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IFN-γ Regulates CD8⁺ Memory T Cell Differentiation and Survival in Response to Weak, but Not Strong, TCR Signals

Diana Stoycheva,*,† Katrin Deiser,*,† Lilian Stärck,‡ Gopala Nishanth,§,¶ Dirk Schlüter,§,¶ Wolfgang Ucket,‡,‖ and Thomas Schüler*‡

In response to primary Ag contact, naive mouse CD8⁺ T cells undergo clonal expansion and differentiate into effector T cells. After pathogen clearance, most effector T cells die, and only a small number of memory T cell precursors (T(Mp)) survive to form a pool of long-lived memory T cells (T(M)). Although high- and low-affinity CD8⁺ T cell clones are recruited into the primary response, the T(M) pool consists mainly of high-affinity clones. It remains unclear whether the more efficient expansion of high-affinity clones and/or cell-intrinsic processes exclude low-affinity T cells from the T(M) pool. In this article, we show that the lack of IFN-γ signaling in CD8⁺ T cells promotes T(M) formation in response to weak, but not strong, TCR agonists. The IFN-γ-sensitive accumulation of T(M) correlates with reduced mammalian target of rapamycin activation and the accumulation of long-lived CD62L⁺Bcl-2⁺Eomes⁺ T(Mp). Reconstitution of mammalian target of rapamycin or IFN-γ-R signaling is sufficient to block this process. Hence, our data suggest that IFN-γ-R signaling actively blocks the formation of T(Mp) responding to weak TCR agonists, thereby promoting the accumulation of high-affinity T cells finally dominating the T(M) pool. The Journal of Immunology, 2015, 194: 000-000.

Functional plasticity of individual CD8⁺ T cells is an in-dispensable prerequisite for the orchestration of adaptive immune responses (1). CD8⁺ T cells are equipped with a huge number of different cell surface molecules that function as receptors for soluble and cell-associated ligands provided by their environment. For example, the TCR-mediated interaction with self-peptide–MHC complexes and consumption of cytokotks, such as IL-7, are essential to maintain survival and function of CD8⁺ naive T cells (T(Na)) (2). However, during the course of an infection, pathogen-related peptide–MHC complexes are produced at high amounts. CD8⁺ T(Na) equipped with appropriate TCRs undergo clonal expansion, convert into CD8⁺ effector T cells (T(Ex)), and mediate pathogen clearance. Finally, most CD8⁺ T(Ex) die during the contraction phase, and only a small fraction of memory precursor T cells (T(Mp)) give rise to long-lived memory T cells (T(M)) (1). There is accumulating evidence that inflammatory cytokines, such as IL-12 and IFN-α/β, directly promote CD8⁺ T cell responses (3). This seems to result from the enhancement of proximal TCR signaling and the subsequent increase in Ag sensitivity of CD8⁺ T cells (4). The relative importance of IFN-γ for CD8⁺ T cell expansion is less clear. Although some studies demonstrated a direct, growth-promoting effect of IFN-γ for some CD8⁺ T cell clones (5), others expand independently of IFN-γ (6, 7). This suggests that IFN-γ-R signaling in CD8⁺ T cells affects their expansion in a context-dependent fashion, probably determined by clone-specific features or the nature of the pathogen.

This assumption is supported by the fact that IFN-γ controls immunodominance during the course of infection. In its absence, CD8⁺ T(M) specific for subdominant epitopes accumulate to unusually high numbers (8). The relative abundance of certain CD8⁺ T(M) clones is also determined by early TCR signals. For example, initial TCR signal strength correlates positively with the likelihood that a certain CD8⁺ T cell clone enters the T(M) pool (9). However, it remains unclear whether a functional link exists among TCR signal strength, IFN-γ-R signaling, and CD8⁺ T(M) differentiation.

