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HIGHLIGHTS

COMMENTARY

Micro RNAs in Tfh regulation: Small molecules with a big impact

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The germinal center (GC) reactions are critical for the production of high-affinity antibodies that comprise the protective humoral response elicited by infection or vaccination. GCs are initiated through the interaction of B cells with T follicular helper (Tfh) cells. While the transcriptional regulation of Tfh differentiation has been studied in great detail, the impact of micro RNAs (miRNAs) on Tfh development and stability has been harder to address. It was previously shown that a complete deletion of miRNAs biogenesis prevents Tfh differentiation. In this issue of the *European Journal of Immunology* [Eur. J. Immunol. 2021. 51: 408-413], Zeiträg et al. use an inducible gene deletion approach to reveal that miRNAs are also required for the maintenance of Tfh cells induced following viral infection in mice. These results provide new clues to the regulation of GC responses through Tfh and T follicular regulatory cells.

Keywords: micro RNAs · T follicular helper cells · T follicular regulatory cells · germinal center

See accompanying article by Zeiträg et al.

The production of high-affinity antibodies requires interactions between B and T cells within lymphoid tissue [1]. T follicular helper (Tfh) cells represent a CD4 T-cell subset with the unique ability to access the B-cell follicle and to provide "help" to B cells [1,2]. In fact, the generation of germinal centers (GC), the anatomic structures where B cells undergo affinity maturation, is initiated following B–Tfh interactions. As a consequence, individuals with a defect in Tfh production or function do not have functional humoral responses [3].

In addition, a specialized population of Foxp3+ CD4 T cells—named T follicular regulatory (Tfr) cells—can gain access to the B cell follicle and regulate the GC response [4–7].

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Given the importance of Tfh cells for humoral immunity, namely the generation of protective neutralizing antibodies following infection or vaccination, the study of Tfh development and function has generated much interest. However, a major hurdle for the study of Tfh development is the multistep process, within distinct anatomic compartments, that is required for an uncommitted CD4 T cell to differentiate into a functional GC-Tfh cell [1]. In the first step, within the T-cell zone of lymphoid tissue, a DC activates a naïve CD4 T cell that acquires a pre-Tfh phenotype; subsequently, the pre-Tfh cell migrates to the T-B border where it has the chance to interact with a B cell presenting the appropriate antigen; finally, the productive interaction with the B cell allows the pre-Tfh to further differentiate into a Tfh cell that gains access to the B-cell follicle where it can initiate a GC. Comprehensibly, it has been extremely difficult to reproduce in vitro the complex site-specific sequential interactions leading to Tfh differentiation.

