Spatiotemporal regulation of type I interferon expression determines the antiviral polarization of CD4⁺ T cells

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Differentiation of CD4⁺ T cells into either follicular helper T (Tfh) or type 1 helper T (Th1) cells influences the balance between humoral and cellular adaptive immunity, but the mechanisms whereby pathogens elicit distinct effector cells are incompletely understood. Here we analyzed the spatiotemporal dynamics of CD4⁺ T cells during infection with recombinant vesicular stomatitis virus (VSV), which induces early, potent neutralizing antibodies, or recombinant lymphocytic choriomeningitis virus (LCMV), which induces a vigorous cellular response but inefficient neutralizing antibodies, expressing the same T cell epitope. Early exposure of dendritic cells to type I interferon (IFN), which occurred during infection with VSV, induced production of the cytokine IL-6 and drove Tfh cell polarization, whereas late exposure to type I IFN, which occurred during infection with LCMV, did not induce IL-6 and allowed differentiation into Th1 cells. Thus, tight spatiotemporal regulation of type I IFN shapes antiviral CD4⁺ T cell differentiation and might instruct vaccine design strategies.

CD4⁺ T cells are key players in adaptive immune responses against pathogens. After priming in secondary lymphoid organs, antigen-specific CD4⁺ T cells undergo clonal expansion and differentiation into specialized effector T cell subsets. Viral infection may result in the generation of Tfh and Th1 cells. Tfh cells, which express the transcription factor Bcl-6, migrate into B cell follicles, where they promote the formation of high-affinity neutralizing antibodies, Tfh cells, which express the transcription factor T-bet, promote activation of macrophages and CD8⁺ T cell responses. As such, Tfh and Th1 cell subsets contribute to adaptive immune responses by specifically supporting humoral and cellular immunity, respectively. Although humoral and cellular immunity can be found in a state of 'competitive coexistence' (ref. 9), one response usually emerges as dominant after viral infection and is responsible for most of the antiviral activity. Viruses that induce direct cell damage (cytopathic viruses), such as VSV, typically induce early, potent neutralizing antibody responses, whereas non-cytopathic viruses, such as LCMV, usually elicit robust cellular responses, but weak and inefficient neutralizing antibody responses. The relative inefficiency of non-cytopathic viruses to induce neutralizing antibodies has been attributed, among other factors, to the properties of viral surface proteins, the frequency of germ line-encoded immunoglobulin variable regions, CD8⁺ T cell-induced immunopathological changes in secondary lymphoid organs and inflammatory monocyte-mediated suppression of B cells. Here we investigated whether CD4⁺ T cell polarization had a role in the dichotomous responses to VSV and LCMV and characterized the spatiotemporal dynamics of the differentiation of antiviral CD4⁺ T cells. We found that CD4⁺ T cells differentiated mostly to Tfh cells upon infection with VSV and mostly to Th1 cells upon infection with LCMV. Regardless of the differentiation outcome, priming of CD4⁺ T cells occurred in the outer paracortex and in the interfollicular areas of lymph nodes in both infections. The dichotomous T cell differentiation could not be explained by distinct cellular composition of the priming niches. Instead, spatiotemporal regulation of type I IFN expression determined whether dendritic cells (DCs) in the lymph node produced the cytokine IL-6 and, consequently, shaped antiviral CD4⁺ T cell polarization.

Results

VSV and LCMV induce distinct antiviral CD4⁺ T cell polarization. First, we compared CD4⁺ T cell polarization upon VSV or LCMV infection in C57BL/6 mice. We adoptively transferred naive VSV-specific (Tg7) or LCMV-specific (SMARTA) transgenic CD4⁺ T cells into C57BL/6 mice 24 h before subcutaneous intrafootpad infection with VSV Indiana (VSV Ind) or LCMV WE, respectively. CD4⁺ T cell polarization into Bcl-6⁺CXCR5⁺ Tfh cells and T-bet⁺CXCR5⁻ Th1 cells was analyzed at 3, 5, 7 and 14 d after infection in the footpad-draining popliteal lymph nodes (dLNs) (Extended Data Fig. 1a). On day 5 after VSV infection, >40% of Tg7 T cells had differentiated into Tfh cells, with little or no differentiation into Th1 cells (Fig. 1a); at the same time point, >80% of SMARTA T cells were Th1 cells and <15% had become Tfh cells (Fig. 1a). This pattern of CD4⁺ T cell polarization during VSV and LCMV infections was also observed when we analyzed

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the endogenous CD45<sup>+</sup>CD4<sup>+</sup> T cell response in the dLNs of mice subcutaneously infected with different viral strains (VSV Ind, VSV New Jersey, LCMV Armstrong, LCMV WE and LCMV clone 13) (Extended Data Fig. 1b,c) and occurred independently of the route of infection (intrafootpad or intravenous infection) (Extended Data Fig. 1d) and the size of the viral inoculum (10<sup>3</sup>–10<sup>7</sup> plaque-forming units (p.f.u.) or focus-forming units (ff.u.) per mouse) (Extended Data Fig. 1e).

T cell antigen receptor (TCR) signal strength influences CD4<sup>+</sup> T cell fate, with high-affinity antigen recognition associated with increased induction of T<sub>FH</sub> cells<sup>11</sup>. To test whether the distinct CD4<sup>+</sup> T cell differentiation during VSV and LCMV infections was due solely to different TCR binding affinities, we infected C57BL/6 mice subcutaneously with recombinant VSV (rVSV) and LCMV (rLCMV) expressing glycoproteins containing the LCMV gp1–40 epitope recognized by SMARTA CD4<sup>+</sup> T cells<sup>12,13</sup>. On day 5, >40% of SMARTA CD4<sup>+</sup> T cells had differentiated into T<sub>FH</sub> cells during infection with rVSV, whereas >60% of these T cells became T<sub>H</sub> cells upon infection with LCMV (Fig. 1b,c and Extended Data Fig. 1f,g), indicating that features of the viral backbone dictated the cell fate of the CD4<sup>+</sup> T cells, independently of TCR affinity.

We next tested the localization and dynamic behavior of CD4<sup>+</sup> T cells in the LN by confocal immunofluorescence histology and intravital multiphoton microscopy (IVM). We imaged the dLNs of wild-type mice receiving CD4<sup>+</sup> T cells from Tg7 or SMARTA TCR-transgenic mice crossed with Actb<sup>+</sup> transgenic mice (Extended Data Fig. 1f,g), indicating that features of the viral backbone dictated the cell fate of the CD4<sup>+</sup> T cells, independently of TCR affinity.

