IFNAR signaling in fibroblastic reticular cells can modulate CD8\(^{+}\) memory fate decision

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CD8\(^{+}\) memory T cells (T\(\text{M}\)) are crucial for long-term protection from infections and cancer. Multiple cell types and cytokines are involved in the regulation of CD8\(^{+}\) T cell responses and subsequent T\(\text{M}\) formation. Besides their direct antiviral effects, type I interferons (IFN-I) modulate CD8\(^{+}\) T cell immunity via their action on several immune cell subsets. However, it is largely unclear how nonimmune cells are involved in this multicellular network modulating CD8\(^{+}\) T\(\text{M}\) formation. Fibroblastic reticular cells (FRCs) form the 3D scaffold of secondary lymphoid organs, express the IFN-I receptor (IFNAR), and modulate adaptive immune responses. However, it is unclear whether and how early IFNAR signals in lymph node (LN) FRCs affect CD8\(^{+}\) T\(\text{M}\) differentiation. Using peptide vaccination and viral infection, we studied CD8\(^{+}\) T\(\text{M}\) differentiation in mice with an FRC-specific IFNAR deletion (FRC\(^{\Delta}\)IFNAR). We show here that the differentiation of CD8\(^{+}\) TCR-transgenic T cells into central memory cells (T\(\text{CM}\)) is enhanced in peptide-vaccinated FRC\(^{\Delta}\)IFNAR mice. Conversely, vesicular stomatitis virus infection of FRC\(^{\Delta}\)IFNAR mice is associated with impaired T\(\text{CM}\) formation and the accumulation of vesicular stomatitis virus specific double-positive CD127\(^{\text{hi}}\)KLRG-1\(^{\text{hi}}\) effector memory T cells. In summary, we provide evidence for a context-dependent contribution of FRC-specific IFNAR signaling to CD8\(^{+}\) T\(\text{M}\) differentiation.

Keywords: FRCs · IFNAR · T cell memory

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

Introduction

Besides diverse subsets of immune cells, secondary lymphoid organs (SLOs) harbor nonhematopoietic stromal cells that constitute the 3D scaffold of the respective organ [1]. The induction of CD8\(^{+}\) T cell responses in SLOs relies on an intact fibroblastic reticular cell (FRC) network [2]. For the initiation of CD8\(^{+}\) T cell responses, FRC-derived chemokines, such as CCL19 and CCL21,
are required to attract sufficient naive CD8+ T cells to SLOs where priming occurs [3]. Furthermore, CCL19 and CCL21 guide CD8+ T cells to areas, where survival factors, such as interleukin-7 (IL-7), are available [4–6]. We have shown recently that FRCs are the major source of IL-7 in lymph nodes (LNs). The FRC-specific deletion of IL-7 caused the selective reduction of CD8+ central memory T cells (T_CM), whereas naive T cells were unaffected [7]. This implies an important contribution of FRCs to CD8+ T_CM differentiation and/or survival [8]. However, the up-stream signalling events involved in the FRC-mediated modulation of IL-7-dependent CD8+ memory T cell (T_M) homeostasis are still unclear.

FRCs are equipped with various cytokine receptors including those for type I interferons (IFN-I) [9–11]. IFN-I receptor (IFNAR) signaling promotes IL7 gene activity [12] and modulates effector CD8+ T cell (T_EFF) responses at multiple levels [13]. For example, it promotes the T cell priming capability of dendritic cells [14], modulates CD8+ T cell homing to SLOs [15], amplifies TCR signals [16], and protects CD8+ T cells from natural killer cell-mediated killing [17]. A recent report demonstrated that FRCs contribute to the IFNAR-dependent regulation of antiviral CD8+ T_EFF responses [18]. However, it remained unclear whether IFNAR signaling in FRCs affects CD8+ T_M formation and/or maintenance.

Here, we analyzed CD8+ T cell responses in mice lacking IFNAR signaling selectively in FRCs (FRC^ΔIFNAR). We show that the development of T_CM is enhanced in FRC^ΔIFNAR mice reconstituted with TCR-transgenic (TCR^EG) CD8+ T cells and subsequent peptide vaccination. In response to vesicular stomatitis virus (VSV) infection, however, CD127^hiKLRG-1^hi CD8+ effector memory (T_EM)-like cells accumulate in FRC^ΔIFNAR mice indicating that the type of immunogen determines the impact of FRC-specific IFNAR signals on CD8+ T_M differentiation. In summary, our data provide evidence for a context-dependent contribution of IFNAR signaling in FRCs to CD8+ T_M formation and maintenance.

Results

IFNAR signaling in FRCs determines OT-1 T_M differentiation

Prx1-Cre mice [19] allow selective gene targeting of LN FRCs [7]. To assess whether IFNAR signaling in FRCs affects T_M differentiation, Prx1-Cre mice were intercrossed with conditional IFNAR knockout (IFNAR^1/2) mice [15] to obtain FRC^ΔIFNAR mice. Prx1-Cre^+ mice harboring intact Ifnar1 alleles served as controls (FRC^WT).

In a first approach, CD8+ TCR^EG OT-1 T cells, which are specific for the ovalbumin-derived peptide SIINFEKL, were adoptively transferred to FRC^ΔIFNAR and FRC^WT mice. The following day, recipient mice were vaccinated intravenously with a mixture of SIINFEKL and LPS, which activates IFNAR signaling within 12 h [9]. The analysis of the spleen, peripheral LNs and bone marrow (BM) on days 7, 14, and 30 post vaccination (dpv) revealed that numbers of OT-I T cells were very similar in FRC^ΔIFNAR and control mice at any given time point (Fig. 1A).

Notably, on day 30 the frequencies of CD44^hiCD62L^hi OT-I T_CM were increased in the spleen of FRC^ΔIFNAR mice (Fig. 1B and C). Correspondingly, the abundance of CD44^hiCD62L^lo OT-I T_M was reduced at this time point (Fig. 1B and C). In contrast, in LNs and BM T_CM/T_EM ratios were overall similar in both mouse lines at all time points analyzed, although LN T_CM frequencies were reduced at 14 dpv in FRC^ΔIFNAR mice. On days 7 and 14, frequencies of CD44^hiCD62L^hi and CD44^hiCD62L^lo OT-I cells were indistinguishable in spleens of FRC^ΔIFNAR mice and controls, similar to PD-1 and Ki-67 levels at 30 dpv (Fig. 1E and F). Interestingly, at this timepoint, elevated frequencies of splenic CD127^hiKLRG-1^hi and IFN-γ^+TNF-α^+ OT-I T_M correlated with increased Bcl-2 expression in FRC^ΔIFNAR mice (Fig. 1D, G, and H). Thus, the lack of IFNAR signaling in FRCs favors the generation of long-lived, polyfunctional OT-I T_CM.

