Immunity to infection

Short Communication

T cell-expressed microRNAs critically regulate germinal center T follicular helper cell function and maintenance in acute viral infection in mice

Julia Zeitrag1, Frank Dahlstrom1, Yinshui Chang1, Dominik Alterauge1, Daniel Richter2, Julia Niemietz1 and Dirk Baumjohann1,3

1 Institute for Immunology, Biomedical Center, Faculty of Medicine, LMU Munich, Planegg-Martinsried, Germany
2 Anthropology and Human Genomics, Department Biology II, Faculty of Biology, LMU Munich, Planegg-Martinsried, Germany
3 Medical Clinic III for Oncology, Hematology, Immuno-Oncology and Rheumatology, University Hospital Bonn, University of Bonn, Bonn, Germany

Constitutive T cell-intrinsic miRNA expression is required for the differentiation of naïve CD4+ T cells into Tfh cells, thus making it difficult to study the role of miRNAs in the maintenance of already established Tfh cells and ongoing germinal center (GC) responses. To overcome this problem, we here used temporally controlled ablation of mature miRNAs specifically in CD4+ T cells during acute LCMV infection in mice. T cell-intrinsic miRNA expression was not only critical at early stages of Tfh cell differentiation, but also important for the maintenance of already established Tfh cells. In addition, CD4+ T cell-specific ablation of miRNAs resulted in impaired GC B cell responses. Notably, miRNA deficiency also compromised the antigen-specific CD4+ T cell compartment, Th1 cells, Treg cells, and Tfr cells. In conclusion, our results highlight miRNAs as important regulators of Tfh cells, thus providing novel insights into the molecular events that govern T cell–B cell interactions and Tfh cell identity.

Keywords: Th cells · T follicular helper cells · microRNA · germinal center · Dgcr8

See accompanying Commentary by Basto and Graca.

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

T follicular helper (Tfh) cells are the primary CD4+ T cell subset that provides help to B cells, for example during germinal center (GC) responses, and thus represent an integral part of the humoral immune response [1, 2]. Tfh cells are characterized by the expression of the master transcriptional regulator Bcl6, high surface expression of the inhibitory receptor PD-1, as well as the chemokine receptor CXCR5, which is important for the migration of Tfh cells toward B cell follicles [1, 2]. The differentiation of Tfh cells is a complex multistep process that involves continuous interaction with different antigen-presenting cells (APCs), cytokine and co-stimulatory signals, and directed migration toward different microanatomical structures [1, 2]. Importantly, a broad network of transcriptional and posttranscriptional regulators is required to ensure optimal Tfh cell differentiation [3, 4]. MicroRNAs (miRNAs) are small endogenously expressed RNAs

Correspondence: Prof. Dirk Baumjohann
e-mail: dirk.baumjohann@uni-bonn.de

© 2020 The Authors. European Journal of Immunology published by Wiley-VCH GmbH
www.eji-journal.eu

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
that function as important posttranscriptional regulators of Th cell differentiation and function, and in particular of Tfh cells [5–7]. Constitutive deletion of essential miRNA biosynthesis factors such as Dicer or Dgcr8, which results in the depletion of almost all mature miRNAs, has been successfully applied to Th cells [8–10]. Using a constitutive CD4-CreERT2/Dgcr8fl/fl mouse model, we have previously shown that global miRNA expression in T cells was absolutely required for the generation of Tfh cells [11]. This phenotype was in remarkable contrast to Th1, Th2, and Th17 cells, which still developed in the absence of mature miRNAs [8, 9].

Since previous in vivo models based on constitutive ablation of global miRNAs in T cells did not allow for studying the contribution of global miRNAs on already established Tfh cells, we here employed a novel tamoxifen-inducible mouse model for the depletion of mature miRNAs specifically in CD4+ T cells to study the role of miRNAs during an ongoing GC response. We observed that induced ablation of miRNAs during acute LCMV infection severely impaired Tfh cell phenotype and function, concomitant with reduced GC B cell responses. Therefore, T cell-expressed miRNAs are not only required for early Tfh cell differentiation, but also important for the maintenance of the Tfh cell phenotype and ongoing GC responses.