Characterization of the antiviral CD4<sup>+</sup> T cell priming niche. Next, we characterized the precise location in the LN where priming and differentiation of antiviral CD4<sup>+</sup> T cells took place. CD4<sup>+</sup> T cell priming is thought to happen within the first 2 d following antigen administration in the outer paracortex and interfollicular areas of LNs<sup>14–16</sup>. In line with this, 48 h after infection with rVSV or rLCMV, SMARTA CD4<sup>+</sup> T cells had upregulated the activation markers CD69 and CD25 (Extended Data Fig. 2c,d) and migrated to the outer paracortex and interfollicular areas of infected LNs (Fig. 2a,b; Extended Data Fig. 2e and Supplementary Video 2), where they started to form clusters (Fig. 2a,c; Extended Data Fig. 2f and Supplementary Video 2). We refer to these areas as CD4<sup>+</sup> T cell ‘priming niches’ hereafter. To test whether the cellular and molecular composition of the niche supporting CD4<sup>+</sup> T cell priming differed between rVSV and rLCMV infections, we used NICHE-seq, which combines photoactivation and single-cell RNA sequencing (scRNA-seq) to spatially reconstruct immune niches<sup>18</sup>. We intravenously transferred CD4<sup>+</sup> T cells from SMARTA TCR-transgenic mice crossed with Actb-cyan fluorescent protein (CFP) mice into transgenic mice ubiquitously expressing photoactivatable GFP (PA-GFP)<sup>19</sup>; we then photoactivated single-cell samples in the dLNs containing SMARTA CD4<sup>+</sup> T cell clusters (that is, the outer paracortex and interfollicular areas) on day 2 after infection with rVSV or rLCMV and performed single-cell sorting to obtain 2,406 photoconverted total GFP<sup>+</sup> endogenous cells (Fig. 2d,e). scRNA-seq indicated that B cells and natural killer (NK) cells were over-represented in the samples from rVSV-infected mice, whereas CD8<sup>+</sup> T cells and CCR2<sup>+</sup> inflammatory monocytes were enriched in the samples from LCMV-infected mice (Fig. 2c,f; cell subsets were identified on the basis of their gene expression signatures; Methods). Confocal microscopy indicated the presence of antigen-specific B cells, CX3CR1<sup>+</sup>CCR2<sup>+</sup> cells, antigen-specific CD8<sup>+</sup> T cells and NKp46<sup>+</sup> cells in the CD4<sup>+</sup> T cell priming niche (Extended Data Fig. 3a–d).

B cells are required for the later stages of differentiation into T<sub>FH</sub> cells<sup>1</sup>. To test whether a higher number of B cells in the CD4<sup>+</sup> T cell priming niche in VSV-infected mice drove differentiation into T<sub>FH</sub> cells, we used V1010<sup>+</sup>em mice, in which B cells express a transgenic B cell receptor (BCR) specific for an irrelevant antigen (VSV Ind glycoprotein)<sup>20</sup>. Adoptively transferred SMARTA CD4<sup>+</sup> T cells differentiated into T<sub>FH</sub> cells and homed to B cell follicles similarly in

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**Fig. 1** VSV and LCMV infections result in distinct antiviral CD4<sup>+</sup> T cell polarization and in vivo dynamics. **a**. Representative flow cytometry plots of transferred CD4<sup>+</sup> T cells or CD4<sup>+</sup> SMARTA CD4<sup>+</sup> T cells (1×10<sup>6</sup> cells each) in the dLNs of CD45.2<sup>+</sup> wild-type recipient mice 5 d after intrafootpad infection with VSV Ind (left) or LCMV WE (right), respectively (see Extended Data Fig. 1a for a schematic of the experimental setup). Numbers indicate percentages within the indicated gates. Plots are representative of at least five independent experiments. **b**. Representative flow cytometry plots of transfected CD45.2<sup>+</sup> SMARTA CD4<sup>+</sup> T cells (1×10<sup>6</sup> cells) in the dLNs of CD45.2<sup>+</sup> wild-type recipient mice 5 d after intrafootpad infection with rVSV (left) or rLCMV (right). Numbers indicate percentages within the indicated gates. Plots are representative of at least five independent experiments. **c**. Quantification of T<sub>b</sub> cells (left) and T<sub>T</sub> cells (right) as percentages of the transferred Tg7 (VSV Ind infection) or SMARTA (rVSV or rLCMV infection) CD4<sup>+</sup> T cells in the dLNs of CD45.2<sup>+</sup> wild-type recipients at 0, 3, 5, 7 and 14 d after infection. The mean±s.e.m. is shown. Day 0: n=3 (VSV) and 4 (rVSV and rLCMV); day 3: n=5 (VSV) and 7 (rVSV and rLCMV); day 5: n=5 (VSV), 9 (rVSV) and 10 (rLCMV); day 7: n=3 (all groups); day 14: n=3 (VSV and rVSV) and 6 (rLCMV). **d**. Representative images on the top except that the channel pertaining to polyclonal B cells was removed to improve clarity. Images were collected 3 d after infection and are representative of at least three independent experiments. Scale bars, 200 μm. **e**. Quantification of follicular antigen-specific CD4<sup>+</sup> T cells in the dLNs of mice shown in **d**. The mean±s.e.m. is shown. n=2 (PBS) and 4 (VSV Ind, rVSV and rLCMV). One-way ANOVA with Bonferroni’s post test was applied: ***P<0.001, f**. Snapshots from multiphoton intravital imaging of dLNs in wild-type mice injected with 1×10<sup>6</sup> purified GFP<sup>+</sup> Tg7 (VSV Ind) or SMARTA (all other groups) CD4<sup>+</sup> T cells (green) and 3×10<sup>5</sup> purified Deep Red-labeled polyclonal B cells (gray) 1 d before injection with PBS, VSV Ind, rVSV or rLCMV. The images on the bottom are the same as the images on the top except that the channel pertaining to polyclonal B cells was removed to improve clarity. Images were collected 3 d after infection and are representative of at least three independent experiments. Scale bars, 200 μm. **f**. Intrafootpad injection with VSV Ind (left) or LCMV WE (right), respectively (see Extended Data Fig. 1a for a schematic of the experimental setup). Numbers indicate percentages within the indicated gates. Plots are representative of at least five independent experiments. **g**. Representative flow cytometry plots of the videos in f showing Tg7 (VSV Ind) or SMARTA (all other groups) CD4<sup>+</sup> T cell xy cell positions over time (top) and meandering index versus mean track speed (bottom; Supplementary Video 1). Gates represent percentages of follicular CD4<sup>+</sup> T cell tracks (top) and CD4<sup>+</sup> T cell tracks with a high meandering index and high mean track speed (bottom). Numbers indicate the percentage of tracks within the indicated gates. Data are representative of at least two independent experiments.
wild-type and V110Yen mice after infection with rVSV (Fig. 2g and Extended Data Fig. 4a), indicating that antigen-specific B cells were not needed for the initial differentiation of CD4⁺ T cells. NK cells have been reported to curtail virus-specific CD4⁺ T cell responses, including T₁H₁ cell responses, during chronic viral infection⁵¹. However, depletion of NKp46⁺ cells, including NK cells, in Ncr1-Cre;
cDC1s were depleted by diphtheria toxin injection starting 2 d before infection. The percentage of T cells out of total transferred cells in dLNs were quantified by flow cytometry. Data are representative of at least two independent experiments. The mean ± s.e.m. is shown. One-way ANOVA with Bonferroni's post test was applied: **P < 0.01, ***P < 0.001.