Early IFNAR signals in FRCs modulate chemokine levels in activated LNs

Within the first few hours after primary antigen contact, CD8+ T_EFF development is programmed [20, 21]. Importantly, transient IFNAR blockade in the early phase of antiviral immune responses improves CD8+ T_M formation [22]. To address whether FRC-intrinsic IFNAR signals in the priming phase affect CD8+ T_M formation [9], we first analyzed the frequencies and phenotypes of FRCs from OT-I-reconstituted FRC^ΔIFNAR and FRC^WT mice 24 h after peptide vaccination (+OT-I). Untreated mice served as controls (-OT-I). As shown in Fig. 2A, LN stroma composition and FRC frequencies were comparable in vaccinated FRC^ΔIFNAR and FRC^WT mice. Similar results were obtained with untreated mice. Hence, altered T_CM/T_EM ratios in FRC^ΔIFNAR mice at 30 dpv do not result from early alterations in FRC frequencies.

Besides the production of CCL19 and CCL21 in the steadystate [4, 23], LN stromal cells upregulate CXCL9 and CXCL10 in response to infection-induced IFNAR signaling [9, 24, 25]. Localization of CD8+ T_EFF within LNs and subsequent memory fate decision is regulated by the CXCL9/CXCL10-binding receptor CXCR3 [26]. However, it was not clear to which extent IFNAR signaling in FRCs contributes to the overall production of CCL19, CCL21, CXCL9, and CXCL10 in LNs. To address this, chemokine mRNA levels were determined in LNs of treated (+OT-I; 24 h post vaccination) and untreated (-OT-I; - vaccination) FRC^ΔIFNAR mice (Fig. 2B). Cxcl9 mRNA was most abundant in untreated mice and declined in treated recipient mice, irrespective of their genotype (Fig. 2B). In contrast, Cxcl10 and Cxcl11 expression increased after vaccination in OT-I-reconstituted FRC^ΔIFNAR and FRC^WT mice, but to a lesser extent in FRC^ΔIFNAR mice than in controls. Hence, vaccination-induced Cxcl21 downregulation is independent of IFNAR signaling in FRCs, whereas the upregulation of Cxcl9 and Cxcl10 is affected by IFNAR triggering. Correspondingly, Il7 expression was slightly lower in LNs of vaccinated FRC^ΔIFNAR mice (Fig. 2C).
Figure 1. IFNAR signaling in FRCs determines OT-I Tc differentiation. (A–H) Twenty-four hours after reconstitution with CD8+ Thy1.1+ OT-I T cells, Thy1.1+ FRCwt and FRCΔIFNAR mice (all Thy1.1−) were immunized with a mixture of SIINFEKL and LPS. 7, 14, and/or 30/31 days post vaccination (dpv) OT-I T cells were isolated from lymph nodes (LN), spleen (Sp), or bone marrow (BM) and analyzed by flow cytometry. (A) Numbers of CD8+ Thy1.1+ OT-I T cells in FRCΔIFNAR and FRCwt mice are shown at 7, 14, and 30/31 dpv. (B, D) Representative contour plots are shown for the (B) CD44/CD62L or (D) CD127/KLRG-1 expression profiles after gating on CD8+ Thy1.1+ OT-I T cells at 30/31 dpv in the spleen. Numbers indicate percentages of the respective population. (C) Frequencies of central memory (TCM; CD44hiCD62Lhi) and effector memory (TEM; CD44hiCD62Llo) after gating on CD8+ Thy1.1+ OT-I T cells are summarized in bar diagrams. (D) Frequencies of CD127hiKLRG-1lo after gating on CD8+ Thy1.1+ OT-I T cells are summarized in bar diagrams. (E–G) The expression of (E) PD-1, (F) Ki-67, and (G) Bcl-2 was analyzed after gating on CD8+ Thy1.1+ CD44+ OT-I T cells at 30/31 dpv. Shown are representative histograms (fluorescence minus one control in gray) and data for gMFIs are summarized in bar diagrams. (H) Splenocytes were restimulated in vitro for 4 h with 1 μM SIINFEKL. Shown are representative contour plots for the IFN-γ/TNF-α expression profile after gating on CD8+ Thy1.1+ OT-I T cells at 30/31 dpv. Numbers indicate percentages. Frequencies of IFN-γ+TNF-α+ cells after gating on CD8+ Thy1.1+ OT-I T cells at 30/31 dpv are summarized in bar diagrams. (A–H) Data are representative of 4–10 mice analyzed in two independent experiments. Bar diagrams represent mean ± SD. Statistical comparisons were made via Mann–Whitney U test and statistically significant values are indicated (*p ≤ 0.05; **p ≤ 0.01). FRCs, fibroblastic reticular cells; IFNAR, IFN-I receptor.
Figure 2. FRC functions are largely unaltered in FRC$^{\Delta IFNAR}$ mice before and during adoptive T cell transfer. (A–D) Untreated (–OT-I, –vaccination) FRC$^{\Delta IFNAR}$ and FRC$^w$ mice or FRC$^{\Delta IFNAR}$ and FRC$^w$ mice receiving OT-I and SIINFEKL/LPS were analyzed 1 dpv (+OT-I, + vaccination; as described in Fig. 1). (A) Frequencies of TER-119$^-$CD45$^-$ LSC subsets were determined in LNs by flow cytometry. Based on their differential expression of gp38 and CD31, live TER-119$^-$CD45$^-$ LN LSCs can be subdivided into gp38$^+$CD31$^-$ FRCs, gp38$^+$CD31$^+$ lymphatic endothelial cells (LECs), gp38$^-$CD31$^+$ blood endothelial cells (BECs), and gp38$^-$CD31$^-$ DNs. LNs of three mice were pooled in each of three independent experiments. Bar diagrams show mean $\pm$ SD from three data points. (B,C) Relative (B) Ccl19, Ccl21, Cxcl9, and Cxcl10 or (C) Il7 mRNA amounts were determined in LNs by RT-qPCR in relation to Hprt. Data (mean $\pm$ SD) are representative of six mice per group analyzed in (B) three or (C) one RT-qPCR experiment(s). (D) Relative fluorescence intensities for H-2kb, CD44, ICAM-1, VCAM-1, and PD-L1, analyzed by flow cytometry on viable TER-119$^-$CD45$^-$ FRCs isolated from LNs. Gray curves indicate fluorescence minus one (FMO) control lacking the H-2kb, CD44, ICAM-1, VCAM-1, or PD-L1 antibody, respectively. Histograms are representative of two independent experiments each with pooled LNs from two to three mice per group. (A–C) Statistical comparisons were made via Mann–Whitney U test and statistically significant values are indicated (*p $\leq$ 0.05). FRCs, fibroblastic reticular cells; LN, lymph node; LSCs, lymphoid stromal cells.