## Results and discussion

### Efficient depletion of mature miRNAs in CD4+ T cells by temporally controlled Dgcr8 ablation

As miRNA expression in T cells is required for Tfh cell formation in vivo [11], mice with a constitutive conditional deletion of Dgcr8 or Dicer are not suitable for studying Tfh cell function and maintenance during ongoing immune responses. To overcome this issue, we crossed Dgcr8+/− and Dgcr8+/− mice to a Cd4-CreERT2 knock-in mouse line [12] (Fig. 1A). This system allowed us to delete Dgcr8 and thus mature miRNAs specifically in CD4+ T cells upon administration of tamoxifen. These mice were further crossed to mice with homozygous Rosa26Δstop–YFP reporter alleles, which allowed for tracking of YFP+ cells that had undergone Cre-mediated recombination. To examine the deletion efficacy, we first applied tamoxifen without any immunization twice daily on two consecutive days and analyzed Dgcr8 mRNA expression in total CD4+ T cells from Cd4-CreERT2+/−/Dgcr8+/−/Rosa26Δstop–YFP (iDgcr8+/−) and Cd4-CreERT2+/−/Dgcr8−/−/Rosa26Δstop–YFP (iDgcr8−/−) mice (Fig. 1A). Eleven days after the initial tamoxifen administration, mice were sacrificed and CD4+ T cells from spleen and LNs were analyzed by flow cytometry and sorted according to their YFP expression. (B) Representative flow cytometry plots showing YFP reporter expression in CD4+ T cells from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). Gated on live CD4+ CD19− lymphocytes.

(C) Relative expression of Dgcr8 mRNA in sorted YFP− and YFP+ CD4+ T cells derived from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). Dgcr8 expression was assessed by qRT-PCR and normalized to the expression of B2m. Each dot represents one mouse. (D) Relative expression of mature miRNAs in YFP− and YFP+ CD4+ T cells derived from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). miRNA expression was assessed by qRT-PCR and normalized to the expression of B2m. Data shown are from one experiment representative of two independently performed experiments, each with six mice per group. Error bars represent mean ± SEM.

Figure 1. Dgcr8 and mature miRNAs are efficiently depleted in CD4+ T cells from iDgcr8+/− mice upon tamoxifen administration. (A) Experimental procedure; Cd4-CreERT2+/−/Dgcr8+/−/Rosa26Δstop–YFP and Cd4-CreERT2+/−/Dgcr8−/−/Rosa26Δstop–YFP mice were treated with tamoxifen by oral gavage twice daily on two consecutive days, which resulted in iDgcr8+/− and iDgcr8−/− mice, respectively. Eleven days after the initial tamoxifen administration, mice were sacrificed and CD4+ T cells from spleen and LNs were analyzed by flow cytometry and sorted according to their YFP expression. (B) Representative flow cytometry plots showing YFP reporter expression in CD4+ T cells from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). Gated on live CD4+ CD19− lymphocytes. (C) Relative expression of Dgcr8 mRNA in sorted YFP− and YFP+ CD4+ T cells derived from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). Dgcr8 expression was assessed by qRT-PCR and normalized to the expression of B2m. Each dot represents one mouse. (D) Relative expression of mature miRNAs in YFP− and YFP+ CD4+ T cells derived from iDgcr8+/− and iDgcr8−/− mice treated as described in (A). miRNA expression was assessed by qRT-PCR and normalized to the expression of B2m. Data shown are from one experiment representative of two independently performed experiments, each with six mice per group. Error bars represent mean ± SEM.

T cell-expressed miRNAs are needed to maintain Tfh phenotype and GC response during viral infection