In this article, we demonstrate that IFN-γ-R signaling in CD8⁺ T cells regulates the differentiation of long-lived T(M). Surprisingly, this effect is only visible after stimulation with weak, but not strong, TCR agonists. In the absence of IFN-γ-R signaling, mammalian target of rapamycin (mTOR) signaling is impaired, correlating with elevated levels of the lymph node–homing molecule CD62L, the antiapoptotic molecule B cell lymphoma protein-2 (Bcl-2), and the T(M)-associated transcription factor eomesodermin (Eomes). As a result, the differentiation of long-lived CD62L⁺Bcl-2⁺Eomes⁺ CD8⁺ T(Mp) and subsequent T(M) formation in response to weak TCR agonists are more efficient for IFN-γ-deficient CD8⁺ T cells. This advantage is overcome by the reconstitution of mTOR signaling. However, the use of specific inhibitors revealed that the IFN-γ-dependent regulation of T(M)-associated factors requires the coordinated action of JAK1, JAK2, and mTOR. In summary, we provide evidence for an unknown link between TCR and IFN-γ-R signaling in CD8⁺ T cells. According to our results, IFN-γ-R signaling, in combination with a weak TCR stimulus, suppresses the differentiation of long-lived CD8⁺ T cells.
CD8\(^+\) T\(_{Me}/T_{M}\) via mTOR-dependent and -independent signaling events. During the course of an immune response, this mechanism might facilitate the enrichment of high-affinity CD8\(^+\) T\(_{M}\) and the loss of low-affinity CD8\(^+\) T\(_{M}\), thereby ensuring both efficient secondary immune responses and the prevention of autoimmunity by low-affinity, self-reactive CD8\(^+\) T\(_{M}\).

**Materials and Methods**

**Mice**

C57BL/6J (B6), B6.SL-Pten\(^{-}\) Pepc\(^{b}/\) B6 (CD45.1-congenic), IFN-\(\gamma\) reporter (B6.129S4-Ifngr\(^{tm}1Agt\)) (CD90.1), and IFN-\(\gamma\)\(^{-}\) (B6.129S7-Ifg\(^{tm}1Ts\)) mice were bred in our animal facility. Bone marrow stem cells were isolated from Rag\(^{-}\) mice and purified with an EasySep Mouse SCA1 Positive Selection Kit (STEMCELL TECHNOLOGIES), according to the manufacturer’s instructions. Mice were crossed to generate CD90.1/-.2-disparate Rag\(^{-}\) mice. Rag\(^{-}\)/- mice were used housed under pathogen-free conditions, and animal experiments were performed according to institutional guidelines (Landesamt für Gesundheit und Soziales Berlin and Landesverwaltungsamt Sachsen-Anhalt).

**Adaptive T cell transfer**

CD8\(^+\) T\(_{M}\) were purified from spleen and lymph nodes of the respective donor mice using CD8a-specific Microbeads and AutoMACS (Miltenyi Biotec). A total of 5 \(\times\) 10\(^{3}\) – 1 \(\times\) 10\(^4\) CD8\(^+\) T\(_{M}\) cells (purity > 97\%) were injected i.v. into the tail vein of recipient mice. For some experiments, mice were immunized i.v. with 50 \(\mu\)g SIINFEKL (N4), SIIVFEKL (V4), SIITFEKL (T4), SIQFEKL (Q4), and SAINFEKL (A2) (Biosynth) 1 d after T cell transfer. Control animals were injected with Dulbecco’s PBS. Rapamycin was injected i.p. at 1 \(\mu\)g/mouse daily.