On the contrary, FRCs derived from untreated FRC$^{\Delta IFNAR}$ and FRC$^w$ mice expressed similar levels of the cell surface molecules H-2Kb, CD44, ICAM-1, VCAM-1, and PD-L1. Upon vaccination, FRCs similarly upregulated these molecules in FRC$^{\Delta IFNAR}$ and FRC$^w$ mice (Fig. 2D). Comparable results were obtained for lymphatic endothelial cells (LECs), blood endothelial cells (BECs), and double-negative cells (DNs) (Supporting information Fig. S1).
Figure 3. IFNAR signaling in FRCs regulates CD8\(^+\) T cell memory differentiation during vesicular stomatitis virus (VSV) infection. (A–E) FRC\(^{+/}\)IFNAR and FRC\(^{+/}\) mice were infected i.v. with 2 × 10\(^6\) plaque forming units of VSV. Mice were bled before and on days 5, 7, 9, and 30 post infection (dpi), and blood was analyzed for CD3\(^+\)CD8\(^+\) T cells (CTLs) by flow cytometry. (A, C) Representative contour plots are shown for (A) CD44/CD62L expression after gating on CD3\(^+\)CD8\(^+\) CTLs or (C) CD127/KLRG-1 expression after gating on CD3\(^+\)CD8\(^+\)CD44\(^{hi}\)CD62L\(^{lo}\) CTLs. Numbers indicate percentages. (B) Bar diagram (mean ± SD) represents frequency of CD44\(^{hi}\)CD62L\(^{lo}\) after gating on CD3\(^+\)CD8\(^+\) CTLs measured before infection (0) or 5, 7, 9, and 30 dpi. (D, E) Frequencies (mean ± SD) of (D) CD127\(^{hi}\)KLRG-1\(^{lo}\) and (E) CD127\(^{lo}\)KLRG-1\(^{hi}\) were determined after gating on CD3\(^+\)CD8\(^+\)CD44\(^{hi}\)CD62L\(^{lo}\) CTLs at 5, 7, 9, and 30 dpi. (A–E) Data are from one to two independent experiments with six to nine mice per group. Statistical comparisons were made via Mann–Whitney U test and statistically significant values are indicated (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001). FRCs, fibroblastic reticular cell; IFNAR, IFN-I receptor.

In summary, the frequencies of lymphoid stromal cells (LSCs), the expression of H-2K\(^b\), CD44, ICAM-1, VCAM-1, and PD-L1 on FRCs, and the abundance of Ccl19 and Ccl21 in LNs were very similar in untreated FRC\(^{+/}\)IFNAR and FRC\(^+/\) mice (Fig. 2A–D) arguing for a rather selective contribution of IFNAR signaling to FRC development and function during steady state. However, after vaccination, Cxcl9, and to a lesser extent Ccl10 and Il7, expression were reduced in FRC\(^{+/}\)IFNAR mice indicating early IFNAR signaling in FRCs in our experimental system [9].

IFNAR signaling in FRCs affects the composition of the antiviral CD8\(^+\) memory T cell pool

As mentioned above, early IFNAR blockade improves the formation of antiviral CD8\(^+\) T cells [22]. Whether the lack of IFNAR signaling in FRCs modulates the differentiation of polyclonal CD8\(^+\) T cells in response to VSV infection was tested next. For this purpose, we first verified that the T cell pools of naïve FRC\(^{+/}\)IFNAR and FRC\(^+/\) mice were overall similar (Supporting information Fig. S2). FRC\(^{+/}\)IFNAR and FRC\(^+/\) mice survived VSV infection equally well (Supporting information Fig. S3) arguing against major differences in B-cell development (data not shown) and anti-VSV antibody responses, which are vital for host survival [27]. However, CD8\(^+\) T\(_m\) development was altered in FRC\(^{+/}\)IFNAR mice. As depicted in Fig. 3A and B, frequencies of CD8\(^+\)CD44\(^{hi}\)CD62L\(^{lo}\) T cells in peripheral blood were comparable at days 5 and 7 post infection (dpi). In contrast, their relative abundance was elevated in FRC\(^{+/}\)IFNAR mice at 9 and 30 dpi (Fig. 3A and B).

To investigate the kinetics of T\(_m\) differentiation in more detail, we analyzed CD127 and KLRG-1 expression in CD8\(^+\)CD44\(^{hi}\)CD62L\(^{lo}\) T cells. In accordance with previous reports [28, 29], frequencies of CD127\(^{hi}\)KLRG-1\(^{lo}\) cells increased progressively in FRC\(^+/\) mice from 5 to 30 dpi. In FRC\(^{+/}\)IFNAR mice, however, elevated frequencies of CD127\(^{hi}\)KLRG-1\(^{lo}\) cells were first observed at 9 dpi. Of note, in FRC\(^{+/}\)IFNAR mice the abundance of CD127\(^{hi}\)KLRG-1\(^{lo}\) cells remained below that observed in FRC\(^+/\) mice at 7–30 dpi (Fig. 3C and D). The delayed formation of
CD127hiKLRG-1lo cells in infected FRCΔIFNAR mice was paralleled by increased frequencies of CD127hiKLRG-1hi cells throughout the entire observation period (Fig. 3C and E). Importantly, the CD127hiKLRG-1hi T cell population declined from 7 to 30 dpi in both mouse lines (Fig. 3E), suggesting a similar half-life of these T cells. Hence, elevated frequencies of CD127hiKLRG-1hi T cells in FRCΔIFNAR mice at day 30 appear to result from their more efficient generation during the early phase of the response.

To analyze the VSV-specific CD8+ T cell response in more detail, we monitored VSV nucleoprotein (NP)-specific CD8+ T cells in peripheral blood of infected FRCΔIFNAR and FRCwt mice (Fig. 4). Frequencies of VSV NP-specific CD8+ T cells were comparable in FRCΔIFNAR and FRCwt mice throughout the observation period, arguing for similar numbers of VSV NP-specific clones in the naive CD8+ T cell compartment in both mouse genotypes (Fig. 4A). At 5 dpi, most VSV NP-specific CD8+ T cells displayed a CD44hiCD62Llo phenotype, which was maintained until the memory phase, irrespective of the genotype of the mice analyzed (Fig. 4B and C).

Despite the aforementioned similarities, we observed differences with respect to KLRG-1 expression. Already at 7 dpi, the frequency of VSV NP-specific CD44hiCD62Llo CD8+ T cells expressing high levels of KLRG-1 was strongly elevated in FRCΔIFNAR mice (Fig. 4D and G). This effect was maintained at 9 dpi and was most pronounced in the memory phase at 30 dpi (Fig. 4D and G). Among the KLRG-1hi cells, a population of double-positive CD127hiKLRG-1hi CD44hiCD62Llo CD8+ T M accumulated in FRCΔIFNAR mice, which was barely detectable in FRCwt mice (Fig. 4D and F). Correspondingly, most NP-specific CD44hiCD62Llo CD8+ T M in FRCwt mice were CD127hiKLRG-1lo, while this population was significantly reduced in FRCΔIFNAR mice (Fig. 4D and E).

Thus, our data demonstrate that the absence of IFNAR signals in FRCs is associated with the accumulation of KLRG-1hi anti-VSV CD8+ T M. This process appears to be programmed in the early phase of the response, as shown by the fact that the frequency of VSV-specific CD8+ KLRG-1hi T cells was already elevated at 7 dpi and further increased until 30 dpi.