To assess the impact of T cell-specific global miRNAs at different stages of Tfh cell differentiation in vivo, we induced the
deletion of Dgcr8 at different time points before and after i.p. infection with LCMV Armstrong (Fig. 2A). The deletion of Dgcr8 could also be reproducibly verified on the protein level by flow cytometry (Supporting Information Fig. S2A). Similar to what we previously observed in constitutive Cd4-Cre<sup>+</sup>B6.Dgcr8<sup>Δ/Δ</sup> mice ([11], loss of miRNAs in naive CD4<sup>+</sup> T cells induced by tamoxifen administration 2 days prior to LCMV infection resulted in an almost complete absence of CXCR5<sup>+</sup>PD-1<sup>hi</sup> Tfh cells 14 days postinfection (p.i.) (Fig. 2B, Supporting Information Fig. S2B). Since Tfh and GC B cell numbers are highly interrelated [13], the deficiency in Tfh cells also led to a defective GC response, as almost no Fas<sup>+</sup>IgD<sup>-</sup> GC B cells (Fig. 2C, Supporting Information Fig. S2B) or CD19<sup>+</sup>CD138<sup>+</sup> plasma cells (Supporting Information Fig. S3A) were observed in Dgcr8<sup>Δ/Δ</sup> mice. When we induced the deletion of mature miRNAs starting three days p.i., which corresponds to early Tfh cell differentiation, this also resulted in severe Tfh cell deficiency (Fig. 2B, Supporting Information Fig. S2B) and reduction in GC B cells and plasma cells (Fig. 2C, Supporting Information Figs. S2B and S3A), indicating that miRNAs were required for Tfh cells at these early differentiation time points. Upon deletion of Dgcr8 starting at 6 days p.i., which corresponds to the peak of the GC response and maximal expansion of the Tfh cell population, the frequencies of Tfh cells (Fig. 2B), GC B cells (Fig. 2C), and plasma cells (Supporting Information Fig. S3A) were still significantly reduced in Dgcr8<sup>Δ/Δ</sup> mice on day 14 p.i. Since LCMV Armstrong is cleared by around 1 week after infection, we believe that the observed reduction in Tfh cells following tamoxifen application starting on day 6 was mostly due to an impact on mature Tfh cells and not due to impaired differentiation of newly generated Tfh cell cohorts at this time point. This implies that T cell-expressed miRNAs were not only essential for early Tfh cell differentiation, but were also required for the maintenance of the Tfh cell phenotype and a functional GC response during acute viral infection.

**T cell miRNAs shape effector and regulatory CD4<sup>+</sup> T cell response quality during viral infection**

Acute viral infections elicit Th1 and Tfh cell responses as well as Th1-like Tfh cells with a mixed phenotype [14–16]. Therefore, we further assessed the quality of the CD4<sup>+</sup> T cell population after induced deletion of mature miRNAs during acute LCMV infection. Almost the entire CXCR5<sup>+</sup> CD4<sup>+</sup> T cell subset co-expressed the chemokine receptor CXCR3, the hallmark chemokine receptor of Th1 cells, thus representing a polarized subset with shared properties of Th1 and Tfh cells. Upon induced deletion of mature miRNAs starting 2 days before or 3 days after LCMV infection, the CXCR3<sup>+</sup>CXCR5<sup>+</sup> Th1-like Tfh cell population was almost completely absent in Dgcr8<sup>Δ/Δ</sup> mice and still severely reduced upon induced deletion of Dgcr8 on day 6 p.i. (Fig. 3A). Interestingly, while the CXCR3<sup>+</sup>CXCR5<sup>+</sup> Tfh cell population was reduced following early Dgcr8 ablation, it was not affected by Dgcr8 ablation starting on days 3 or 6. In contrast, CXCR3<sup>+</sup>CXCR5<sup>+</sup> Th1 cells were not only reduced in frequency when Dgcr8 was deleted...
Figure 3. T cell-expressed miRNAs shape the quality of the effector CD4+ T cell response during viral infection. (A) Representative flow cytometry plots showing CXCR3 and CXCR5 expression of CD4+ T cells derived from mice treated as depicted in Fig. 2A. Gated on live CD4+CD19−CD44hi lymphocytes. Quantification of CXCR3+CXCR5−, CXCR3+CXCR5+, and CXCR3−CXCR5+ cell frequencies is shown in the bar graphs. (B) Representative flow cytometry plots showing antigen-specific CD4+ T cells derived from iDgcr8+/− and iDgcr8Δ/Δ mice treated as depicted in Fig. 2A. Gated on live CD4+CD19−CD44hi lymphocytes. Quantification of tetramer-specific CD4+ T cell frequencies and numbers. Data shown are from one experiment per time point of tamoxifen treatment, each representative of one to three independently performed experiments with two to seven mice per group per experiment. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant; Mann–Whitney test.

before infection (Fig. 3A), which is in accordance with previous studies [9, 10], but also following later tamoxifen treatment time points, although with a smaller magnitude than for Th1-like Tfh cells.