**Cell culture**

Spleen and lymph node cells of the respective OT-I mice were pooled and cultured at 5 \(\times\) 10\(^3\) – 10\(^4\) T\(_{M}\) cells/ml in round-bottom 96-well plates in RPMI 1640 medium supplemented with 10% FCS, 1% penicillin/streptomycin, 2-ME (50 \(\mu\)M), nonessential amino acids, sodium pyruvate, and glutamate at 37\(^{\circ}\)C with 5% CO\(_2\) for the indicated period. Where indicated, 1 \(\mu\)g of the respective altered peptide ligand (APL), 100 ng/ml rmIFN-\(\gamma\) (R&D Systems/BioLegend), 5 \(\mu\)g/ml purified anti-IFN-\(\gamma\) (from R4-6A4 hybridoma supernatant), 20 ng/ml rapamycin (Sigma-Aldrich), or 1 \(\mu\)M ruxolitinib (JAK2 inhibitor; Selleck Chemicals) was added. Supernatants of in vitro–cultured cells were collected and analyzed by IFN-\(\gamma\)-specific ELISA (eBioscience), according to the manufacturer’s instructions.

**Flow cytometry**

The following Abs were used: anti-CD8a (53-6-8; BioLegend/eBioscience), anti-CD90.1 (OX-7; BioLegend/BD), anti-CD90.2 (53-2-1; BioLegend/BD), anti-CD62L (MEL-14; eBioscience/BD), anti-Ki67 (SolA15; eBioscience), anti-Bcl-2 (10C4; BioLegend), anti–T-bet (4B10; BioLegend/eBioscience), anti-Eomes (Dan11Mag; eBioscience), anti–IFN-\(\gamma\) (XMG1.2; BioLegend/eBioscience), anti-CD107a (1D4B; BD), and anti–p-mTORC1 (Ser248) (Cell Signaling). For intracellular cytokine staining, cells were restimulated for 3–4 h with 1 \(\mu\)M SIINFEKL in the presence of brefeldin A, monensin A, and anti-CD107a, fixed with the intracellular staining kit (BioLegend) according to the manufacturer’s instructions, and stained with anti–IFN-\(\gamma\). For staining of nuclear Ags, cells were fixed using the Foxp3 Fixation Kit (eBioscience), according to the manufacturer’s instructions. Samples were measured on a FACSCalibur, FACSCanto, or LSR II flow cytometer and analyzed by FlowJo software.

**Statistical analysis**

Statistical analysis and graphical representations were done using Prism5 software (GraphPad Software). Statistical significance was determined using a nonparametric two-tailed Mann–Whitney or paired Student t test.

**Results**

The lack of IFN-\(\gamma\)\(^{-}\) signaling promotes the accumulation of memory CD8\(^+\) T cells in response to weak, but not strong, TCR signals.

Self-PEptide–MHC complexes are weak TCR agonists that usually do not activate mature peripheral CD8\(^+\) T cells in lymphocyte-competent mice. However, under lymphopenic conditions, self-PEptide–MHC complexes, together with IL-7, promote CD8\(^+\) T\(_{M}\) activation, lymphopenia-induced proliferation (LIP), and subsequent differentiation of T\(_{M}\) (12–15).