Discussion

Based on their homing patterns, CD8+ T M can be divided into different subsets. For example, CD8+ T CM and CD8+ T TM recirculate between SLOs and nonlymphoid tissues, respectively. This functional diversity enables long-lived CD8+ T M to provide systemic protection against recurrent infections, irrespective of the pathogen entry site [30].

So far, most studies focused on the cell-intrinsic pathways controlling CD8+ T M diversification and maintenance [30–32]. However, CD8+ T M are part of multicellular networks sensing pathogen-associated tissue perturbations [33]. Various immune and nonimmune cells contribute to these signaling hubs, which can be found in most if not all tissues in the body [33]. In LNs, chemokines and cytokines produced by FRCs regulate various aspects of T cell responses including CD8+ T M differentiation and survival [8]. For example, in the steady state, CCL19 and CCL21 attract naïve T cells into LNs to increase the likelihood of productive T-APC interactions in case of infection [34]. Furthermore, the IL-7-dependent survival of CD8+ T CM in LNs relies on FRCs, which contribute to CCL19/21 gradients guiding CD8+ T CM into niches rich in IL-7 [4, 6, 7]. In case of viral infections, protective CD8+ T cell responses rely on IFNAR signaling in multiple cell types [13], which must be well balanced to provide optimal protection [35]. Suboptimal IFNAR signaling in particular cell types may affect the entire network of IFNAR-dependent cellular interactions controlling CD8+ T M differentiation and/or maintenance.

Of note, IFN-γ-dependent immunomodulation already occurs before pathogen contact [36]. In the steady state, the commensal microflora promotes low-level IFN-γ production, which results in tonic IFNAR signaling. This elevates the activation thresholds of innate immune cells thereby increasing their responsiveness to subsequent infections [14, 37]. As shown in Fig. 2B and C, neither chemokine nor IL-7 production differed significantly between untreated FRCΔIFNAR and FRCΔIFNAR mice. Similarly, (1) the frequencies of FRCs (Fig. 2A), (2) the composition of the T cell repertoire, and (3) the abundance of T CM/T M were indistinguishable between untreated FRCΔIFNAR and FRCΔIFNAR mice (Supporting information Fig. S2). The same was true for FRC surface markers (Fig. 2D) indicating that the steady state regulation of the aforementioned parameters is independent of tonic IFNAR signaling in FRCs.

As a result of infection, CCL21 expression is downregulated in SLOs thereby restricting recruitment and further priming of naïve T cells [38]. This mechanism is IFN-γ dependent and remained unaffected in vaccinated FRCΔIFNAR mice (Fig. 2B). In accordance with previous reports [24, 25], we observed the early upregulation of CXCL9 and CXCL10 in activated LNs, which was less pronounced in vaccinated FRCΔIFNAR mice (Fig. 2B). Although we cannot exclude a contribution of other immune and/or nonimmune cells, IFNAR+ FRCs appear critical for chemokine induction in the early phase of an immune response [26]. In activated LNs, CXCL9/CXCL10 gradients guide primed CD8+ T cells to specialized niches where differentiation proceeds [26]. Consequently, CD8+ T EM migration and the subsequent generation of KLRG-1hi cells are impaired in CXCL10- and IFNAR-deficient mice [26]. Similar findings were obtained in lymphocytic choriomeningitis virus-infected FRCΔIFNAR mice [18] as well as in our OT-I system, though at later time points (at 30 dpi; Fig. 1D).

Besides tissue localization, the type of pathogen determines the differentiation program of CD8+ T EM [31]. For example, the generation and subsequent accumulation of KLRG-1lo CD8+ T cells in response to Listeria monocytogenes infection are strongly delayed [39]. On the contrary, high frequencies of KLRG-1lo CD8+ T cells are generated early after VSV infection and are maintained in long term [39]. CD8+ T EM expressing low levels of KLRG-1 and high levels of the IL-7 receptor α (IL-7Rα; CD127) typically give rise to long-lived T M expressing high levels of the anti-apoptotic molecule B-cell lymphoma 2 (Bcl-2) [28, 40]. However, cell-fate-mapping experiments revealed that CD8+ T EM expressing high
Figure 4. IFNAR signaling in FRCs regulates the differentiation of nucleoprotein (NP)-specific CD8\(^+\) memory T cells. (A–G) FRC\(^{ΔIFNAR}\) and FRC\(^{wt}\) mice were infected as described in Fig. 3 and blood was analyzed by flow cytometry. (A, B) Bar diagrams show (A) frequencies of total VSV NP-specific (CD3\(^+\)CD8\(^+\)) CTLs as well as (B) frequencies and total numbers of CD44\(^{hi}\)CD62L\(^{lo}\) NP-specific CTLs. (C, D) Representative contour plots are shown for (C) CD44/CD62L expression on NP-specific CTLs or (D) CD127/KLRG-1 expression after gating on CD3\(^+\)CD8\(^+\)CD44\(^{hi}\)CD62L\(^{lo}\) NP-specific CTLs at 5, 7, 9, and 30 dpi. Numbers indicate percentages. (E–G) Frequencies of (E) CD127\(^{hi}\)KLRG-1\(^{lo}\), (F) CD127\(^{hi}\)KLRG-1\(^{hi}\), and (G) CD127\(^{lo}\)KLRG-1\(^{hi}\) after gating on CD44\(^{hi}\)CD62L\(^{lo}\) NP-specific CTLs are summarized in bar diagrams. (A, B, E–G) Bar diagrams show mean ± SD from five to six mice analyzed per group in one experiment. Statistical comparisons were made via Mann–Whitney U test and statistically significant values are indicated (*\(p \leq 0.05\); **\(p \leq 0.01\)). FRCs, fibroblastic reticular cells; IFNAR, IFN-1 receptor.
levels of both KLRG-1 and CD127 are also a potent source of T\textsuperscript{M} [41]. As shown here, the formation of VSV NP-specific KLRG-1\textsuperscript{lo} CD8\textsuperscript{+} T\textsuperscript{M} was strongly impaired in FRC\textsuperscript{ΔIFNAR} mice. Instead, and as opposed to FRC\textsuperscript{wt} mice, CD127\textsuperscript{hi/lo} KLRG-1\textsuperscript{hi} CD8\textsuperscript{+} T\textsuperscript{M} accumulated in FRC\textsuperscript{ΔIFNAR} mice. Despite these phenotypic differences, the VSV NP-specific CD8\textsuperscript{+} T\textsuperscript{M} pool was similar in size in both hosts. This suggests that IFNAR signaling in FRCs does not affect the frequency but rather the phenotype of VSV NP-specific CD8\textsuperscript{+} T\textsuperscript{M} precursors and their progeny, at least with respect to KLRG-1 and CD127. However, different patterns of CD8\textsuperscript{+} T\textsuperscript{M} differentiation were observed after peptide vaccination of OT-I-reconstituted mice. This may be due to FRC-independent differences between both experimental systems. For instance, CD8\textsuperscript{+} T\textsuperscript{M} differentiation correlates with the number of naive CD8\textsuperscript{+} T cells activated upon primary antigen contact [42, 43]. The precursor frequencies generated by adoptive transfer of naive CD8\textsuperscript{+} OT-1 T cells are much higher compared with those of VSV-specific cells in the naive polyclonal CD8\textsuperscript{+} T cell repertoire. Furthermore, the amount of antigen affects T\textsubscript{CM}/T\textsubscript{EM} fate decision [44]. Given that IFNAR signaling limits viral replication in FRCs [18] and mouse embryonic fibroblasts [45], we cannot fully exclude elevated levels of viral replication and subsequent antigen accumulation in VSV-infected FRC\textsuperscript{ΔIFNAR} mice. Although this potential effect did not impair the clearance of primary VSV infections (Supporting information Fig. S3), we cannot exclude that altered dynamics of viral replication and subsequent antigen availability affected CD8\textsuperscript{+} T\textsuperscript{M} differentiation in FRC\textsuperscript{ΔIFNAR} mice. As opposed to viruses, peptide vaccines do not replicate and are rapidly removed from the system. Hence, a direct comparison of the data obtained with peptide vaccination and VSV infection is of limited value. Nevertheless, our results imply that the relative contribution of IFNAR\textsuperscript{+} FRCs to CD8\textsuperscript{+} T\textsuperscript{M} differentiation is context-dependent and thus varies between experimental systems.