We further assessed the size of the antigen-specific CD4+ T cell population by using an I-Ab GP66-77 tetramer. Upon miRNA depletion starting 2 days before or 3 days after viral infection, the antigen-specific CD4+ T cell population was largely absent in iDgcr8Δ/Δ mice, indicating that the clonal expansion of antigen-specific CD4+ T cells was severely affected by the loss of T cell-expressed miRNAs. This effect was specific to the iDgcr8Δ/Δ genotype and most likely not due to a potentially generalized tamoxifen-induced toxicity issue that has been ascribed to other Cd4-CreERT2 mouse lines [17]. Notably, not only effector T cells, but also regulatory T cells (Tregs) and T follicular regulatory (Tfr) cells were reduced in response to induced miRNA depletion (Supporting Information Fig. S3B and C). However, similarly to Th1 cells, several Treg and Tfr cells were still present after ablation of T cell-expressed miRNAs (Supporting Information Fig. S3B and C), showing that miRNAs are important, but not essential for the differentiation and maintenance of Treg subsets. Since Tfr cells appear to differentiate predominantly from Tregs [18], the less severe impact of miRNA deficiency on Tfr cells as compared to Tfh cells may be explained by a potential effect on Treg precursors, which would be indicative of a differential regulation of Tfr versus Tfh cell differentiation.
Concluding remarks

In summary, with our new system of temporally guided, CD4+ T cell-specific global miRNA ablation, we were able to answer the long-standing question of whether miRNAs are important regulators of Tfh cells beyond their earliest differentiation steps. We showed that T cell-expressed miRNAs were not only essential for the generation of Tfh cells, but also critical for the maintenance of their phenotype and function during an ongoing immune response and, thus, for an optimal GC response. In addition, we revealed that miRNAs regulate the antigen-specific CD4+ T effector cell response as well as other Th cell subsets such as Th1, Th2, and Treg cells during acute LCMV infection, further stressing the importance of these small regulatory molecules. Future studies should aim at deciphering the contributions of individual miRNAs to the maintenance and function of established Tfh cells and whether these miRNAs act alone or as part of larger miRNA-target networks.

Materials and methods

Mice

Cd4-CreERT2 (Cd4tm1(cre/ERT2)Thbu) knock-in mice [12] were crossed to Rosa26Gfp/dsRed knock-in mice (006148; The Jackson Laboratory) and Dgcr8flo/flo mice [19] to generate inducible mouse lines, with each expressing heterozygous Cd4-CreERT2 and homozygous Rosa26Gfp/dsRed alleles together with either two WT Dgcr8 alleles (referred to as iDgcr8+/+ after tamoxifen application) or two LoxP site-containing Dgcr8 alleles (iDgcr8Δ/Δ). Sex- and age-matched mice were used for experiments. All animal experiments were approved and performed in accordance with the regulations of the Regierung von Oberbayern.

LCMV infection

Mice were injected i.p. with 2 × 10^5 PFU LCMV Armstrong in 100 μL DMEM without supplements using an insulin syringe (BD Biosciences).

Tamoxifen administration

Tamoxifen treatment of mice was performed as described previously [17] at different time points as indicated in the figure legends.

Flow cytometry

Mice were sacrificed by CO2 asphyxiation and subsequent cervical dislocation. Dissected spleens were disrupted between the frosted ends of microscope slides and single-cell suspensions were filtered through a 70 μm cell strainer. Antigen-specific CD4+ T cells were detected with an 1-A6GP66–77 tetramer (NIH Tetramer Core Facility, Atlanta, GA). Tfh cells were stained and analyzed as previously described [20, 21]. Nonspecific binding was blocked with anti-CD16/CD32 (BioLegend) plus 2% normal mouse serum and 2% normal rat serum. Antibodies used were as follows: anti-CD4 (clone RM4-5), anti-CD8α (53-6.7), anti-CD19 (1D3), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-B220 (RA3-6B2), anti-IgD (11-26c), anti-PD-1 (J43 or RMP1-30) and anti-Foxp3 (FJK-16s; all from eBioscience); anti-Fas (Jo2) and anti-CD138 (281-2; from BD Biosciences); anti-CXCR3 (CXCR3-173; from BioLegend); anti-GITR (DTA-1; from BD Horizon); and anti-Dgcr8 (EPR18757, from Abcam). Biotinylated anti-CXCR5 (L138D7; BioLegend) was visualized with streptavidin–Brilliant Violet 421 (BioLegend). Intracellular Dgcr8 and Foxp3 were stained with the Foxp3 Staining Set (eBioscience). Dead cells were excluded with 7-aminoactinomycin or Fixable Viability Dye eFluor780 (both from eBioscience). Samples were acquired on an LSR II Fortessa cytometer (BD Biosciences) and were analyzed with FlowJo 10 software (Tree Star). Gating strategies are described in Supporting Information Fig. S1. Samples for qRT-PCR were sorted using an FACS Aria Fusion cell sorter (BD Biosciences).

