acharya S, Lewis DB. Human Neonatal Naive CD4+ T Cells Have Enhanced Activation-Dependent Signaling Regulated by the MicroRNA miR-181a. J Immunol. 2013
43. Rusca N, et al. miR-146a and NF-kappaB1 Regulate Mast Cell Survival and T Lymphocyte Differentiation. Mol Cell Biol. 2012; 32:4432–4444. [PubMed: 22927641]
44. Curtale G, et al. An emerging player in the adaptive immune response: microRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. Blood. 2010; 115:265–273. [PubMed: 19965651]
45. Zhao JL, et al. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci U S A. 2011; 108:9184–9189. [PubMed: 21576471]
46. Yang L, et al. miR-146a controls the resolution of T cell responses in mice. J Exp Med. 2012; 209:1655–1670. [PubMed: 22891274]
47. Wu T, et al. Temporal expression of microRNA cluster miR-17-92 regulates effector and memory CD8+ T-cell differentiation. Proc Natl Acad Sci U S A. 2012; 109:9965–9970. [PubMed: 22665768]
48. Xiao C, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008; 9:405–414. [PubMed: 18327259] Elegant study showing that overexpression of the miR-17~92 cluster in lymphocytes leads to lymphoproliferative disease and autoimmunity.
49. Loeb GB, et al. Transcriptome-wide miR-155 Binding Map Reveals Widespread Noncanonical MicroRNA Targeting. Mol Cell. 2012 Transcripome-wide analysis of miRNA binding sites that revealed an unexpectedly high frequency of functional non-canonical target sites.
50. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell. 2010; 39:493–506. [PubMed: 20797623]
51. Huffaker TB, et al. Epistasis between MicroRNAs 155 and 146a during T Cell-Mediated Antitumor Immunity. Cell Rep. 2012; 2:1697–1709. [PubMed: 23200854] This paper revealed epistasis between miR-155 and miR-146a in T cell-mediated anti-tumour immunity.
52. Banerjee A, Schambach F, DeJong CS, Hammond SM, Reiner SL. Micro-RNA-155 inhibits IFN-gamma signaling in CD4+ T cells. Eur J Immunol. 2010; 40:225–231. [PubMed: 19877012]
53. Thai TH, et al. Regulation of the germinal center response by microRNA-155. Science. 2007; 316:604–608. [PubMed: 17463289] These two studies were the first to show that genetic disruption of a single miRNA in vivo can have adverse effects on immune cell homeostasis and function.
54. Rodriguez A, et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007; 316:608–611. [PubMed: 17463290] These two studies were the first to show that genetic disruption of a single miRNA in vivo can have adverse effects on immune cell homeostasis and function.
55. O'Connell RM, et al. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity. 2010; 33:607–619. [PubMed: 20888269]
56. Oertli M, et al. MicroRNA-155 is essential for the T cell-mediated control of Helicobacter pylori infection and for the induction of chronic Gastritis and Colitis. J Immunol. 2011; 187:3578–3586. [PubMed: 21880981]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