Our data strongly suggest that FRC-specific IFNAR signaling modulates early FRC-CD8\textsuperscript{+} T cell interactions in LNs and helps to adapt subsequent CD8\textsuperscript{+} T\textsuperscript{M} differentiation to the inflammatory context. This is in agreement with a recent report showing that transient blockade of IFNAR signaling strongly increases CD8\textsuperscript{+} T\textsuperscript{M} formation [22]. This could be determined by a combination of pathogen-related parameters including the amount of IFN-I induced in the priming phase of the response [46]. Using bone marrow chimeras and FRC-specific IFN-β luciferase reporter mice [47], we observed the induction of IFN-I responses in radio-resistant stromal cells already 24 h after VSV infection (Supporting information Fig. S3A). Among these stromal cells, FRCs contributed to the IFN-I response in LNs (Supporting information Fig. S5B and C), which are mandatory for the priming of VSV-specific CD8\textsuperscript{+} T cells [3]. Whether the autocrine action of FRC-derived IFN-I affects the immunomodulatory function of FRCs remains to be elucidated. In FRC\textsuperscript{ΔIFNAR} mice, the Cre expression is not restricted to the LN but also active in BM stromal cells [48]. Since naive CD8\textsuperscript{+} T cells can be primed in the BM [49], which also serves a survival niche for CD8\textsuperscript{+} T\textsuperscript{M} [50, 51], we cannot formally exclude a contribution of IFNAR signaling in BM FRCs to our results. Nevertheless, our data suggest an important contribution of early IFNAR signaling in FRCs programming subsequent VSV-specific CD8\textsuperscript{+} T\textsuperscript{M} differentiation, a process that is mainly initiated in LNs [3].

In summary, we provide evidence for an early, context-dependent contribution of FRC-specific IFNAR signals to CD8\textsuperscript{+} memory fate decision. Hence, efforts aiming at the optimization of vaccination strategies should not only focus on CD8\textsuperscript{+} T cell intrinsic pathways but also consider the multicellular interactions involved in the early events of an immune response.

**Materials and methods**

**Mice and viruses**

B6.Cg-Tg(Prrx1-cre)1Cjt/J (Prrx1-Cre) [19] (stock no. 005584) mice were purchased from The Jackson Laboratory. C57BL/6J mice were purchased from Envigo. Together with IFNAR\textsuperscript{fl/fl} [15], Thy1.1\textsuperscript{+} Rag1\textsuperscript{−/−} OT-I [52], B6.Bruce4-ifnb1tm2.2Lien (short: IFN-β\textsuperscript{Δlox-lox}; reporter expressed ubiquitously) [47], and B6.Bruce4-ifnb1tm2.2Lien (short: IFN-β\textsuperscript{floxed-lox/floxed-lox}; conditional reporter mice) [47] were maintained under specific pathogen-free conditions at the central animal facility of the Medical Faculty of the Otto-von-Guericke-University Magdeburg and the TWIN-CORE, Centre for Experimental and Clinical Infection Research, Hannover. Whenever possible, control littermates were used. VSV-Indiana (Mudd-Summers isolate), originally obtained from D. Kolakofsky (University of Geneva, Geneva, Switzerland), was grown on BHK-21 cells. Virus was harvested from conditioned culture medium and titers were determined by plaque formation on Vero cells as previously described [53]. Virus was injected intravenously.

**Cell isolation**

Single-cell suspensions from peripheral LNs and spleen were obtained as previously described [7]. Organs were forced through a metal strainers in PBS/2 mM EDTA (Carl Roth) and erythrocytes were lysed in the spleen. BM was flushed out using PBS/2 mM EDTA (Carl Roth), 27G needles (B. Braun), and syringes (BD Biosciences). For erythrocyte lysis, spleen and BM cells were re-suspended in ammonium-chloride-potassium lysis buffer for 90 s followed by the addition of RPMI 1640 (Biochrom) containing 10% (v/v) FCS (PAN Biotech) and 1% (v/v) penicillin/streptomycin (P/S; Gibco). After centrifugation, all organs were re-suspended in PBS/2 mM EDTA and filtered through 40 μm cell strainers (Corning, Durham, NC).

For LSC isolation, peripheral LNs were digested as previously described [7]. In brief, fat-free LNs were cut into small (1 × 1 mm) pieces in RPMI 1640/10% FCS/1% P/S. LN fragments were vortexed and the supernatant was removed after the organ pieces had settled. This process was repeated three times. LN fragments were transferred into 12-well plates containing 1 mL digestion...
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**Flow cytometry**