Quantitative RT-PCR

Sorted cells were washed twice with PBS, counted, and frozen in Qiazol (Qiagen) at −80°C. Total RNA isolation was performed using the RNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions. RNA concentration was determined with a SimpliNano (GE Healthcare). For detection of Dgcr8, cDNA was generated with the SensiFAST cDNA Synthesis Kit (BioLine) according to the manufacturer’s instructions. Quantitative real-time PCR was performed on a LightCycler (Roche) using PrimeTime Gene Expression Master Mix and PrimeTime probes for Dgcr8 (Primer Sequences: 1. AGAGACAAGTG-GATTAGGAGG; 2. CATGATCCATCCATCAGGCA; Probe: 5′-FAM/AAAACAGTG/ZEN/CTCAAAAGTCGTGGCC/3′IABkFQ/′; Integrated DNA Technologies). For detection of mature miRNAs, cDNA was generated using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher). Quantitative real-time PCR was performed on a LightCycler (Roche) using TaqMan™ Fast Advanced Master Mix and TaqMan™ miRNA Probes (Assay IDs: miR-92a: 477827_mir; miR-361: 478056_mir; miR-17: 478447_mir; miR-20a: 478586_mir; all from Thermo Fisher). Data were quantified using the ΔΔCT method and normalized to expression of B2m.

Statistics

Data were analyzed with Prism 8 (GraphPad Software). The two-tailed nonparametric Mann–Whitney test was used for comparison of two unpaired groups.

Acknowledgements: This work was supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation).
— Emmy Noether Programme BA 5132/1-1 and BA 5132/1-2 (252623821) as well as SFB 1054 Project B12 (210592381); D.B. is a member of Germany’s Excellence Strategy EXC2151 (390873048). We thank Thorsten Buch for providing CD4-CreERT2 mice through Vigo Heissmeyer; Robert Bleiloch for providing Dgcr8<sup>−/−</sup> mice through K. Mark Ansel, the BMC Core Facility Flow Cytometry of the LMU Munich for providing equipment, the NIH Tetramer Core Facility for providing tetramers, and Dana Matzek and Benjamin Knust (BMC Core Facility Animal Models) for help with experiments. Open access funding enabled and organized by Projekt DEAL.

Author contributions: J.Z. designed, performed, and analyzed most of the experiments, interpreted data, and wrote the manuscript; E.D. performed and analyzed the qRT-PCR experiments. Y.C., D.A., D.R., and J.N. contributed to the performance of some of the experiments; DB conceived the project, interpreted data, wrote the manuscript, and provided overall direction of the study; all authors approved the final version of the manuscript.

Conflicts of interest: The authors have no commercial or financial conflicts of interest to declare.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202048867.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1 Crotty, S., T follicular helper cell differentiation, function, and roles in disease. *Immunity* 2014. 41: 529–542.

2 Vinuesa, C. G., Linteman, M. A., Yu, D. and MacLennan, I. C., Follicular helper T cells. *Annu. Rev. Immunol.* 2016. 34: 335–368.

3 Baumjohann, D. and Heissmeyer, V., Posttranscriptional gene regulation of T follicular helper cells by RNA-binding proteins and microRNAs. *Front. Immunol.* 2018. 9: 1794.

4 Qin, L., Waseem, T. C., Sahoo, A., Bieerkehazhi, S., Zhou, H., Galkina, E. V. and Nurieva, R., Insights into the molecular mechanisms of T follicular helper-mediated immunity and pathology. *Front. Immunol.* 2018. 9: 1884.

5 Maul, J., Alterauge, D. and Baumjohann, D., MicroRNA-mediated regulation of T follicular helper and T follicular regulatory cell identity. *Immunol. Rev.* 2019. 288: 97–111.

6 Maul, J. and Baumjohann, D., Emerging roles for MicroRNAs in T follicular helper cell differentiation. *Trends Immunol.* 2016. 37: 297–309.

7 Baumjohann, D. and Ansel, K. M., MicroRNA-mediated regulation of T helper cell differentiation and plasticity. *Nat. Rev. Immunol.* 2013. 13: 666–678.