57. Liao W, Lin JX, Leonard WJ. Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. Immunity. 2013; 38:13–25. [PubMed: 23352221]
58. Thiele S, Wittmann J, Jack HM, Pahl A. miR-9 enhances IL-2 production in activated human CD4(+) T cells by repressing Blimp-1. Eur J Immunol. 2012; 42:2100–2108. [PubMed: 22585398]
59. Seddiki N, et al. The microRNA-9/B-lymphocyte-induced maturation protein-1/IL-2 axis is differentially regulated in progressive HIV infection. Eur J Immunol. 2013; 43:510–520. [PubMed: 23129528]
60. Stittrich AB, et al. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. Nat Immunol. 2010; 11:1057–1062. [PubMed: 20935646]
61. Lu LF, et al. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell. 2010; 142:914–929. [PubMed: 20850013]
62. Lu LF, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. 2009; 30:80–91. [PubMed: 19144316]
63. Jiang S, 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; 118:5487–5497. [PubMed: 21972292]
64. Lazarevic V, Glimcher LH. T-bet in disease. Nat Immunol. 2011; 12:597–606. [PubMed: 21685955]
65. Lu TX, Munitz A, Rothenberg ME. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. J Immunol. 2009; 182:4994–5002. [PubMed: 19342679]
66. Lu TX, et al. MicroRNA-21 limits in vivo immune response-mediated activation of the IL-12/IFN-gamma pathway, Th1 polarization, and the severity of delayed-type hypersensitivity. J Immunol. 2011; 187:3362–3373. [PubMed: 21849676]
67. Jeker LT, Bluestone JA. MicroRNA regulation of T-cell differentiation and function. Immunol Rev. 2013; 253:65–81. [PubMed: 23550639]
68. Beaulieu AM, et al. MicroRNA function in NK-cell biology. Immunol Rev. 2013; 253:40–52. [PubMed: 23550637]
69. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol. 2011; 11:375–388. [PubMed: 21610741]
70. Locksley RM. Asthma and allergic inflammation. Cell. 2010; 140:777–783. [PubMed: 20303868]
71. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol. 2010; 10:225–235. [PubMed: 20336151]
72. Sawant DV, Wu H, Kaplan MH, Dent AL. The Bcl6 target gene microRNA-21 promotes Th2 differentiation by a T cell intrinsic pathway. Mol Immunol. 2013; 54:435–442. [PubMed: 23416424]
73. Guerau-de-Arellano M, et al. Micro-RNA dysregulation in multiple sclerosis favours pro-inflammatory T-cell-mediated autoimmunity. Brain. 2011; 134:3578–3589. [PubMed: 22088562]
74. Seumois G, et al. An integrated nano-scale approach to profile miRNAs in limited clinical samples. Am J Clin Exp Immunol. 2012; 1:70–89. [PubMed: 23304658]
75. Solberg OD, et al. Airway epithelial miRNA expression is altered in asthma. Am J Respir Crit Care Med. 2012; 186:965–974. [PubMed: 22955319]
76. Mattes J, Collison A, Plank M, Phipps S, Foster PS. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. Proc Natl Acad Sci U S A. 2009; 106:18704–18709. [PubMed: 19843690]
77. Collison A, et al. Altered expression of microRNA in the airway wall in chronic asthma: miR-126 as a potential therapeutic target. BMC Pulm Med. 2011; 11:29. [PubMed: 21605405]
78. Collison A, Mattes J, Plank M, Foster PS. Inhibition of house dust mite-induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment. J Allergy Clin Immunol. 2011; 128:160–167. e4. [PubMed: 21571357]
79. Polikepahad S, et al. Proinflammatory role for let-7 microRNAS in experimental asthma. J Biol Chem. 2010; 285:30139–30149. [PubMed: 20630862]
80. Kumar M, et al. Let-7 microRNA-mediated regulation of IL-13 and allergic airway inflammation. J Allergy Clin Immunol. 2011; 128:1077–1085. e1–e10. [PubMed: 21616524]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

81. Swaminathan S, et al. Differential regulation of the Let-7 family of microRNAs in CD4+ T cells alters IL-10 expression. J Immunol. 2012; 188:6238–6246. [PubMed: 22586040]
82. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009; 27:485–517. [PubMed: 19132915]
83. Weaver CT, Elson CO, Fouser LA, Kolls JK. The Th17 pathway and inflammatory diseases of the intestines, lungs, and skin. Annu Rev Pathol. 2013; 8:477–512. [PubMed: 23157335]
84. Thamilarasan M, Koczan D, Hecker M, Paap B, Zettl UK. MicroRNAs in multiple sclerosis and experimental autoimmune encephalomyelitis. Autoimmun Rev. 2012; 11:174–179. [PubMed: 21621006]
85. Du C, et al. MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol. 2009; 10:1252–1259. [PubMed: 19838199]
86. Takahashi H, et al. TGF-beta and retinoic acid induce the microRNA miR-10a, which targets Bcl-6 and constrains the plasticity of helper T cells. Nat Immunol. 2012; 13:587–595. [PubMed: 22544395]
87. Oestreich KJ, Huang AC, Weinmann AS. The lineage-defining factors T-bet and Bcl-6 collaborate to regulate Th1 gene expression patterns. J Exp Med. 2011; 208:1001–1013. [PubMed: 21518797]
88. Nurieva RI, et al. Bcl6 mediates the development of T follicular helper cells. Science. 2009; 325:1001–1005. [PubMed: 19628815]
89. Yu D, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity. 2009; 31:457–468. [PubMed: 19631565]
90. Lazarevic V, et al. T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgammat. Nat Immunol. 2011; 12:96–104. [PubMed: 21151104]
91. Murugaiyan G, Beynon V, Mittal A, Joller N, Weiner HL. Silencing microRNA-155 ameliorates experimental autoimmune encephalomyelitis. J Immunol. 2011; 187:2213–2221. [PubMed: 21788439]
92. Mycko MP, et al. MicroRNA-301a regulation of a T-helper 17 immune response controls autoimmune demyelination. Proc Natl Acad Sci U S A. 2012; 109:E1248–E1257. [PubMed: 22517757]
93. Basu R, Hatton RD, Weaver CT. The Th17 family: flexibility follows function. Immunol Rev. 2013; 252:89–103. [PubMed: 23405897]
94. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyster JG. In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. J Exp Med. 1999; 190:1123–1134. [PubMed: 10523610]
95. Breitfeld D, 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; 192:1545–1552. [PubMed: 11104797]
96. Schaerli P, et al. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med. 2000; 192:1553–1562. [PubMed: 11104798]
97. Crotty S. Follicular Helper CD4 T Cells (T(FH)). Annu Rev Immunol. 2011; 29:621–663. [PubMed: 21314428]
98. Craft JE. Follicular helper T cells in immunity and systemic autoimmunity. Nat Rev Rheumatol. 2012; 8:337–347. [PubMed: 22549246]
99. Johnston RJ, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science. 2009; 325:1006–1010. [PubMed: 19608860]
100. Choi YS, et al. ICOS Receptor Instructs T Follicular Helper Cell versus Effector Cell Differentiation via Induction of the Transcriptional Repressor Bcl6. Immunity. 2011; 34:932–946. [PubMed: 21636296]
101. Baumjohann D, Okada T, Ansel KM. Cutting Edge: Distinct Waves of BCL6 Expression during T Follicular Helper Cell Development. J Immunol. 2011; 187:2089–2092. [PubMed: 21804014]
102. Haynes NM, et al. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. J Immunol. 2007; 179:5099–5108. [PubMed: 17911595]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