Spatiotemporal regulation of type I IFN expression determines CD4⁺ T cell polarization. Next, we analyzed the transcriptome of cells in the photoactivated CD4⁺ T cell priming niche of rSVS- and rLCMV-infected mice. NICHE-seq analysis indicated an increased type I IFN signature in cells isolated from the CD4⁺ T cell priming niche of rLCMV-infected mice as compared to cells from the CD4⁺ T cell priming niche of rSVS-infected mice on day 2 after infection (Fig. 3a). Although sensing of type I IFN is known to influence CD4⁺ T cell polarization, the effect of type I IFN on the activation and differentiation of antiviral CD4⁺ T cells remains poorly defined, with conflicting reports, possibly depending on the context of infection. Kinetic analysis of Ifna2, Ifna5, Ifna6, Ifna7, Ifna9, Ifna12, Ifna13, Ifna14, Ifnb and two representative IFN-stimulated genes (ISGs), Igf15 and Oas2, in total RNA isolated from LN at 4, 8, 16, 24 and 48 h after infection with rSVS or rLCMV indicated that, although the magnitude of type I IFN induction did not significantly differ between rSVS and rLCMV infection, rSVS induced an earlier wave of type I IFN, which peaked at 8 h after infection, whereas rLCMV induced a delayed (24 h) and prolonged peak (Fig. 3b and Extended Data Fig. 6), possibly reflecting the different kinetics of viral replication in vivo. REX3 reporter mice express red fluorescent protein (RFP) and blue fluorescent protein (BFP) under the control of the promoters of the ISGs Cxcl9 and Cxcl10, respectively. We detected stronger expression of Cxcl9-RFP and Cxcl10-BFP in the outer paracortical, cortical ridge and interfollicular areas in the LNs of rSVS-infected REX3 mice as compared to rLCMV-infected mice 12 h after infection, corresponding to areas where SMARTA CD4⁺ T cells accumulated (Fig. 3c).

To test the effect of the two profiles of type I IFN induction on the differentiation of CD4⁺ T cells, we transferred SMARTA CD4⁺ T cells into wild-type mice and treated them with an antibody that blocks signaling through the type I IFN receptor (IFNAR1) 1 d before infection with rSVS or rLCMV. Whereas CD4⁺ T cells transferred in IFNAR1-blocked rLCMV-infected mice differentiated into Tfol i cells, induction of Tfol i cells in rSVS-infected mice was severely compromised, with transferred CD4⁺ T cells differentiating into Tbet⁺CXCR5⁺Tfol i cells, which were excluded from B cell follicles and induced fewer germinal center B cells and GP-specific IgG1 antibodies (Fig. 3d,e, Extended Data Fig. 7a,b and Supplementary Video 3), suggesting that type I IFN affected CD4⁺ T cell polarization at early time points after infection. Administration of the blocking antibodies against IFNAR1 on day 1 after rSVS infection did not affect the differentiation of SMARTA cells into Tfol i cells in comparison to untreated controls (Fig. 3f and Extended Data Fig. 7c), indicating that type I IFN sensing within the first 24 h after infection was essential for the induction of Tfol i cells. To assess whether an early wave of type I IFN increased the differentiation of
TFH cells in LCMV-infected mice, we injected poly(I:C), which binds TLR3 to induce type I IFN, simultaneously with rLCMV. On day 5 after infection, we observed a significant increase in TFH cell differentiation and a reduction in Th1 cells in the LNs of poly(I:C)-treated mice as compared to untreated mice (Fig. 3g). Together, these results indicate that early (<24 h) type I IFN sensing drives TFH cell differentiation.

Early type I IFN sensing by DCs and IL-6 are essential for TFH cell differentiation. Type I IFN sensing by different cell types has been reported to influence CD4+ T cell differentiation in a variety of experimental settings, with data pointing to a stimulatory or inhibitory role in the polarization toward TFH and/or Th1 cells. To investigate the role of type I IFN receptor signaling in CD4+ T cells, we adoptively transferred wild-type or Ifnar1−/− SMARTA CD4+ T cells.
Fig. 3 | Spatiotemporal regulation of type I IFN expression determines antiviral CD4^+ T cell polarization. a, Expression profile of selected ISGs (Ifit7, Cxcl9, Cxcl10, Oasl1, Ifitm3, Oas2 and Isg15) in 2,406 single cells from the photoactivated CD4^+ T cell priming niches described in Fig. 2d–f. Data were pooled from two independent experiments. Kolmogorov-Smirnov test was applied: \(*\*\* P < 0.0001\). b, Analysis of Ifna4, Ifnb, Isg15 and Oas2 gene expression in dLNs at 0, 4, 8, 16, 24 and 48 h after rVSV (blue) or rLCMV (red) infection by qPCR. \(n = 3\) (0 h), 4 (4 h), 4 (8 h), 4 (16 h), 3 (24 h, rLCMV), 4 (24 h, rVSV) and 4 (48 h). Data were pooled from two independent experiments. The mean ± s.e.m. is shown. Two-way ANOVA with LSD post test was applied: **P < 0.01. c, Confocal micrographs of dLNs in Rex3 reporter mice infected with 1 × 10^7 purified GFP^+ antigen-specific (SMARTA) CD4^+ T cells (green) and 3 × 10^7 purified Deep Red-labeled polyclonal B cells (gray), 12 h after rVSV or rLCMV infection. CXCL10^+ (blue) and CXCL9^+ (red) cells are depicted. Data are representative of at least two independent experiments. d, Representative flow cytometry plots showing T_{FH} and T_{H1} cells among antigen-specific CD4^+ T cells, 5 d after infection of CD45.2^+ wild-type recipients injected with 1 × 10^6 purified CD45.1^+ SMARTA CD4^+ T cells and treated with anti-IFNAR1 blocking antibody (or isotype control) 1 d before rVSV (blue, left) or rLCMV (red, right) infection. Numbers represent the percentage of cells within the indicated gate. e, Quantification of T_{FH} (top) and T_{H1} (bottom) cells, expressed as percentages of antigen-specific CD4^+ T cells out of total transferred cells, in dLNs of the mice described in d. The mean ± s.e.m. is shown. \(n = 6\) (rVSV) and 8 (rLCMV). Data are representative of at least two independent experiments. One-way ANOVA with Bonferroni’s post test was applied: ***P < 0.001. f, Quantification of the percentages of T_{FH} (left) and T_{H1} (right) antigen-specific CD4^+ T cells (out of total transferred cells) in dLNs 5 d after infection of CD45.2^+ wild-type recipients injected with 1 × 10^6 purified CD45.1^+ antigen-specific (SMARTA) CD4^+ T cells and treated with anti-IFNAR1 blocking antibody either 1 d before (light blue) or 1 d after (yellow) rVSV infection. Data are representative of at least two independent experiments. The mean ± s.e.m. is shown; \(n = 4\). One-way ANOVA with Bonferroni’s post test was applied: ***P < 0.001. g, Quantification of the percentages of T_{FH} (left) and T_{H1} (right) antigen-specific CD4^+ T cells (out of total transferred cells) in dLNs 5 d after infection of CD45.2^+ wild-type recipients injected with 1 × 10^6 purified CD45.1^+ antigen-specific (SMARTA) CD4^+ T cells, infected with rLCMV and treated or not with poly(I:C). Data are representative of at least two independent experiments; \(n = 7\) (PBS) and 9 (poly(I:C)). An unpaired two-tailed t test was applied: **P < 0.01.
transfer showed significantly impaired differentiation into T<sub>FH</sub> cells on day 5 after rVSV infection (Fig. 4b), indicating that type I IFN sensing by DCs is essential for antiviral T<sub>FH</sub> induction.