The following reagents were purchased from BioLegend: Bcl-2-FITC (BCL/10C4), CD3-APF700 (17A2), CD3ε-PE/CY7 (145-2C11), CD4-V505 (RM4-5), CD4-APC/CY7 (GK1.5), CD8α-PE/CY5 (53-6.7), CD8αε-APC/CY7 (53-6.7), CD44-APC/CY7 (IM7), CD44-FITC (IM7), CD44-PE (IM7), CD45-APC/CY7 (10F.12), CD62L-FITC (MEL-14), CD90.1-PE (OX-19), CD106/A2B5 (429 MVCAM.A), CD127/IL-7Rα (A7R34), CD274/PD-L1-PE (11F.159), CD279/PD-1-PE-Cy5 (29F.1A12), gp38/Podoplanin-APF488 (8.1.1), H-2Kd/MHC-I-APF (AP6-85.85), IFN-γ-APC (XMG1.2), Ki-67-PE (16A8), KLK-1-APC (2F1/KLRG-1), KLK-1-PE/CY7 (2F1/KLRG-1), KLK-1-APC (2F1/KLRG-1), TER-119-PE/CY7 (123B8), TNF-α-PE (MP6-XT22), 7-AAD viability staining solution, and streptavidin-BV510. CD127/IL-7Rα-PE/CY5 (A7R34), CD31-PE/CY7 (390), Ki-67-PE/CY7 (SolA15), and CD279/IL-10-PE (J43) were purchased from eBioscience. CD8α-BUV395 (53-6.7), CD62L-APC (MEL-14), and the anti-mouse TCR Vβ screening panel were purchased from BD Biosciences. CD44-FITC (IM7) and CD127/IL-7Rα-PE/CY5 were purchased from Invitrogen. The extent of expanding VSV-specific nucleoprotein 52-59 (H-2Kd – RGYYVYGL)-positive T lymphocytes was assessed by pentamer immunolabeling (Proimmune). For analyses of blood lymphocytes, 25 μL blood (obtained through retro-bulbar bleeding with microhematocrit capillaries (Hirschmann)) was stained with VSV-pentamer for 10 min at 4°C. Afterward, fluorochrome-labeled antibodies were added and the samples were incubated for 15 min at 4°C. Blood cell lysis and fixation were performed using 1 mL FACS lysing solution (BD Biosciences) for 20 min at room temperature in the dark. Cells were washed with staining buffer (2% BSA in DPBS, 20 mM EDTA, 0.2% natriumzid, 1 × PBS in ddH2O).

Before staining with fluorochrome-labeled antibodies, single-cell suspensions of LNs, spleens, and BM were incubated with 50 μL of anti-mouse CD16/32 (purified from 2.4G2 ATCC HB-197) in staining buffer for 10 min at 4°C. Afterward, cells were incubated with 50 μL of fluorochrome-labeled antibodies diluted in anti-CD16/32 containing staining buffer. After incubation for 30 min at 4°C, cells were washed with 200 μL PBS/2 mM EDTA. For LSC analyses, 7-AAD was added 5 min before data acquisition. For intranuclear staining of Bcl-2, samples were processed using the FoxP3/transcription factor staining buffer set (eBioscience, Thermo Fisher Scientific) according to the manufacturer’s recommendations. For intracellular cytokine staining, cells were re-stimulated for 4 h with 1 μM SIINFEKL (Biosyntan) in the presence of brefeldin A (BioLegend) and monensin A (BioLegend), stained with surface antibodies as described above, fixed with the intracellular staining kit (BioLegend) according to the manufacturer’s instructions, and stained with anti–IFN-γ or anti-TNF-α. Samples were measured on an LSFRFortessa and LSRII flow cytometer (Becton Dickinson) and analyzed with FlowJo 10 software (FlowJo, LLC) according to the “Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition)” [54]. Individual gating strategies are depicted in Supporting information Figs. S6–S8.

**Reverse transcriptase PCR and real-time quantitative PCR**

Peripheral LNs were homogenized in CK14 0.5-mL tubes (Peqlab/VWR) containing 200 μL TRizol reagent (Invitrogen) in a Precells 24 homogenizer (Peqlab/VWR). Total RNA was extracted using chloroform (Sigma-Aldrich) according to the manufacturer’s instructions (Invitrogen). Isolated RNA was quantified by photometric Nanodrop (Thermo Fisher Scientific) and reverse-transcribed using random hexamer primers and the advantage RT-for-PCR kit (Takara Clontech) according to the manufacturer’s instructions. For real-time quantitative PCR (RT-qPCR) analyses, the Taqman gene expression assays (Thermo Fisher Scientific) were used according to the manufacturer’s instructions: Ccl19 (FAM-MGB probe Mm00016686_m1), Ccl21 (FAM-MGB probe Mm00434946_m1), Cxcl10 (FAM-MGB probe Mm00445235_m1), Il7 (FAM-MGB probe Mm01295805_m1), and Hprt (FAM-MGB probe Mm00446968_m1). Samples were analyzed in triplicates and the Ct values were exported from the ABI PRISM 7000 (Applied...
Biosystems) sequence detection system. The relative quantifications were calculated according to the \( \Delta C_T \) method and data points represent triplicate averages.

**Generation of bone marrow chimeric mice**

Mice were lethally irradiated with 9 Gy and the following day, they were i.v. reconstituted with \( 1 \times 10^7 \) BM cells in PBS of the indicated genotype. BM cells were isolated by flushing femur and tibia with RPMI 1640 medium supplemented with 10% FCS, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM Glutamax, 100 U/mL penicillin, 100 \( \mu \)g/mL streptomycin (all from Life Technologies), and 0.1 mM \( \beta \)-mercaptoethanol. Cells were then treated with RBC lysis buffer (Sigma-Aldrich) and washed with PBS. BM chimeric mice were used for experiments after at least 8 weeks of recovery.

**Detection of bioluminescence in vitro and by in vivo imaging**

One day before in vivo imaging, mice were shaved for better signal detection. Immediately after i.v. injection of 100 \( \mu \)L luciferin (30 mg/mL in PBS/20 g mouse weight), mice were anesthetized using isoflurane and analyzed in an IVIS live imaging instrument (IVIS Spectrum CT) under transient isoflurane anesthesia. The acquired images were analyzed using Living Image 4.3.1 software.

For the detection of luciferase activity in different organs in vitro, the respective organs were prepared and weighed at the indicated times after infection and stored at \(-80^\circ\)C until analysis. The samples were thawed on ice and calculated amounts of Glo Lysis buffer (Promega) in relation to the organ weight was added to the samples. Tissues were homogenized in Lysing Matrix A tubes for 60 s (4 m/s) in an organ homogenizer (MP Biomedicals). Each homogenate (20 \( \mu \)L/well) was pipetted to a 96-well plate. For luciferase measurements, 20 \( \mu \)L/well of Bright Glo Luciferin (Perkin Elmer) was added to determine bioluminescence activity using a plate reader (BioTek). Data were normalized to background values obtained before adding luciferin. All steps were prepared on ice. The luciferase activity was measured with an integration time of 10 s.

**Statistical analyses**

Statistical analyses and graphical representations were performed using Prism 8 (GraphPad Software Inc.). Statistical significances were determined using nonparametric one-tailed (Supporting information Fig. S5) or two-tailed Mann–Whitney \( U \) tests (Figs. 1–4, Supporting information Figs. S2 and S4); \( *p \leq 0.05; \; **p \leq 0.01; \; ***p \leq 0.001; \; ****p \leq 0.0001 \).

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Author contributions: TS and UK designed and supervised the study with the help of LK and JS; LK, JS, PKL, AW, and UB performed experiments and analyzed data together with IRD, UK, and TS; TS wrote the manuscript with the help of the other co-authors.

Ethics statement: Experimental procedures were approved by the relevant animal experimentation committee and performed in compliance with international and local animal welfare legislations (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, permit AZ. 33.12-42502-04-12/1025 and 13/1072 and Landesverwaltungsamt Sachsen-Anhalt, permit no. 42502-2-1288 UniMD).