103. Deenick EK, et al. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. Immunity. 2010; 33:241–253. [PubMed: 20691615]
104. Baumjohann D, et al. Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. Immunity. 2013; 38:596–605. [PubMed: 23499493]
105. Baumjohann D, et al. The microRNA cluster miR-17~92 promotes TFH cell differentiation and represses subset-inappropriate gene expression. Nat Immunol. 2013 in press.
106. Vinuesa CG, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature. 2005; 435:452–458. [PubMed: 15917799]
107. Yu D, et al. Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. Nature. 2007; 450:299–303. [PubMed: 18172933]
108. Glasmacher E, et al. Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression. Nat Immunol. 2010; 11:725–733. [PubMed: 20639877]
109. Kang, et al. Nat Immunol. 2013 in press.
110. Pepper M, Pagan AJ, Igyarto BZ, Taylor JJ, Jenkins MK. Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. Immunity. 2011; 35:583–595. [PubMed: 22018468]
111. Nakayamada S, et al. Early Th1 Cell Differentiation Is Marked by a Tfh Cell-like Transition. Immunity. 2011; 35:919–931. [PubMed: 22195747]
112. Oestreich KJ, Mohn SE, Weinmann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. Nat Immunol. 2012; 13:405–411. [PubMed: 22406686]
113. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol. 2004; 22:745–763. [PubMed: 15032595]
114. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nat Immunol. 2010; 11:7–13. [PubMed: 20016504]
115. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–564. [PubMed: 22224781]
116. Zhou X, et al. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. J Exp Med. 2008; 205:1983–1991. [PubMed: 18725525] These three papers described that miRNA expression in Treg cells is required to prevent autoimmunity.
117. Liston A, Lu LF, O'Carroll D, Tarakhovsky A, Rudensky AY. Dicer-dependent microRNA pathway safeguards regulatory T cell function. J Exp Med. 2008; 205:1993–2004. [PubMed: 18725526] These three papers described that miRNA expression in Treg cells is required to prevent autoimmunity.
118. Zheng Y, et al. Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature. 2007; 445:936–940. [PubMed: 17237761]
119. Marson A, et al. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature. 2007; 445:931–935. [PubMed: 17237765]
120. de Kouchkovsky D, et al. miR-17~92 regulates interleukin-10 production by Tregs and control of experimental autoimmune encephalomyelitis. J Immunol. 2013 in press.
121. Jeker LT, et al. MicroRNA 10a Marks Regulatory T Cells. PLoS One. 2012; 7:e36684. [PubMed: 22629323]
122. Tsuji M, et al. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. Science. 2009; 323:1488–1492. [PubMed: 19286559]
123. Ansel KM. RNA regulation of the immune system. Immunol Rev. 2013; 253:5–11. [PubMed: 23550634]
124. Pagani M, et al. Role of microRNAs and long-non-coding RNAs in CD4(+) T-cell differentiation. Immunol Rev. 2013; 253:82–96. [PubMed: 23550640]
125. Dooley J, Linterman MA, Liston A. MicroRNA regulation of T-cell development. Immunol Rev. 2013; 253:53–64. [PubMed: 23550638]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