IL-6 is known to promote early T<sub>FH</sub> differentiation<sup>33,34</sup>, and its induction is known to be dependent on type I IFN<sup>35,36</sup>. Kinetic analysis of Il6 expression in LNs from wild-type mice infected with rVSV or rLCMV indicated that induction of Il6 mRNA had an early peak (8 h after infection) during VSV infection and a delayed peak (16 h after infection) during LCMV infection (Fig. 4c), mirroring the type I IFN signature. Ifnar<sup>1−/−</sup> mice had no upregulation of Il6 mRNA 8 h after rVSV infection (Fig. 4d), indicating that the VSV-induced early expression of Il6 requires type I IFN sensing. Blocking IL-6 before, but not 24 h after, rVSV infection significantly impaired induction of T<sub>FH</sub> cells and virus-specific antibody titers and increased induction of T<sub>TH1</sub> cells (Fig. 4e and Extended Data Fig. 9a,b), whereas it did not affect T helper cell polarization upon rLCMV infection (data not shown). These observations identify IL-6 as a critical early determinant of the differentiation of antiviral T<sub>FH</sub> cells.

To determine whether type I IFN induced expression of IL-6 in DCs, we assessed the composition and transcriptional state of DC subsets after early and late type I IFN sensing. scRNA-seq on 2,179 CD11c<sup>+</sup>MHC-II<sup>−</sup> DCs passing quality control sorted from the LNs of wild-type or Ifnar<sup>1−/−</sup> mice at 8 or 48 h after infection with rVSV or rLCMV and unbiased analysis using the MetaCell package<sup>37</sup> indicated that CD11c<sup>+</sup>MHC-II<sup>−</sup> DCs could be subsetted into migratory cDC2s, cDC1s, cDC2s, monocyte-derived DCs (moDCs) and a small subset of contaminant macrophages (Fig. 5a). Experimental conditions where type I IFN signaling was maximal (that is, 8 h after rVSV infection and 48 h after rLCMV infection of wild-type mice as compared to the same time points in Ifnar<sup>1−/−</sup> mice or 8 h after rLCMV infection) showed an enrichment of DC subsets (migratory cDC2s and moDCs) that can support T<sub>FH</sub> cell differentiation (Fig. 5b). Furthermore, ISGs induced by type I IFN were upregulated in DCs from wild-type, but not Ifnar<sup>1−/−</sup> mice, particularly in experimental conditions (8 h after rVSV and 48 h after rLCMV infection) where type I IFN signaling was maximal (Fig. 5c,d and Extended Data Fig. 10), indicating that DCs responded to type I IFN. Notably, migratory cDC2s and moDCs produced Il6 at 8 h after rVSV infection of wild-type but not Ifnar<sup>1−/−</sup> mice (Fig. 5e); by contrast, after rLCMV infection, Il6 mRNA had very low expression in migratory cDC2s and moDCs from wild-type and Ifnar<sup>1−/−</sup> mice, even at 48 h after infection when other ISGs were maximally induced (Fig. 5e). Thus, DCs produce Il6 and drive T<sub>FH</sub> cell polarization in response to early (rVSV) but not late (rLCMV) type I IFN signaling.

**Discussion**

Here we identified tight spatiotemporal regulation of the expression of type I IFN as a critical determinant of CD4<sup>+</sup> T cell fate upon viral infection. When DCs were exposed to an early (<24 h) wave of type I IFN, they made IL-6, thus promoting the differentiation of T<sub>FH</sub> cells and, consequently, enhancing humoral immunity; in contrast, when DCs were exposed to late type I interferon (>24 h), they no longer produced IL-6 and CD4<sup>+</sup> T cells differentiated into a non-T<sub>FH</sub> cell fate. These results might explain why many non-cytopathic, slow-replicating viruses fail to induce or interfere with the generation of neutralizing antibodies<sup>38</sup>.

The notion that type I IFN influences the differentiation of CD4<sup>+</sup> T cells is not without precedent<sup>39</sup>. However, the role of type I IFN in CD4<sup>+</sup> T cell polarization has remained controversial. While some
studies have shown that type I IFN promoted T\textsubscript{FH} cell differentiation, others reported that type I IFN suppressed the formation of T\textsubscript{FH} cells or induced T\textsubscript{H}1 cells\cite{footnote1}. Our results suggest that spatial and temporal regulation of type I IFN is critical for its effect on DCs, thus providing a potential explanation for the above-mentioned conflicting results.

The cellular source of type I IFN in VSV and LCMV infections is an interesting question but, unfortunately, one that is technically difficult to address because of the many isofoms of type I IFN and the lack of a sensitive, robust type I IFN reporter mouse that would allow for unambiguous identification of cells producing small amounts of these cytokines\cite{footnote2, footnote3}. VSV induces production of type I IFN in subcapsular sinus macrophages and plasmacytoid DCs\cite{footnote4}. However, the relative contribution of each LN cell type to the production of type I IFN after subcutaneous infection with LCMV or the recombinant viral strains used in this study is unknown\cite{footnote5}. Nevertheless, it is tempting to speculate that subcapsular sinus macrophages might be the critical cellular source of the early type I IFN response necessary for T\textsubscript{FH} cell differentiation. Pertinent to this, it is worth noting that viral infections induce relocalization of subcapsular sinus macrophages to inner follicular areas and toward the anatomical niche where antiviral CD4\textsuperscript{+} T cell priming takes place\cite{footnote6}.

Our data identify DCs as the necessary platform that integrates early type I IFN signaling to promote T\textsubscript{FH} cell differentiation. Mechanistically, type I IFN is necessary for induction of the T\textsubscript{FH} cell-promoting cytokine IL-6 (ref. \textsuperscript{5}). Our scRNA-seq data identified subsets of DCs (migratory cDC2s and moDCs) that produced IL6 mRNA in response to early, but not late, type I IFN. One experimental caveat is that we analyzed DCs from the entire LN, rather than from the CD4\textsuperscript{+} T cell priming niche, thus potentially underestimating the localized production of IL-6 by DCs. The relative contribution of DC-derived IL-6, as compared to IL-6 from other cellular sources, in shaping antiviral CD4\textsuperscript{+} T cell polarization remains to be determined.

We found that CD4\textsuperscript{+} T cells that differentiated into T\textsubscript{FH} cells after rVSV infection showed relatively high velocity and a high meandering index, whereas CD4\textsuperscript{+} T cells that differentiated into T\textsubscript{H}1 cells after rLCMV infection were characterized by reduced mean speed and a lower meandering index. A mechanistic explanation...
for these different motility behaviors remains elusive and might potentially include intrinsic differences in motility between T_{FH} and T_{H1} cells, differences in spatial constraints provided by the different anatomical niches they occupy (B cell follicles versus interfollicular and T cell areas), different adhesion molecule expression and different antigen levels.

Blockade of early type I IFN signaling or of IL-6 resulted in reduced generation of T_{FH} cells and, consequently, reduced antiviral antibody responses. Of note, whereas the main effect of type I IFN blockade was observed on antiviral IgG1 titers, IL-6 blockade affected mostly the levels of antiviral IgG2b. The reason for this discrepancy is unknown but might lie with additional T cell-independent roles of IL-6 that affect B cell differentiation.