Previously, we studied the impact of IFN-\(\gamma\) on CD8\(^+\) T\(_{M}\) generation and differentiation (7, 16). We showed that IFN-\(\gamma\)-deficient (IFN-\(\gamma\)^{-/-}) and wild-type (WT) CD8\(^+\) TCR-tg OT-I cells responding to their cognate peptide SIINFEKL did not differ with regard to CD262 expression, expansion, or T\(_{M}\) formation (7). In contrast, the frequency of CD62L\(^{hi}\) IFN-\(\gamma\)^{-/-} OT-I T\(_{M}\) cells was strongly increased when both OT-I T cells populations underwent LIP in Rag-deficient (Rag^{-/-}) mice (7). Because the self-peptides driving LIP of OT-I T cells are weak TCR agonists (13), we hypothesized that the lack of IFN-\(\gamma\) signaling in CD8\(^+\) T\(_{M}\) facilitates the differentiation of CD62L\(^{hi}\) T\(_{M}\) in response to weak, but not strong, TCR agonists. To test this assumption, we performed adoptive T cell–transfer experiments. Equal numbers of purified CD8\(^+\) T\(_{M}\) cells from Rag^{-/-} CD90.1\(^{+}\) WT OT-I and Rag^{-/-} CD90.1\(^{+}\)/CD90.2\(^{+}\) IFN-\(\gamma\)^{-/-} OT-I (IFN-\(\gamma\)^{-/-}/OT-I) mice were mixed and adoptively transferred into Rag^{-/-} recipient mice. Different cohorts of mice were immunized with APLs of N4, which induce gradually increasing TCR signal intensities (V4 [SIIVFEKL] < T4 [SIITFEKL] < T4 [SIQFEKL] < A2 [SAINFEKL] < N4 [SIINFEKL]) in OT-I T cells (9). Control mice were injected with PBS, allowing LIP in response to self-PEptide–MHC complexes. Twelve days later, most IFN-\(\gamma\)^{-/-}/CD8\(^+\) OT-I cells undergoing LIP in PBS-treated recipients expressed high levels of CD62L as opposed to WT CD8\(^+\) OT-I cells. This difference was still apparent in response to V4, but it finally disappeared after vaccination with stronger TCR agonists (Fig. 1A). Hence, the lack of IFN-\(\gamma\) signaling in CD8\(^+\) T cells favors the generation of CD62L\(^{hi}\) cells in response to weak, but not strong, TCR signals. Early CD62L\(^{hi}\) CD8\(^+\) T cells contain T\(_{M}\), which also express elevated levels of the antiapoptotic molecule Bcl-2 and the T\(_{M}\)-associated transcription factor Eomes (17–19). Correlating with their high levels of CD62L, IFN-\(\gamma\)^{-/-}/CD8\(^+\) OT-I cells undergoing LIP also expressed elevated levels of Bcl-2 and Eomes compared with their WT counterparts. These differences remained stable after vaccination with the weakest APL V4, indicating that self-PEptide–MHC complexes driving LIP
FIGURE 1. IFN-γR signaling regulates CD8+ T cell memory formation in response to weak, but not strong, TCR signals. (A–F) Equal numbers of WT and IFN-γR<sup>−/−</sup> OT-I T cells were transferred simultaneously into Rag<sup>−/−</sup> mice. One day later, mice were injected i.v. with PBS or the indicated APLs. (A) Twelve days after T cell transfer, splenocytes were analyzed by flow cytometry. Shown are relative fluorescence intensities for WT and IFN-γR<sup>−/−</sup> OT-I T cells. Data are representative of two independent experiments with three to five mice/group. (B and C) After PBS or N4 injection, the relative abundance (± SEM) of WT and IFN-γR<sup>−/−</sup> cells within the OT-I T cell pool was determined by flow cytometry at the indicated time points. Data are representative of two independent experiments with three to five mice/group. (D–F) Sixty days after T cell transfer, the phenotype of splenic WT (black) and IFN-γR<sup>−/−</sup> (red) memory OT-I T cells was determined by flow cytometry. (D) Splenocytes were restimulated with N4 for 3–4 h prior to the measurement of CD107a and IFN-γ. (E and F) CD62L and Ki67 levels were determined directly after isolation. Shown are relative fluorescence intensities for the indicated molecules. Data are representative of two independent experiments with three or four mice/group. *p < 0.05.