126. Petrocca F, Lieberman J. Promise and challenge of RNA interference-based therapy for cancer. J Clin Oncol. 2011; 29:747–754. [PubMed: 21079135]

127. Janssen HL, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013; 368:1685–1694. [PubMed: 23534542]

128. Peer D. A daunting task: manipulating leukocyte function with RNAi. Immunol Rev. 2013; 253:185–197. [PubMed: 23550647]

129. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol. 2012; 19:586–593. [PubMed: 22664986]

130. Leshkowitz D, Horn-Saban S, Parmet Y, Feldmesser E. Differences in microRNA detection levels are technology and sequence dependent. RNA. 2013; 19:527–538. [PubMed: 23431331]

131. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 2011; 39:D152–D157. [PubMed: 21037258]

132. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009; 19:92–105. [PubMed: 18955434]

133. Hsu SD, et al. miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res. 2011; 39:D163–D169. [PubMed: 21071411]

134. Prosser HM, Koike-Yusa H, Cooper JD, Law FC, Bradley A. A resource of vectors and ES cells for targeted deletion of microRNAs in mice. Nat Biotechnol. 2011; 29:840–845. [PubMed: 21822254]

135. Park CY, et al. A resource for the conditional ablation of microRNAs in the mouse. Cell Rep. 2012; 1:385–391. [PubMed: 22570807]

136. Brown BD, Naldini L. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. Nat Rev Genet. 2009; 10:578–585. [PubMed: 19609263]

137. Thomas M, Lieberman J, Lal A. Desperately seeking microRNA targets. Nat Struct Mol Biol. 2010; 17:1169–1174. [PubMed: 20924405]

138. Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. Nature. 2009; 460:479–486. [PubMed: 19536157]

139. Hafner M, et al. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell. 2010; 141:129–141. [PubMed: 20371350]

Glossary terms

T helper cell	Effector T cell that develops from naïve $CD4^+$ T cells. Upon activation in the periphery, Th cells produce cytokines that orchestrate cellular and humoral immunity.
secondary lymphoid organs	Organized lymphoid structures in which adaptive immune responses are elicited, e.g. spleen and lymph nodes.
positive selection	Immature T cells are selected in the thymus for expression of a functional TCR.
negative selection	Immature T cells that harbor high affinity for self-antigens are deleted in the thymus to prevent egress of auto-reactive T cells into the circulation.
germinal center	Specialized anatomic structure in secondary lymphoid organs in which T_{FH} cells provide help to B cells to generate high affinity antibodies, memory B cells, and long-lived plasma cells.
processing bodies	Molecular structures within the cytoplasm that are major sites for mRNA turnover.
miRISC	The *miR*NA-*i*nduced *s*ilencing *c*omplex consists of a miRNA bound to an Argonaute protein. The miRNA provides sequence specificity

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

	for the complex's function in translational repression and decreasing target mRNA stability.
miRNA cluster	miRNAs are often found within clusters in the genome, and these clusters are typically transcribed together to form primary miRNA transcripts that are processed to yield multiple mature miRNAs.
miRNA family	miRNAs are classified into 'families' that share the same seed sequence, and are therefore predicted to share many of the same target mRNAs. For example, the polycistronic miR-17~92 cluster comprises six miRNAs representing four separate miRNA families: the miR-17 family (miR-17, miR-20a), the miR-18 family (miR-18a), the miR-19 family (miR-19a, miR-19b), and the miR-25 family (miR-92a).
miRNA sponge	A genetically engineered construct containing several miRNA binding sites that compete with endogenous miRNA binding sites, reducing specific miRNA availability and function.
miRNA mimics	Small transfectable synthetic RNAs that mimic endogenous miRNAs used to study the effects of miRNA overexpression.
antagomir	Also known as anti-miR, miRNA inhibitor, and miRNA antagonist. Small synthetic nucleic acid oligonucleotides that bind to endogenous miRNAs and inhibit their function. Antagomirs are often chemically modified to promote their stability and/or entry into target cells.
locked nucleic acids (LNA)	RNA oligonucleotides bearing a modification of the ribose moiety in their backbone that "locks" them in a favorable conformation for base pairing, increasing their binding affinity. LNAs are used in various applications for miRNA detection and in miRNA inhibitors for experimental and therapeutic use.
experimental autoimmune encephalitis (EAE)	An animal model of multiple sclerosis — a chronic demyelinating disease in humans. In animals, EAE is induced by the injection of several different antigens that are derived from the myelin sheath, including myelin basic protein, proteolipid protein or myelin oligodendrocyte glycoprotein, together with a potent adjuvant.