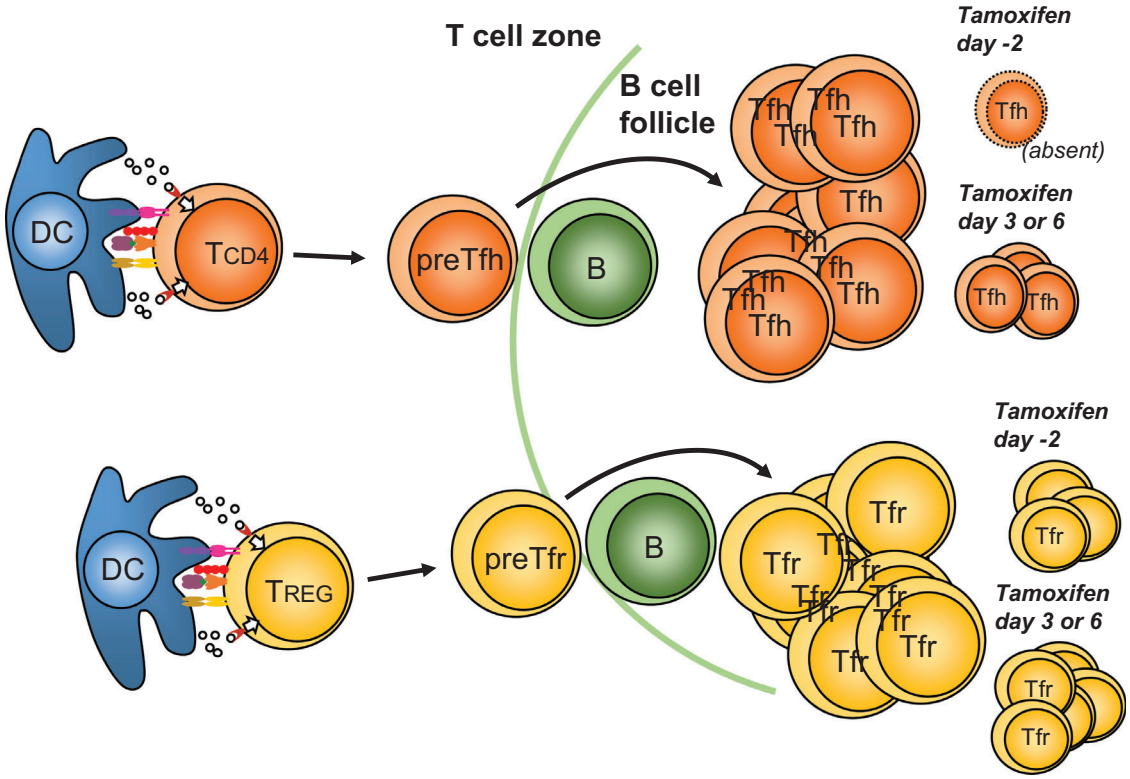

Figure 1. *Differential impact of miRNAs abrogation on Tfh and Tfr cell differentiation.* Tfh cells differentiate from naïve CD4 in a two-step process: first, through a dendritic cell (DC)-mediated activation, and then the resulting pre-Tfh cell interacts with a B cell in the T–B border leading to the final differentiation toward a mature Tfh cell that can access the B-cell follicle and participate in the germinal center (GC) reaction. Tfr cells differentiate from Treg cells following a similar two-step process (bottom). When miRNA production is ablated before infection (tamoxifen administration at day -2), it leads to an absence of mature Tfh cells without affecting the number of naïve CD4 T cells significantly. Under the same conditions, there is a partial reduction of mature Tfr cells together with a slight reduction of Treg cells from which they differentiate. When miRNA biogenesis is ablated following LCMV infection, at the time of GC initiation (day 3), or when GCs are already mature (day 6), it leads to a reduction of Tfh cells, albeit with a smaller magnitude, as well as a small decrease in Tfr cells.

As a consequence, while Th1, Th2, and Th17 polarization can be studied, to a large extent, with in vitro models, Tfh differentiation has been mainly studied in vivo due to the unavailability of appropriate in vitro models. In this respect, the study of human Tfh and Tfr cells has been especially challenging given the additional limitations regarding in vivo studies with lymphoid tissue where GC reaction occurs [8,9].

The study of the role of microRNAs (miRNAs) on Tfh differentiation and maintenance is a good example of the technical difficulties described above.

In addition to the transcriptional regulation of T-cell differentiation, it was described that miRNAs can provide an additional layer of regulation at the posttranscriptional level [10]. One way to study miRNA requirement for T-cell differentiation is through the use of mice with constitutive deletion of *Dicer* or *Dgcr8*, factors that are critical for miRNA production. It was shown that miRNAs are abundantly present on naïve T cells to prevent spontaneous differentiation into effector cells where miRNAs are downregulated [11]. It was also shown that miRNAs are important for the optimal development of CD8 T cells [12] and Foxp3+ regulatory T (Treg) cells [13]. Indeed, mice deficient in *Dicer* displayed a major block in CD8 T cells differentiation, a moderate reduction of effector CD4 T cells, and a partial defect of Treg cells and Treg polarization [12,13]. However, when Tfh differentiation was assessed independently, it was found that miRNAs were essential for Tfh development [14,15]. Global elimination of miRNAs in CD4 T cells through *Dgcr8* ablation led to a very profound Tfh deficiency following immunization, that could be partially explained by the expression of a miRNA cluster (miR-17∼92) [14].