8 Cobb, B. S., Hertweck, A., Smith, J., O’Connor, E., Graf, D., Cook, T., Smale, S. T. et al., A role for Dicer in immune regulation. *J. Exp. Med.* 2006. 203: 2519–2527.

9 Muljo, S. A., Ansel, K. M., Kanellopoulou, C., Livingston, D. M., Rao, A. and Rajewsky, K., Aberrant T cell differentiation in the absence of Dicer. *J. Exp. Med.* 2005. 202: 261–269.

10 Steiner, D. F., Thomas, M. F., Hu, J. K., Yang, Z., Babiarz, J. E., Allen, C. D., Matloubian, M. et al., MicroRNA-29 regulates T-box transcription factors and interferon-gamma production in helper T cells. *Immunity* 2011. 35: 169–181.

11 Baumjohann, D., Kageyama, R., Clingan, J. M., Morar, M. M., Patel, S., de Kouchkovsky, D., Bannard, O. et al., The microRNA cluster miR-17–approximately 92 promotes Tfh cell differentiation and represses subset-inappropriate gene expression. *Nat. Immunol.* 2013. 14: 840–848.

12 Sleedzinska, A., Hemmers, S., Mair, F., Gorka, O., Ruland, J., Fairbairn, L., Nissler, A. et al., TGF-beta signalling is required for CD4(+) T cell homeostasis but dispensable for regulatory T cell function. *PLoS Biol.* 2013. 11: e1001674.

13 Baumjohann, D., Preite, S., Reboldi, A., Ronchi, F., Ansel, K. M., Lanzavecchia, A. and Sallusto, F., Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. *Immunity* 2013. 38: 596–605.

14 Hale, J. S., Youngblood, B., Latner, D. R., Mohammed, A. U., Ye, L., Akody, R. S., Wu, T. et al., Distinct memory CD4+ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. *Immunity* 2013. 38: 805–817.

15 Liang, H., Tang, J., Liu, Z., Liu, Y., Huang, Y., Xu, Y., Hao, P. et al., ZIKV infection induces robust Th1-like Tfh cell and long-term protective antibody responses in immunocompetent mice. *Nat. Commun.* 2019. 10: 3859.

16 Weinstein, J. S., Laidlaw, B. J., Lu, Y., Wang, J. K., Schulz, V. R., Li, N., Herman, E. I. et al., STAT4 and T-bet control follicular helper T cell development in viral infections. *J. Exp. Med.* 2018. 215: 337–355.

17 Zeitrag, J., Alterauge, D., Dahlstrom, F. and Baumjohann, D., Gene dose matters: considerations for the use of inducible CD4-CreER(T2) mouse lines. *Eur. J. Immunol.* 2020. 50: 603–605.

18 Maceiras, A. R., Almeida, S. C. P., Mariotti-Ferrandiz, E., Chaara, W., Jебbawi, F., Six, A., Hori, S. et al., T follicular helper and T follicular regulatory cells have different TCR specificity. *Nat. Commun.* 2017. 8: 15067.

19 Rao, P. K., Toyama, Y., Chiang, H. R., Gupta, S., Bauer, M., Medvid, R., Reinhardt, F. et al., Loss of cardiac microRNA-mediated regulation leads to dilated cardiomyopathy and heart failure. *Circ. Res.* 2009. 105: 585–594.

20 Baumjohann, D. and Ansel, K. M., Tracking early T follicular helper cell differentiation in vivo. *Methods Mol. Biol.* 2015. 1291: 27–38.

21 Cosarizzi, A., Chang, H. D., Radbruch, A., Acs, A., Adam, D., Adam-Klages, S., Agace, W. W. et al., Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *Eur. J. Immunol.* 2019. 49: 1457–1973.

Abbreviations: p.i.: postinfection · Tfh: T follicular helper

Full correspondence: Prof. Dirk Baumjohann, Medical Clinic III for Oncology, Hematology, Immuno-Oncology and Rheumatology, University Hospital Bonn, University of Bonn, Venusberg-Campus 1, 53127 Bonn, Germany
e-mail: dirk.baumjohann@uni-bonn.de

See accompanying commentary: https://doi.org/10.1002/eji.202049086

Received: 6/7/2020
Revised: 25/8/2020
Accepted: 28/9/2020
Accepted article online: 30/9/2020