Biographies

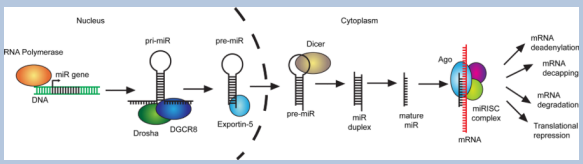
Dirk Baumjohann is currently a postdoctoral fellow in the laboratory of Dr. K. Mark Ansel at the University of California, San Francisco. He received his Ph.D. in Cell Biology/ Immunology from the University of Bern, Switzerland, for work with Dr. Federica Sallusto at the Institute for Research in Biomedicine in Bellinzona, Switzerland. His recent studies focus on understanding how miRNAs and transcription factors regulate T helper cell differentiation.

K. Mark Ansel is an Assistant Professor of Microbiology & Immunology in the Sandler Asthma Basic Research Center at the University of California, San Francisco (UCSF). He completed his Ph.D. in Biomedical Sciences in Dr. Jason Cyster's laboratory at UCSF, and conducted postdoctoral research with Dr. Anjana Rao at Harvard Medical School. His laboratory studies miRNA regulation in the immune system.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Box 1 | miRNA biogenesis and function

MicroRNA genes are transcribed into primary miRNAs (pri-miRNAs) by RNA polymerase II. Pri-miRNAs are bound by Dgcr8 and processed by the RNase III activity of Drosha into hairpin structures called pre-miRNAs. Exportin-5 shuttles pre-miRNAs from the nucleus into the cytoplasm where the RNase III Dicer cleaves off the pre-miRNA's hairpin loop. The resulting duplex segregates and the mature single-stranded miRNA associates with Argonaute and other accessory proteins to form the miRNA-induced silencing complex (miRISC), which mediates translational repression and increased degradation of its mRNA targets. A mature miRNA bound to an Argonaute (Ago) protein forms the core of the miRISC. Ago recruits other protein complexes that antagonize translation and deadenylate the targeted mRNA[129]. This ultimately leads to mRNA decapping and degradation, so the effect of miRNA repression can be observed at both the protein and mRNA level. The miRNA provides specificity through complementary base pairing with target mRNAs[11]. Nucleotides in positions 2–8 from the 5′ end of a miRNA, termed the seed sequence, are a major determinant of target recognition. However, complementarity in the 3′ half of the miRNA does contribute to binding, and 'seedless' targets that rely on non-seed sequences for binding also exist. Most functional miRNA binding sites occur in the 3′ UTR of target mRNAs, and many of these are deeply conserved, indicating co-evolution of miRNAs and their targets. These principles have been exploited to develop algorithms for bioinformatic prediction of miRNA targets. Though useful for hypothesis generation, these programs remain imperfect. Predicted targets must be confirmed experimentally, and many true targets are missed.



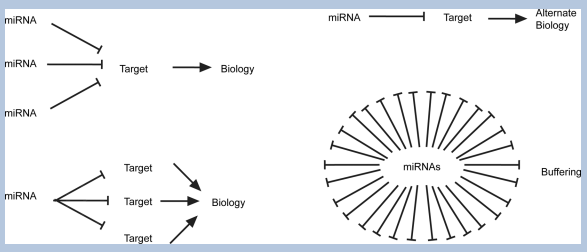
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Box 2 | Regulation of gene expression networks by miRNAs

The magnitude of repression of direct miRNA targets is relatively low – less than 50% at both the protein and mRNA level in the vast majority of cases. Nevertheless, miRNAs can have big effects on biological processes. Target genes are frequently inhibited by a combination of several miRNAs. Similarly, each miRNA targets many genes at the same time. In some cases, miRNA effects are amplified through coordinated repression of several target mRNAs in a common pathway. Small changes in individual targets can also have big effects in systems governed by feedback mechanisms and activation thresholds. In this context, miRNAs can be important to maintain effective gene silencing in the face of “leaky” transcription that produces a functionally significant amount of mRNA in an inappropriate cell type or biological condition. Finally, the combined effect of miRNAs acting on many target genes can buffer noise associated with transcriptional processes and effectively set thresholds for signals that induce programmed changes in gene expression, such as those that mediate T_H cell differentiation and plasticity.



NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Box 3 | Methods for studying miRNAs and their function

Several methods exist to profile and quantify miRNA expression in cells, tissues and body fluids. These range from measuring single miRNAs by conventional or multiplexed qPCR in purified T cell subsets to genome-wide analysis of miRNA expression by microarrays and RNA-sequencing [74, 130]. Online databases catalog miRNA sequences and annotation[131], and both bioinformatically predicted[132] and experimentally validated[133] miRNA targets.

MicroRNA function can be studied by a variety of gain- and loss-of-function technologies. Conditional deletion of key molecules of the miRNA biogenesis pathway showed that miRNAs as a whole regulate many aspects of $CD4^+$ T cell behavior, but the current challenge is to understand how each miRNA contributes to these effects. Two consortia have generated freely available miRNA-deficient ES cells[134] and conditional knock-out mice[135] that will be very helpful in advancing our understanding of miRNA function *in vivo*. Specific miRNA inhibition can also be achieved using a variety of transfectable or self-deliverable synthetic antagonists (often called 'antagomirs'), or by overexpressing target mRNAs that act as 'sponges' of miRNAs, decreasing their activity against endogenous targets[136]. Overexpression of specific miRNAs can be achieved with transfectable synthetic miRNA mimics, expression constructs, viral vectors, or transgenesis in mice.

Understanding the mechanism of miRNA function requires methods to identify its targets[137]. Genome-wide transcriptional profiling of specific miRNA-deficient or overexpressing cells and controls can be used to assess global target gene expression changes, though it is often difficult to distinguish direct and indirect targets with this approach. Direct targeting can be validated with 3′ UTR luciferase reporter assays, but validating functional relevance requires genetic rescue experiments. Recently, biochemical techniques such as high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP)[138] and photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP)[139], have been developed to identify RNA sequences that bind to RNA-binding proteins such as Argonaute proteins.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Online summary

- T helper (T_H) cells represent a central component of the adaptive immune system. They coordinate cellular and humoral responses by producing cytokines and growth factors. Several T_H cell subsets have been described, including T_H1, T_H2, T_H17, T_H9, T_H22 cells, regulatory T (T_{Reg}) cells, and T follicular helper (T_{FH}) cells.
- MicroRNAs are small evolutionarily conserved nucleotide sequences that regulate gene expression by interfering with mRNA translation and stability.
- MicroRNA-deficient $CD4^+$ T cells display impaired survival and proliferation, but also exhibit increased sensitivity to signals that induce effector T_H cell differentiation and cytokine production.
- An expanding list of individual microRNAs and co-expressed microRNA clusters have been shown to have significant effects on T_H cell fate decisions and immune functions.
- MicroRNAs are critical for the proper regulation of Treg development, homeostasis, plasticity, and the maintenance of immune tolerance.
- Research on microRNA function can be used as a tool for the discovery of novel pathways that regulate T_H cell biology, and may identify novel targets for the treatment of conditions in which T_H cell functions are impaired or exaggerated.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