Although the data presented in this study link T_{FH} cell differentiation to early type I IFN sensing by DCS, they do not fully explain the strong T_{FH} cell differentiation observed during LCMV infection. In addition to a lack of early type I IFN and IL-6, as reported here, LCMV might promote T_{FH} cell differentiation through induction of T_{FH} cell-polarizing cytokines, such as IL-12 and IFN-γ (refs. 35,44). Although IL-12 is dispensable for T_{FH} cell polarization upon LCMV infection45,46, the role of other cytokines such as IFN-γ on antiviral CD4+ T cell differentiation, their cellular source and their potential interference with IL-6 induction warrant further investigation.

In conclusion, we have characterized the cellular and molecular composition of the LN niches where CD4+ T cell differentiation occurs, delineated the spatiotemporal dynamics of the ensuing helper T cell subsets and identified tight spatiotemporal regulation of type I IFN expression as a critical regulator of antiviral CD4+ T cell polarization. Our model predicts that, if viruses induce an early wave of type I IFN (a situation that typically occurs with fast-replicating, highly cytopathic viruses that are controlled by neutralizing antibodies), this cytokine acts on migratory cDC2s and/or moDCs to induce IL-6 (and possibly other T_{FH} cell-promoting cytokines), drive T_{FH} cell differentiation and ultimately enhance humoral immunity; by contrast, if type I IFN is induced later upon viral infection (a situation that is typical of slow-replicating, non-cytopathic viruses that are controlled by the CD8+ T cell response), DCS no longer make IL-6 and helper T cell polarization is biased toward non-T_{FH} cell fates such as T_{H1} cells, which favor cellular immunity at the expense of B cell responses.

Online content
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Niche-seq was performed as previously described18. Briefly, an
and SMARTA
propagated and quantified as described12,28 and were diluted in 25
or with 1
SMARTA TCR-transgenic and KL25 BCR-transgenic cells instead of the VSV GP)
footpad injection or in 200 l of PBS before intravenous injection.

In infections and immunizations. Unless otherwise indicated, mice were infected
the Institutional Animal Committee of the San Raffaele Scientific Institute.
Mice were anesthetized with 5% isoflurane (Abbott) through a nose cone also delivering oxygen at 1 liter min

T and B cell isolation, adoptive transfer and in vivo treatments. Naive CD4
T cells from the spleens of SMARTA CD45.1
, SMARTA GFP
, SMARTA CFP
, and SMARTA Iftar1
 transgenic mice, CD8
 T cells from the spleens of LCMV-
− (ref. 51) mice were obtained

Cell isolation and flow cytometry. Single-cell suspensions of spleens and LNs were
regenerating index (the ratio of a migrating cell's linear displacement to the total path
time-lapse movies with Imaris 9.0.2 (Bitplane). The mean track speed and

Cell isolation and flow cytometry. Single-cell suspensions of spleens and LNs were

Confocal immunofluorescence histology. Confocal microscopy analysis of

Intravital multiphoton microscopy. Mice were anesthetized with 5% isoflurane (Abbott) through a nose cone also delivering oxygen at 1 liter min

Intravital microscopy. Intravital microscopy was performed by adapting a method

Niche-seq. NICHE-seq was performed as previously described6. Briefly, an

Single-cell RNA sequencing. DCs were sorted with a BD FACSaria fusion (BD Biosciences). In order to

Images were acquired on an inverted Leica microscope (SP8, Leica Microsystems) with a motorized stage for tiled imaging using an HC PL APO CS2 x20 objective (NA 0.75). To minimize fluorophore spectral spillover, we used the Leica sequential laser excitation and detection modality. B cell follicles were defined on the basis of the presence or absence of polyclonal B cell staining. For barcoding fluorescence imaging, ten xy stacks (1,024 x 1,024 pixels) sampled with 2-µm z spacing were acquired to provide image volumes that were 20 µm in depth.

Infections and immunizations. Unless otherwise indicated, mice were infected

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Intravital multiphoton microscopy. Mice were anesthetized with 5% isoflurane (Abbott) through a nose cone also delivering oxygen at 1 liter min

Methods
Mice. C57BL/6, CD45.1 (inbred C57BL/6), Actb-CFP (B6.129(CRF)-Tg(CAG-ECFP)
CK6Nagy/J), Actb-CFP (B6.C57BL/6-Tg(CAG-ECFP)Osb/J), Ccr5
2/2 (B6.129-Ccr5tm1 targeting to the CCR5 locus). Rosa26-CXCR4tm1 targeting the murine CXCR4 locus (Jax Mice 003835) and PA-GFP
PA-GFP
were purchased from Charles River or the Jackson Laboratory.

Methods
Mice. C57BL/6, CD45.1 (inbred C57BL/6), Actb-CFP (B6.129(CRF)-Tg(CAG-ECFP)
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spun down to ensure cell immobilization in the lysis solution, snap frozen on dry ice and stored at -80°C until processing.

Massively parallel single-cell RNA sequencing library preparation. Single-cell libraries were prepared as previously described. Briefly, mRNA from cells sorted into cell capture plates was barcoded, converted to cDNA and pooled with an automated pipeline. The pooled sample was then linearly amplified by T7 in vitro transcription, and the resulting RNA was fragmented and converted to a sequencing-ready library by tagging the samples with pool barcodes and illumina sequences during ligation, reverse transcription and PCR. Each pool of cells was tested for library quality, and the concentration was assessed as described.

All RNA-seq libraries (pooled at equimolar concentrations) were sequenced on the Illumina NextSeq 500 platform at a median sequencing depth of 38,323 reads per cell. Sequences were mapped to the mouse genome (mm9), demultiplexed and filtered as described, extracting a set of unique molecular identifiers that defined distinct transcripts in single cells for further processing. We estimated the level of spurious UMIs in the data with statistics on empty MARS-seq wells and excluded all plates with estimated noise of >5%. Mapping of reads was done with HISAT (v0.1.6); reads with multiple mapping positions were discarded from the analysis. Reads were associated with genes if they were mapped to an exon, were excluded. Reads were associated with genes if they were mapped to an exon, shared a genomic position on the same strand were considered to represent a single gene with a concatenated gene symbol. Cells with fewer than 500 UMIs were discarded from the analysis.

Clustering of infected lymph nodes. For clustering of scRNA-seq data from infected LNs, we used the R package MetaCell. MetaCell was used to derive Clustering of infected lymph nodes. Three clusters were discarded from the analysis. We used the R package MetaCell as previously described, as previously described. Cells from rVSV- and rLCMV-infected LNs, from all time points and genetic backgrounds, were clustered together. Meta-cells were annotated by pooled differential expression of marker genes, using the FPgene,mc metric, which signifies for each gene and meta-cell the fold change between the geometric mean of the gene within the meta-cell and the geometric mean across all meta-cells. Each gene was given an FP threshold and a priority index, such that coloring for activated T cells by GzmB was favored over coloring for general T cells by Trbc2. The selected genes, priorities and fold change threshold parameters were as follows:

| Group | Gene | Priority | Fold change |
|-------|------|----------|-------------|
| T     | Trbc2| 1        | 2           |
| T     | Cd3e | 1        | 2           |
| CD8T  | Cd8a | 2        | 2.5         |
| NK    | KlrBc| 3        | 2           |
| ActT  | Gzma | 4        | 2           |
| Unknown | Gm23935 | 3 | 2 |
| DC4T  | C4d  | 3        | 1.5         |
| DC    | Cxcl16| 5       | 2           |
| Mono  | Fcer1g| 2       | 3           |
| B     | Cd79b| 3        | 0.6         |