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References

1. Krishnamurty, A. T. and Turley, S. J., Lymph node stromal cells: cartographers of the immune system. Nat. Immunol. 2020. 21: 369–380.

2. Scandella, E., Bolinger, B., Lattmann, E., Miller, S., Favre, S., Littman, D. R., Finke, D. et al., Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. Nat. Immunol. 2008. 9: 667–675.

3. Karrer, U., Althage, A., Odermatt, B., Roberts, C. W. M., Korsmeyer, S. J., Miyawaki, S., Hengartner, H. et al., On the key role of secondary lymphoid organs in antiviral immune responses studied in lymphoplastic (aly/aly) and spleenless (Host11/−/−) mutant mice. J. Exp. Med. 1997. 185: 2157–2170.

4. Link, A., Vogt, T. K., Favre, S., Britschgi, M. R., Acha-Orbea, H., Hinz, B., Cyster, J. G. et al., Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. Nat. Immunol. 2007. 8: 1255–1265.

5. Cui, G., Staron, M. M., Gray, S. M., Ho, P.-C., Amezquita, R. A., Wu, J. and Kaech, S. M., IL-7-Induced Glycerol Transport and TAG Synthesis Promotes Memory CD8(+) T Cell Longevity. Cell 2015. 161: 750–761.

6. Jung, Y. W., Kim, H. G., Perry, C. J. and Kaech, S. M., CCR7 expression alters memory CD8 T-cell homeostasis by regulating occupancy in IL-7- and IL-15-dependent niches. Proc. Natl. Acad. Sci. USA 2016. 113: 8278–8283.

7. Knop, L., Deiser, K., Bank, U., Witte, A., Mohr, J., Philipsen, L., Fehling, H. J. et al., IL-7 derived from lymph node fibroblastic reticular cells is
dispensable for naïve T cell homeostasis but crucial for central memory T cell survival. Eur. J. Immunol. 2020. 50: 846–857.

8 Alexandre, Y. O. and Mueller, S. N., Stromal cell networks coordinate immune response generation and maintenance. Immunol. Rev. 2018. 283: 77–85.

9 Malhotra, D., Fletcher, A. L., Astarić, J., Lukac-Kornek, V., Tayalia, P., González, S. F., Elpek, K. G. et al., Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. Nat. Immunol. 2012. 13: 499–510.

10 Gregory, J. L., Walter, A., Alexandre, Y. O., Hor, J. L., Liu, R., Ma, J. Z., Devi, S. et al., Infection Programs Sustained Lymphoid Stromal Cell Responses and Shapes Lymph Node Remodeling upon Secondary Challenge. Cell Rep. 2017. 18: 406–418.

11 Rodda, L. B., Lu, E., Bennett, M. L., Sokol, C. L., Wang, X., Luther, S. A., Barres, B. A. et al., Single-Cell RNA Sequencing of Lymph Node Stromal Cells Reveals Niche-Associated Heterogeneity. Immunology 2018. 48: 1014–1028.

12 Sawa, Y., Arima, Y., Ogura, H., Kitabayashi, C., Jiang, J.-J., Fukushima, T., Kamimura, D. et al., Hepatic interleukin-7 expression regulates T cell responses. Immunology 2009. 30: 447–457.

13 Crouse, J., Kalinke, U. and Oxenius, A., Regulation of antiviral T cell responses by type I interferons. Nat. Rev. Immunol. 2015. 15: 231–242.

14 Schaupp, L., Muth, S., Rogell, L., Kofoed-Branzuk, M., Melchior, F., Lienen-Klaus, S., Ganal-Vonarburg, S. C. et al., Microbiota-Induced Type I Interferons Instruct a Paired Basal State of Dendritic Cells. Cell 2020. 181: 1080–1096.e19.

15 Kamphuis, E., Junt, T., Waible, Z., Forster, R. and Kalinke, U., Type I interferons directly regulate lymphocyte recirculation and cause transient blood lymphopenia. Blood 2006. 108: 3253–3261.

16 Richer, M. J., Nolz, J. C. and Harty, J. T., Pathogen-specific inflammatory milieu tunes the antigen sensitivity of CD8+ T cells by enhancing T cell receptor signaling. Immunology 2013. 138: 140–152.

17 Crouse, J., Bedenikovic, G., Wiesel, M., Ibberison, M., Xenarios, I., Von Laer, D., Kalinke, U. et al., Type I interferons protect T cells against NK cell attack mediated by the activating receptor NCR1. Immunology 2014. 40: 961–973.

18 Perez-Shibayama, C., Islander, U., Lüthe, M., Cheng, H. W., Onder, L., Ring, S. S., De Martin, A. et al., Type I interferon signaling in fibroblastic reticular cells prevents exhaustive activation of antiviral CD8+ T cells. Sc. Immunol. 2020. 5.

19 Logan, M., Martin, J. F., Nagy, A., Lobe, C., Olson, E. N. and Tabin, C. J., Expression of Cre Recombinase in the developing mouse limb bud driven by a Prxl enhancer. Genesis 2002. 33: 77–80.

20 Keach, S. M. and Ahmed, R., Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells. Nat. Immunol. 2001. 2: 415–422.

21 van Stipdonk, M. J., Lemmens, E. E. and Schoenberger, S. P., Naïve CTLs require a single brief period of antigenic stimulation for clonal expansion and maturation. Nat. Immunol. 2001. 2: 423–429.

22 Palacio, N., Dangui, T., Chung, Y. R., Wang, Y., Loredano-Varela, J. L., Zhang, Z. and Penaloza-MacMaster, P., Early type I IFN blockade improves the efficacy of viral vaccines. J. Exp. Med. 2020. 217: e20191220.

23 Woolf, E., Grigorova, I., Sagiv, A., Grabovsky, V., Feigelson, S. W., Shulman, Z., Hartmann, T. et al., Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. Nat. Immunol. 2007. 8: 1076–1085.

24 Kastenmüller, W., Brandes, M., Wang, Z., Herz, J., Egen, J. G. and Germain, R. N., Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. Immunity 2013. 38: 502–513.

25 Sung, J. H., Zhang, H., Moseman, E. A., Alvarez, D., Iannacone, M., Henrickson, S. E., de la Torre, J. C. et al., Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. Cell 2012. 150: 1249–1263.

26 Duckworth, B. C., Lafouresse, F., Wimmer, V. C., Broomfield, J. B., Dalit, L., Alexandre, Y. O., Sheikh, A. A. et al., Effector and stem-like memory cell fates are imprinted in distinct lymph node niches directed by CXCR3 ligands. Nat. Immunol. 2021. 22: 434–444.

27 Bründler, M., Aichele, P., Bachmann, M., Kitamura, D., Rajewsky, K. and Zinkernagel, R. M., Immunity to viruses in B cell-deficient mice: influence of antibodies on virus persistence and on T cell memory. Eur. J. Immunol. 1996. 26: 2257–2262.