While miRNA requirement for Tfh development could be readily assessed with animals lacking miRNA production on CD4 T cells, the importance of miRNAs for Tfh maintenance remained unaddressed. These questions can be addressed with the help of mouse models that could allow discrimination between the two time-points. In this issue, Zeiträg et al. investigated this issue with mice in which *Dgcr8* ablation of CD4 T cells could be induced at the desired time through tamoxifen administration [16]. This way, it became possible to investigate the importance of miRNAs for the induction and the maintenance of Tfh cells by ablating the miRNA biogenesis before or after an infection leading to Tfh differentiation and GC reaction.

The authors found that the administration of tamoxifen before LCMV infection led to a near-complete absence of Tfh cells and

GCs (assessed using quantification of GC-B cells by flow cytometry as a surrogate indicator). When *Dgcr8* ablation was induced through tamoxifen administration on days 3 or 6 postinfection, there was still a very marked reduction of Tfh cells and GCs, albeit not as profound as when miRNAs were absent before infection (Figure 1). These results show that miRNAs are not only required for the differentiation of Tfh cells, but also for the maintenance of mature Tfh cells within ongoing GCs.

As LCMV infection is known to lead to Th1 responses [17,18], the authors continued to investigate the consequences of miRNAs ablation at different times on Th1 and Tfh1 differentiation using the chemokine receptor CXCR3 as a type-1 marker. Tamoxifen administration before infection led to the near-complete abrogation of Tfh1 development (CXCR3+CXCR5+ cells) and a very significant reduction of Th1 cells (CXCR3+CXCR5- cells) [16]. When tamoxifen was administered 3 or 6 days postinfection, the reduction of Tfh and Th1 cells was still present but not as profound. The authors found that CXCR3- Tfh cells (i.e., non-Tfh1) did not seem to be affected by the absence of miRNAs postinfection [16]. It should be noted, however, that LCMV infection strongly polarizes the response toward Tfh1, leaving only a very minor population of Tfh cells with a different phenotype. As a consequence, it is unclear whether, in a different type of response, the emerging polarized Tfh cells (Tfh2, Tfh13, or Tfh17) could be more dependent on miRNAs.

An immunization or infection not only leads to the differentiation of Tfh cells, from naïve T cells, but also to the development of Tfr cells, predominantly from Treg cells [7,19]. In both cases, the process involves an initial interaction of the T or Treg cell with a DC, followed by a T-B interaction [20–22]. It appears, however, that the requirements for Tfh and Tfr development are not exactly the same. One good example is the observation that individuals with a B-cell deficiency display a profound reduction of circulating Tfh cells, while immature Tfr cells can be found in circulation in normal numbers [23].

The results from the study by Zeiträg et al. suggest that Tfr development may be less dependent on miRNAs than Tfh development [16]. Indeed, while the absence of miRNAs induced prior to LCMV infection abrogates the emergence of Tfh cells, lack of miRNAs causes a significant but incomplete reduction of Tfr cells (**Figure 1**). The administration of tamoxifen postinfection also leads to a smaller reduction of Tfr cells than what was observed for Tfh cells.

It should be also noted that the number of Treg cells, the immediate precursors of Tfr cells, is reduced in the absence of miRNAs [13,16]. It needs to be established the extent by which the Tfr reduction in the absence of miRNAs is a consequence of a lower number of precursors. In any case, it appears that the Tfh sensitivity to miRNA levels by far exceeds the Tfr dependency on miRNAs.

The magnitude of the impact of miRNAs ablation of Tfh cells suggests it represents a level of regulation of Tfh polarization that has not received sufficient attention. To a large extent, the dissection of miRNAs' impact on Tfh and Tfr cell fate has been hampered by the lack of appropriate tools. The development of a strategy to abrogate miRNAs biogenesis at a specific time has the potential to pave the way to the investigation of miRNAs regulation of Tfh and Tfr cell fate in vivo under different physiological conditions.

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Abbreviations: **Tfh**: T follicular helper · **Tfr**: T follicular regulatory

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