Clustering of dendritic cells. For clustering of scRNA-seq data from infected LNs, we used the R package MetaCell, as previously described. Cells from rVSV- and rLCMV-infected LNs, from all time points and genetic backgrounds, were clustered. Meta-cells were annotated by pooled differential expression of marker genes, as described above. The selected genes, priorities and fold change threshold parameters were as follows:

| Group | Gene  | Priority | Fold change |
|-------|-------|----------|-------------|
| Migratory cDC2 | Fscn1 | 1        | 5           |
| Migratory cDC2 | Il18b | 1        | 2           |
| cDC1 | Noaa | 1        | 3           |
| cDC1 | Cd24a| 1        | 2.5         |
| cDC2 | Cd209a| 2       | 5           |
| cDC2 | Cd209d| 2       | 1.8         |
| MoDC | Csf1r| 4        | 3           |
| MoDC | TgbB1| 4        | 2           |
| MoDC | Fcer1g| 4       | 2           |
| Macrophage | C1qB  | 5        | 4           |

qPCR. Total RNA was isolated from frozen LNs with the ReliaPrep RNA Miniprep system (Promega), following the manufacturer's instructions. One microgram of total RNA was reverse transcribed before qPCR analyses for Ifnar (Mm00393369_S1), Ifna4 (Mm0033969_S1), Ifna5 (Mm00833976_S1), Ifna6 (Mm01703458_S1), Ifna7 (Mm02525960_S1), Ifna9 (Mm00833983_S1), Ifna12 (Mm00616655_S1), Ifna13 (Mm01731013_S1), Ifna14 (Mm01703465_S1), Ifnb (Mm00439532_S1), Ifna15 (Mm01705338_S1), Ox26 (Mm00469691_M1) and Il6 (Mm0046190_M1). Ifna15, Ifnb, and Il6 were tested for library quality, and the concentration was assessed as described.

Statistical analyses. Results are expressed as the mean ± s.e.m. All statistical analyses were performed in Prism 5 (GraphPad Software). Means between two groups were compared with unpaired two-tailed t tests. Means among three or more groups were compared with one-way or two-way ANOVA. Bonferroni post test was used to correct for multiple comparisons, and in some experiments Fisher’s LSD post test was used when correction for multiple comparisons was not necessary.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data are available in the main text or the Supplementary Information. RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession GSE130089. Source data for Figs. 1–4 and Extended Data Figs. 1, 2, 5–7 and 9 are presented with the paper.

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Author contributions
M.D.G., V.C. and M.K. designed and performed experiments, analyzed data, performed the statistical analyses and drafted the manuscript. E.S., C.G.M., P.D.L., E.B., C.C., E.C., L.G. and A.F. performed experiments and analyzed data. A.G., C.M. and I.A. performed the NICHE-seq and scRNA-seq analyses and prepared the related figures. S.E. and W.K. performed the Xcr1-DTR experiments and analyzed data. M.K. provided funding, conceptual advice and supervision. M.I. designed and coordinated the study, provided funding and wrote the manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to M.K. or M.I.
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Extended Data Fig. 1 | VSV and LCMV infections result in distinct antiviral CD4+ T cell polarization and in vivo dynamic behavior, independently of viral strain, viral dose, infection route and TCR signal strength. a, Schematic representation of experimental procedure for the results described in Fig. 1a–c. 1 × 10^6 purified Ag-specific (Tg7 when VSV-Ind was used, SMARTA cells in all other cases) CD45.1+ CD4+ T cells were injected into CD45.2+ WT recipients 1 day before intrafootpad infection. dLNs were collected at the indicated time points after infection and analyzed by flow cytometry. TFH were defined as either Bcl-6+ CXCR5+ or CXCR5+ T-bet+ cells (in the latter case we always verified that cells were also Bcl-6+); Th1 were defined as T-bet+ CXCR5- cells. b, Representative flow cytometry plots showing TFH and Th1 cells (out of CD44high endogenous CD4+ T cells) in dLNs 7 days after footpad infection with the indicated virus. Numbers indicated the percentage of cells within the indicated gate. Results are representative of at least 2 independent experiments. c, Quantification of TFH (top) and Th1 cells (bottom)—expressed as percentages of CD44high endogenous CD4+ T cells—in dLNs of mice described in b. Results are representative of at least 2 independent experiments. Mean±SEM is shown. PBS n = 2, all other conditions n = 3. A one-way ANOVA test was applied; *** p value < 0.001. d, Quantification of TFH (left) and Th1 (right) cells (expressed as percentages out of total transferred cells) in the spleens of CD45.2+ WT recipients injected with 1×10^6 Ag-specific (Tg7 when VSV-Ind was used, SMARTA in all other cases) CD45.1+ T cells one day prior to intravenous infection with VSV-Ind (left), LCMV-Arm (right) or LCMV-Ci13 (not shown), respectively. Mean±SEM is shown. An unpaired two-tailed t test was applied. *** p value < 0.001. e, Quantification of TFH (left) and Th1 (right) cells (expressed as percentages out of total transferred cells) in dLNs 5 days after infection of CD45.2+ WT recipients injected with 1×10^6 Ag-specific (Tg7 for VSV-Ind, SMARTA for LCMV-WE) CD45.1+ T cells one day prior to intrafootpad infection with the indicated doses of VSV-Ind (black) or LCMV-WE (red). Results are pooled from 2 independent experiments. Mean±SEM is shown. n = 6. A one-way ANOVA test was applied. *** p value < 0.001. f, Quantification of TFH (left) and Th1 (right) cell absolute numbers at 0, 3, 5, 7 and 14 days after VSV-Ind (black), rVSV (blue) or rLCMV (red) infection. Mean±SEM is shown. Day 0 n = 3 (VSV), 4 (rVSV and rLCMV); Day 3 n = 5 (VSV), 7 (rVSV and rLCMV); Day 5 n = 5 (VSV), 9 (rVSV), 10 (rLCMV); Day 7 n = 3; Day 14 n = 3 (VSV and rVSV), 6 (rLCMV). Black and blue stars indicate significance of respectively VSV and rVSV samples towards LCMV samples. A two-way ANOVA with LSD post-test was applied. * p value < 0.05; ** p value < 0.01; *** p value < 0.001; **** p value < 0.0001.
Extended Data Fig. 2 | Spatiotemporal dynamics and activation of Ag-specific CD4+ T cells within dLN upon VSV or LCMV infection. (a, b) Track speed mean (a) and meandering index (b) of GFP+ Ag-specific (Tg7 when VSV-Ind was used, SMARTa in all other cases) CD4+ T cells in the mice described in Fig. 1d–g and Supplementary Movie 1, 3 days after PBS, VSV-Ind, rVSV or rLCMV injection. Data are pooled from 2 independent experiments. PBS, n = 395; VSV-Ind n = 11219; rVSV n = 6692; rLCMV n = 3537. One-way Anova test was applied. **** p value < 0.0001; (c, d) Mean fluorescent intensity of CD69 (c) and CD25 (d) within Ag-specific (SMARTa) CD4+ T cells in dLN, 2 days after PBS, rVSV or rLCMV injection. Data are representative of 2 independent experiments. Mean +/− SEM is shown. n = 2. One-way Anova test was applied. ** p value < 0.01; *** p value < 0.001 (e, f) Methods used to determine the normalized distance from T area centre (e) and percentages of clustered T cells / section (f). e, T cell area volume was defined based on polyclonal B cell positioning and its centre was geometrically identified in Imaris. Ag-specific CD4+ T cells were localized using Imaris built-in spot detection function and distance from T cell area centre was calculated and normalized for T cell area volumes. f, A T cell cluster was defined as a minimum of 3 T cells aggregating within closest distance of 15 μm measured from cell centroids (see Materials and Methods). Cell clusters of less than 3 cells were manually removed.
Extended Data Fig. 3 | Confocal analysis of the CD4+ T cell priming niche. Confocal imaging of murine dLNs collected 2 days after rVSV or rLCMV infection. Dashed lines represent the edges of B cell follicles and were depicted based on B220 staining. a, Ag-specific GFP+ CD4+ T cells (SMARTA, depicted in purple) and Ag-specific CFP+ B cells (KL25, depicted in cyan) were adoptively transferred into WT mice. b, Ag-specific CFP+ CD4+ T cells (SMARTA, depicted in purple) were adoptively transferred into CX3CR1-GFP x CCR2-RFP mice. A colocalization channel for GFP and RFP was used to depict inflammatory monocytes (cells positive for both CX3CR1 and CCR2, in green). c, Ag-specific CFP+ CD4+ T cells (SMARTA, depicted in purple) and Ag-specific GFP+ CD8+ T cells (P14, depicted in green) were adoptively transferred into WT mice. d, Ag-specific CFP+ CD4+ T cells (SMARTA, depicted in purple) were adoptively transferred into NKp46-ZsGreen mice. Scale bars represent 50 μm or 30 μm (zoom). The dotted square represents the zoomed area in the IFA where CD4+ T cell clusters are found. All images are representative of at least 2 independent experiments.
Extended Data Fig. 4 | Early antiviral CD4+ T cell localization is independent of Ag-specific B cells, Ag-specific CD8+ T cells and CCR2+ monocytes.