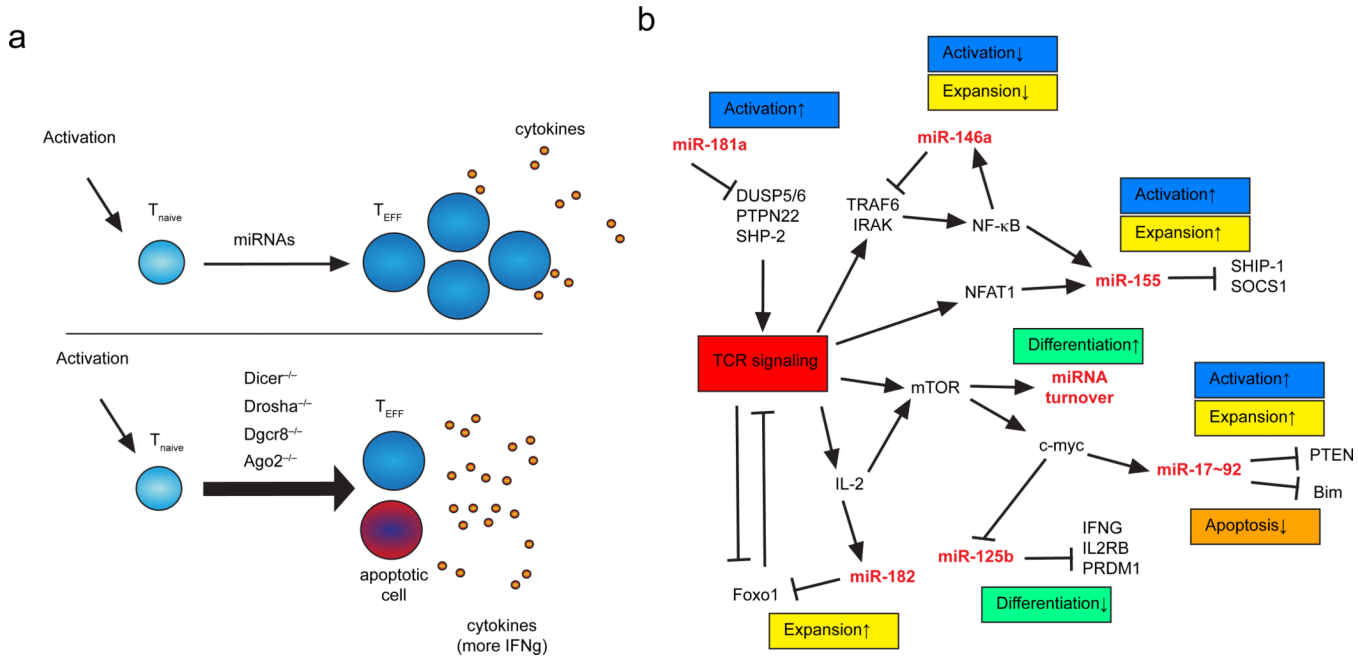


Figure 1. miRNA regulation of T helper cell activation

a | MicroRNAs are important regulators of effector T cell differentiation, including T cell activation, acquisition of effector functions such as cytokine production, and T cell proliferation (top panel). Genetic ablation of key molecules of the miRNA biogenesis pathway in $CD4^+$ T cells underlines the importance of miRNAs for these processes (lower panel). Activation of microRNA-deficient $CD4^+$ T cells results in increased and aberrant cytokine production, reduced cell proliferation. **b** | Mechanistic overview illustrating miRNA participation in regulatory networks that control T cell activation, expansion, and effector cell differentiation. Note that specific miRNAs are both regulated targets and upstream regulators of signaling pathways that govern T cell behavior. Selected miRNA target genes are indicated. T_{EFF}, effector T cell; T_{naive}, naïve T cell.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