28 Kaech, S. M., Tan, J. T., Wherry, E. J., Konieczny, B. T., Surh, C. D. and Ahmed, R., Selective expression of the interleukin 7 receptor identifies effector CD8+ T cells that give rise to long-lived memory cells. Nat. Immunol. 2003. 4: 1191–1198.

29 Joshi, N. S., Cui, W., Chandele, A., Lee, H. K., Urso, D. R., Hagman, J., Gapin, L. et al., Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity 2007. 27: 281–295.

30 Jameson, S. C. and Masopust, D., Understanding Subset Diversity in T Cell Memory. Immunity 2018. 48: 214–226.

31 Chang, J. T., Wherry, E. J. and Goldrath, A. W., Molecular regulation of effector and memory T cell differentiation. Nat. Immunol. 2014. 15: 1104–1115.

32 Henning, A. N., Roychoudhuri, R. and Restifo, N. P., Epigenetic control of CD8+ T cell differentiation. Nat. Rev. Immunol. 2018. 18: 340–356.

33 Masopust, D. and Soorens, A. G., Tissue-Resident T Cells and Other Resident Leukocytes. Annu. Rev. Immunol. 2019. 37: 521–546.

34 Qi, H., Kastenmüller, W. and Germain, R. N., Spatiotemporal Basis of Innate and Adaptive Immunity in Secondary Lymphoid Tissue. Annu. Rev. Cell Dev. Biol. 2014. 30: 141–167.

35 Yao, C., Bora, S. A., Parimon, T., Zaman, T., Friedman, O. A., Palatinus, J. A., Surapaneni, N. S. et al., Cell-Type-Specific Immune Dysregulation in Severely ill COVID-19 Patients. Cell Rep. 2021. 34: 108590.

36 Gough, D. J., Messina, N. L., Clarke, C. J., Johnstone, R. W. and Levy, D. E., Constitutive type I interferon modulates homeostatic balance through tonic signaling. Immunity 2012. 36: 166–174.

37 Abt, M. C., Osborne, L. C., Monticelli, L. A., Doering, T. A., Alenghat, T., Sonnenberg, G. F., Paley, M. A. et al., Commensal bacteria calibrate the activation threshold of innate antiviral immunity. Immunity 2012. 37: 158–170.

38 Mueller, S. N., Hosiaawa-Meagher, K. A., Konieczny, B. T., Sullivan, B. M., Bachmann, M. F., Locksley, R. M., Ahmed, R. et al., Regulation of homeostatic chemokine expression and cell trafficking during immune responses. Science 2007. 317: 670–674.

39 Ober, J. J., Jellison, E. R., Sheridan, B. S., Blair, D. A., Pham, Q. M., Zickovick, J. M. and Lefrançois, L., Pathogen-induced inflammatory environment controls effector and memory CD8+ T cell differentiation. J. Immunol. 2011. 187: 4967–4978.

40 Grayson, J. M., Zajac, A. J., Altman, J. D. and Ahmed, R., Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. J. Immunol. 2000. 164: 3950–3954.

41 Herndler-Brandstetter, D., Ishigame, H., Shinnakasu, R., Plajer, V., Stecher, C., Zhao, J., Lietzmann, M. et al., KLRE1+ Effector CD8+ T Cells Lose KLRE1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity. Immunity 2018. 48: 716–729.e8.
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42 Marzo, A. L., Klonowski, K. D., Le Bon, A., Borrow, P., Tough, D. F. and Lefrançois, L., Initial T cell frequency dictates memory CD8+ T cell lineage commitment. Nat. Immunol. 2005. 6: 793–799.

43 Badovinac, V. P., Haring, J. S. and Harty, J. T., Initial T cell receptor transgenic cell precursor frequency dictates critical aspects of the CD8(+) T cell response to infection. Immunity 2007. 26: 827–841.

44 Jameson, S. C. and Masopust, D., Diversity in T cell memory: an embarrassment of riches. Immunity 2009. 31: 859–871.

45 Stirnweiss, A., Ksienzyk, A., Klages, K., Rand, U., Grashoff, M., Hauser, H. and Kröger, A., IFN regulatory factor-1 bypasses IFN-mediated antiviral effects through viperin gene induction. J. Immunol. 2010. 184: 5179–5185.

46 Thompson, L. J., Kolumam, G. A., Thomas, S. and Murali-Krishna, K., Innate inflammatory signals induced by various pathogens differentially dictate the IFN-I dependence of CD8 T cells for clonal expansion and memory formation. J. Immunol. 2006. 177: 1746–1754.

47 Lienenklaus, S., Cornitescu, M., Zietara, N., Lysztkiewicz, M., Gekara, N., Jablonska, J., Edenhofer, F. et al., Novel reporter mouse reveals constitutive and inflammatory expression of IFN-beta in vivo. J. Immunol. 2009. 183: 3229–3236.

48 Greenbaum, A., Hsu, Y.-M. S., Day, R. B., Schuettelpelz, L. G., Christopher, M. J., Borgerding, J. N., Nagasawa, T. et al., CXCL12 in early mesenchymal progenitors is required for hematopoietic stem-cell maintenance. Nature 2013. 495: 227–230.

49 Feuerer, M., Beckhove, P., Garbi, N., Mahnke, Y., Limmer, A., Hommel, M., Hämmerling, G. J. et al., Bone marrow as a priming site for T-cell responses to blood-borne antigen. Nat. Med. 2003. 9: 1151–1157.

50 Alp, Ö. S., Durianik, S., Schulz, D., McGrath, M., Grün, J. R., Bardua, M., Ikuta, K. et al., Memory CD8(+) T cells colocalize with IL-7(+) stromal cells in bone marrow and rest in terms of proliferation and transcription. Eur. J. Immunol. 2015. 45: 975–987.

51 Chang, H. D., Tokoyoda, K. and Radbruch, A., Immunological memories of the bone marrow. ImmunoL Rev. 2018. 283: 86–98.

52 Stoycheva, D., Deiser, K., Stärck, L., Nishanth, G., Schlüter, D., Uckert, W. and Schuler, T., IFN-γ regulates CD8+ memory T cell differentiation and survival in response to weak, but not strong, TCR signals. J. Immunol. 2015. 194: 553–559.

53 Spanier, J., Lienenklaus, S., Pajio, J., Kessler, A., Borst, K., Heindorf, S., Baker, D. P. et al., Concomitant TLR/RLH signaling of radioresistant and radiosensitive cells is essential for protection against vesicular stomatitis virus infection. J. Immunol. 2014. 193: 3045–3054.

54 Gossarizza, A., Chang, H. D., Radbruch, A., Abrignani, S., Addo, R., Akdis, M., André, I. et al., Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). Eur. J. Immunol. 2021. 51: 2708–3145.

Abbreviations: dpi: day post infection · dpv: day post vaccination · FRC: fibroblastic reticular cell · IFNAR: IFN-I receptor · LSC: lymphoid stromal cell · NP: nucleoprotein · SLO: secondary lymphoid organ · TCM: central memory T cell · TEFF: effector T cell · TEM: effector memory T cell · TM: memory T cell · VSV: vesicular stomatitis virus

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