Confocal imaging of dLNs of VI10YEN (a), Cor93 Tg TCR (b), and CCR2−/− (c) mice collected either 3 (a) or 5 (b, c) days after rVSV (left) or rLCMV (right) infection. Ag-specific CD4+ T cells are depicted in green. Dashed lines represent the edges of B cell follicles and were depicted based on polyclonal B cell positioning (b, c) or B220 staining (a) (both in grey). Scale bars represent 50 μm. Results are representative of at least 2 independent experiments.
Extended Data Fig. 5 | Antiviral CD4+ T cell are primed by cDC2 cells and differentiate independently of NK cells. 

**a.** Schematic representation of the experimental procedure for the results described in panels b and c. 1×10⁶ purified CD45.1+ Ag-specific (SMARTA) CD4+ T cells were injected into Nkp46-DTR mice treated with PBS or DT as indicated. dLNs were collected 5 days after rVSV (blue) or rLCMV (red) infection. Percentages of NK cells (b), Th1 (c, left) and TFh (c, right) in dLNs were quantified by flow cytometry. Data are representative of 2 independent experiments. Mean±SEM is shown. n=3. A one-way Anova with Bonferroni’s post-test was applied. * p value < 0.05; **** p value < 0.0001 (d) Schematics of the experimental setup for the results described in panel e. 1×10⁶ purified CD45.1+ Ag-specific (SMARTA) CD4+ T cells were transferred to WT mice treated with anti-ICOS blocking antibody or with isotype control, as indicated, prior to rVSV (blue) or rLCMV (red) infection. dLNs were collected 3 days after infection. e, ICOSL expression (mean fluorescent intensity) within CD11c+ MHC-II⁺ CD8⁺ (cDC1) and CD11c+ MHC-II⁺ CD11b⁺ (cDC2) cell subsets in dLNs of the mice described in d. Data are representative of 2 independent experiments. Mean±SEM is shown. PBS conditions n=2, all other conditions n=3. A one-way Anova with Bonferroni’s post-test was applied. ** p value < 0.01; **** p value < 0.0001 f. 1×10⁶ purified CD45.1+ Ag-specific (SMARTA) CD4+ T cells were transferred to WT and DT-treated XCR1-DTR mice prior to rVSV (blue, left) or rLCMV (red, right) infection. Quantification of TFh (left) and Th1 (right)—expressed as percentages of the total transferred cells—in dLNs 5 days after infection is shown. Mean±SEM is shown. Data are representative of 2 independent experiments. n=4-6.
Extended Data Fig. 6 | Measurement of IFN-α isoforms upon rVSV and rLCMV infection. Analysis of Ifna2, Ifna5, Ifna6, Ifna7, Ifna9, Ifna12, Ifna13, Ifna14 gene expression in dLN at 0, 4, 8, 16, 24 and 48 hours after rVSV (blue) or rLCMV (red) infection by qPCR. Data are pooled from 2 independent experiments. Mean± SEM is shown. 0 hours n = 3; 4 hours n = 4; 8 hours n = 3 (rLCMV), 4 (rVSV); 16 hours n = 3 (rLCMV), 4 (rVSV); 24 hours n = 2 (rLCMV), 4 (rVSV); 48 hours n = 4. A two-way Anova with LSD post-test was applied. * p value < 0.05. The same sample was measured repeatedly for the 4 genes.
Extended Data Fig. 7 | Early type I IFN signalling promotes germinal centre B cells and antiviral antibody responses.  

**a.** Quantification of IgD⁻ CD95⁺ germinal centre (GC) B cells (left)—expressed as percentage of B220⁺ cells—and of IgG1⁺ cells (right)—expressed as percentage of B220⁺ B cells—in the dLN of mice treated with anti-IFNAR blocking antibody (or isotype control), and infected with rVSV 14 days earlier. Mean +/− SEM is shown. n = 3. An unpaired two-tailed t test was applied. *** p value < 0.001.  

**b.** GP–binding IgG1 Abs (expressed as fold induction over uninfected controls) were measured in the sera of mice described in panel a, 14 days after rVSV infection. Data are pooled from 2 independent experiments. Mean ± SEM is shown. n = 7. An unpaired two-tailed t test was applied. * p value < 0.05.  