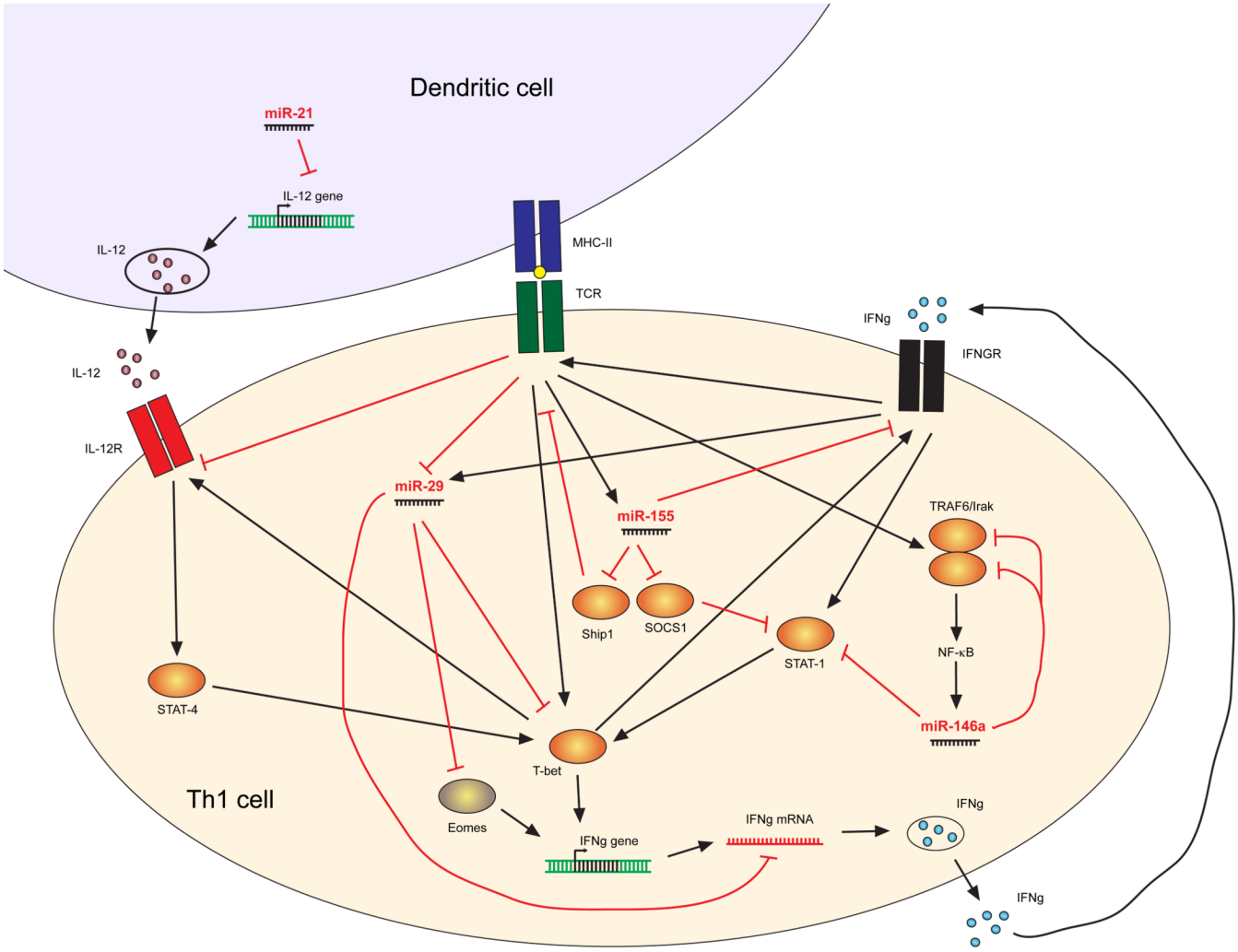


Figure 2. miRNA regulation of IFNγ production

IFN-γ production and signaling in Th1 cells is regulated by several miRNAs at distinct levels. The lineage determining transcription factor T-bet induces expression of the Th1 hallmark cytokine IFN-γ. T-bet is induced by TCR signaling, by IL-12 via STAT4 signaling, and by IFN-γ itself in a positive feedback loop via STAT1. miR-146a directly targets STAT1 and the NFκB signaling molecules TRAF6 and IRAK1. TCR activation induces miR-155, which in turn downregulates negative regulators of cytokine signaling SHIP1 and SOCS1. However, miR-155 has also been proposed to induce downregulation of IFNγR1. miR-29 limits Th1 cell differentiation and IFN-γ production by targeting the mRNAs encoding T-bet, Eomes, and IFN-γ itself.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

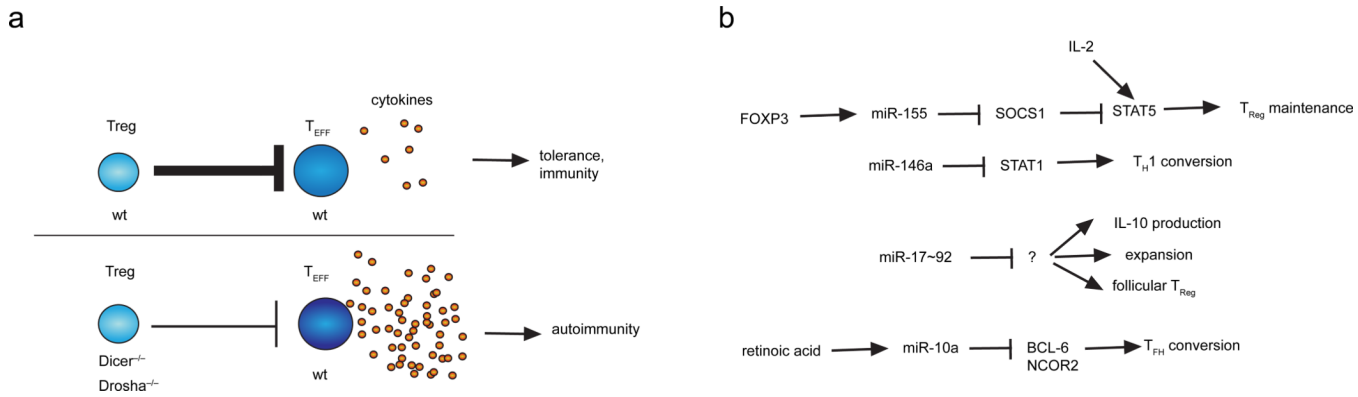


Figure 3. miRNA regulation of T_{Reg} function and plasticity

a | Treg-specific miRNA expression is required to restrain effector T cell responses. In the absence of miRNA expression in T_{Reg} cells, for example due to deletion of essential components of the miRNA biogenesis pathway, such as DICER or DROSHA, T_{Reg} cells fail to maintain tolerance, which might result in autoimmunity. **b** | Examples of miRNA pathways that contribute to the regulation of T_{Reg} cell function and plasticity. T_{EFF}, effector T cell; T_{Reg}, regulatory T cell.