**c.** Schematic representation of experimental procedure for the results described in Fig. 3d–f. 1 × 10⁶ purified CD45.1⁺ ag-specific (SMARTa) CD4⁺ T cells were transferred to CD45.2⁺ WT recipients and treated anti-IFNAR1 blocking antibody either 1 day prior to (light blue) or 1 day after (yellow) rVSV infection.
Extended Data Fig. 8 | Expression of interferon stimulated genes within the cellular components of the CD4+ T cell priming niche. Expression of the indicated interferon-stimulated genes (ISGs) within the cellular components of the photoactivated CD4+ T cell priming niches of the mice described in Fig. 2d–f. The colour bar on the bottom indicates each cell’s origin (blue: photoactivated cells from rVSV; red: photoactivated cells from rLCMV).
Extended Data Fig. 9 | Blocking IL-6 impairs antiviral antibody responses. a, WT mice were treated with anti-IL-6 blocking antibody (or isotype control) and sera were collected 14 days after rVSV infection. GP-binding IgG2b Abs were measured in the sera and expressed as fold induction over uninfected controls. Data are representative of 2 independent experiments. Mean ± SEM is shown. An unpaired two-tailed t test was applied. *** p value < 0.001. b, Schematic representation of experimental procedure for the results described in Fig. 4e. 1 x 10^6 purified CD45.1^+ Ag-specific (SMARTa) CD4^+ T cells were transferred to CD45.2^+ WT recipients and treated with anti-IL-6 blocking antibody starting either 1 day prior to (yellow) or 1 day after rVSV infection (orange).
Extended Data Fig. 10 | Expression of interferon stimulated genes in dendritic cells. Expression of different ISGs across 2179 single QC positive CD11c+ MHC-IIhigh cells grouped in 5 clusters as described in the legend to Fig. 5a.
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- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- BD FACSDiva Software v8.0.2 (BD Pharringen)
- Leica Application Suite X (Leica Microsystems)
- ImSpector 6.4 (LaVision BioTec)

Data analysis

- FlowJo 9.3 and FlowJo X (Treestar)
- Prism 5 (GraphPad Software)
- Imaris 9.0.2 (Bitplane)
- R package 5 MetaCell
- Python 3.5.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

- All data is available in the main text or the supplementary materials.
- RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE130009.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were chosen based on prior research conducted in our laboratories to provide sufficient numbers of mice in each group to provide informative results and perform statistical testing.

Data exclusions
In Niche-Seq experiments sequenced reads with multiple mapping positions were excluded, because their genomic origin could not be determined.

Replication
Biological replicates were used to ensure reproducibility of this study. All presented data are representative of at least 2 independent experiments with similar results. All result described in the study could be reproduced.

Randomization
Mice were matched for age and sex before randomization.

Blinding
Blinding was not performed as not relevant in this study, because experiments with human samples and subjective measurement were not involved.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☑ | ☑ |
| Antibodies | Involved in the study |
| Eukaryotic cell lines | | |
| Palaeontology | | |
| Animals and other organisms | | |
| Human research participants | | |
| Clinical data | | |

Antibodies

- Antibodies used for flow cytometry or confocal microscopy were the following: anti-Bcl 6 (clone K112-91, Pharmingen #561524, 1:60), anti-T-bet (clone 4B10, Biolegend #644805, 1:60), anti-CXCR5 (clone 2G8, Pharmingen #560615, 1:100), anti-CD4 (clone RM4-5, eBioscience #48-0042, 1:100), anti-CD45.1 (clone A20, Biolegend #110716, 1:100), anti-CD45.2 (clone K104, Pharmingen #561874, 1:100), anti-CD44 (clone IM7, Biolegend #103032, 1:100), anti-IgD (clone 11-26C.2A, Biolegend #101224, 1:100), anti-NK1.1 (clone PK136, Biolegend #8207543, 1:100), anti-TCRb (clone H57-597, Biolegend #109229, 1:100), anti-CD8 (clone K53-6.7, Biolegend #100714, 1:100), anti-CD11c (clone N418, eBioscience #25-0144-82, 1:100), anti-CD11b (clone M1/70, Pharmingen #55/960, 1:100), anti-I-Ab (MHC-II) (clone AF6-120.1, Biolegend #116416, 1:100), anti-IgG1 (clone MOPC-21, Biolegend #400119, 1:100), anti-CD95 (clone J02, Pharmingen #557653, 1:100), anti-B220 (clone RA3-6B2, Biolegend #103229, 1:100), anti-CD69 (clone H1.2F3, Biolegend #104518, 1:100) and anti-CD25 (clone PC61, Biolegend #102008, 1:100).

- Purified antibodies used in vivo were purchased from Bioxcell and included: InVivoMab anti-IFNα (clone MAR-1 5A3, Bioxcell #BE0241, 1 mg i.v. one day prior to or one day after infection), Mouse IgG1 isotype control (clone MOPC-21, Bioxcell #BE0083), Anti-I-Ab (clone MOPC-21, Bioxcell #BE0046, 0.5 mg i.v. one day prior to or one day after infection and 0.25 mg every other day), Rat IgG1 isotype control (clone HRPN, Bioxcell #BE0088), InVivoMab anti-ICOS (clone 7E.17G9, Bioxcell #BE0059; 0.5mg i.v. two and one days prior to infection), Rat IgG2b isotype control (clone LTF-2, Bioxcell #BE0092).

Validation

All antibodies were obtained from commercial vendors and we based specificity on descriptions and information provided in corresponding Data Sheets available and provided by the Manufacturers. Representative flow panels were shown in Fig. 1a, 1b, 3d, and Extended Data Fig. 1b.
Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

| CS7BL/6, CD45.1 (inbred CS7BL/6), β-actin-CFP (B6.129I(CR)-Tg(CAG-ECFP)CK6Nagyl/J), β-actin-GFP (C57BL/6-Tg(CAG-EGFP)1Osob/J), Ccr2−/− (B6.129S4-Ccr2tm1ifc/J), Rosa26-ZsGreen (B6.Cg-Gt(Rosa)26Sortm6(CAG-ZsGreen)1Hze/J) and PA-GFP mice were purchased from Charles River or from the Jackson Laboratory. LCMV-P14, SMARTA, Tg7, and Ifnar1−/− mice were obtained through the Swiss Immunological Mouse Repository (SwissMR, Zurich, Switzerland). Heavy chain knock-in and light chain BCR transgenic mice specific for VSV Indiana (V10YEN) were obtained through the European Virus Archive. BCR transgenic mice specific for LCMV WE (KL25) bred against β-actin-DsRed were described. Cor93 TCR transgenic mice (lineage BC103.1, inbred CD45.1), in which > 99% of the splenic CD8+ T cells recognize a Kb-restricted epitope located between residues 93−100 in the HBV core protein (MGLKFRQL), have been previously described. XCR1-DTR-Venus, CD11c-cre and Ifnar1−/− have been previously described. Nkp46-Cre mice were obtained from Eric Vivier. Rosa26-DTR mice were obtained from Marco Bacigaluppi. CX3CR1GFP/+ and CCR2RFP/+ mice were provided by I. Charo (University of California, San Francisco) by way of B. Engelhardt (University of Bern). Bone marrow chimeras were generated by irradiation of CS7BL/6 mice with ~900 rad and reconstitution with the indicated bone marrow; mice were allowed to reconstitute for at least 8 weeks prior to use. Mice were housed under specific pathogen-free conditions and used at 8−10 weeks of age, unless otherwise indicated. In all experiments, mice were matched for age and sex before experimental manipulation. |

Wild animals

| No wild animals were used in the study |

Field-collected samples

| No field collected samples were included in the study |

Ethics oversight

| All experimental animal procedures were approved by the Institutional Animal Committee of the San Raffaele Scientific Institute. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

| Sample preparation is described in the Materials & Methods section. |

Instrument

| BD FACS Canto or BD FACSaria III |

Software

| BD FACS DIVA for acquisition and FlowJo (Treestar) for analyses |

Cell population abundance

| Cells were always at least 98% pure |

Gating strategy

| Gating strategies is indicated in the Figure legends and the Materials & Methods section. |

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.