and V4 induce comparable differentiation programs in CD8<sup>+</sup> OT-I T cells. However, with increasing TCR signal strength, the expression of Bcl-2 and Eomes became indistinguishable in the OT-I populations, whereas T-bet levels were independent of IFN-γR signaling and TCR signal strength at this time point. High levels of CD62L, Bcl-2, and Eomes and low levels of T-bet indicate the long-term survival potential of CD8<sup>+</sup> TMs (19, 20). Hence, our results suggested that IFN-γR signaling blocks the generation of long-lived CD8<sup>+</sup> TMs in response to weak TCR agonists. To test this hypothesis, both T cell populations were transferred simultaneously into Rag<sup>−/−</sup> mice, which were either vaccinated with N4 or injected with PBS to allow LIP. As shown in Fig. 1B, WT and IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells undergoing LIP in PBS-treated recipients were equally abundant until day 12. However, from days 30 to 60, the frequency of IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells increased significantly, from ~60 to 80%. This survival advantage of IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells was not observed after vaccination with N4. Under these conditions, WT and IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells had similar frequencies at any given time point (Fig. 1C). Importantly, 60 d after adoptive transfer into PBS-treated Rag<sup>−/−</sup> mice, IFN-γ<sup>+</sup> CD107a<sup>+</sup> cells had similar frequencies among WT and IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells (Fig. 1D). Furthermore, the majority of WT and IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I cells expressed high levels of CD62L (Fig. 1E), and the frequency of Ki67<sup>+</sup> proliferating cells was indistinguishable in the OT-I populations at this time point of LIP (Fig. 1F). Hence, although different in number (Fig. 1B), WT and IFN-γR<sup>−/−</sup> CD8<sup>+</sup> OT-I TMs differentiated equally well into day-60 TMs. This suggested that early, rather than late, IFN-γR signals determine the size of CD8<sup>+</sup> TM clones primed by weak TCR agonists. Therefore, we hypothesized that the lack of early IFN-γR signaling facilitates the differentiation of long-lived CD62L<sup>+</sup>Bcl-2<sup>+</sup>Eomes<sup>+</sup> TMs, after contact with weak, but not strong, TCR agonists (Fig. 1A), thereby determining the size of the resulting TM pool (Fig. 1B).

To test this assumption, we established an in vitro priming system. For this purpose, naive WT and IFN-γR<sup>−/−</sup> OT-I cells were cocultured with V4 or N4 for 72 h. Flow cytometry revealed that IFN-γR<sup>−/−</sup> OT-I cells primed with V4 expressed significantly higher levels of Bcl-2 and Eomes compared with WT OT-I T cells (Fig. 2A, 2B). Importantly, expression levels of Bcl-2 and Eomes were indistinguishable between WT and IFN-γR<sup>−/−</sup> OT-I cells after stimulation with N4. Hence, the lack of IFN-γR signaling was associated with Bcl-2 and Eomes upregulation in response to weak, but not strong, TCR agonists. After primary Ag contact, IFN-γ promotes the rapid upregulation of T-bet in CD8<sup>+</sup> T cells (20). In accordance with this, T-bet
levels were significantly lower in IFN-γR−/− OT-I cells, irrespective of whether they had been activated by V4 or N4 (Fig. 2C). However, T-bet is downregulated again in the course of memory CD8+ T cell differentiation (20, 21). This might explain why T-bet levels were identical in WT and IFN-γR−/− OT-I cells at later time points (Fig. 1A), whereas they differed significantly 72 h after priming (Fig. 2C).

K66T proliferating cells had similar frequencies in the OT-I cell populations after stimulation with either peptide (Fig. 2D). In contrast, CD62L levels on WT and IFN-γR−/− OT-I cells correlated inversely with TCR signal strength. Importantly, however, CD62L was always more abundant on IFN-γR−/− OT-I cells stimulated with weak TCR agonists. This difference disappeared with increasing TCR signal strength and was not apparent after stimulation with N4 (Fig. 2E). Similar results were obtained for Eomes (Fig. 2F). In summary, our in vitro experiments suggest that early IFN-γR signals limit the differentiation of long-lived CD8+ CD62LhiBcl-2hiEomeshi TMPs in response to weak, but not strong, TCR agonists.

To test the in vivo function of V4-primed WT and IFN-γR−/− OT-I cells, B6 mice were challenged s.c. with 10^6 EG7 tumor cells expressing N4. Three days later, mice were reconstituted with 10^6 WT or IFN-γR−/− OT-I cells activated in vitro with V4 for 72 h. Compared with WT OT-I cells, IFN-γR−/− OT-I cells efficiently delayed tumor growth (Fig. 2G). This was associated with their accumulation in the spleens of tumor-bearing mice (Fig. 2H). Thus, the improved survival capabilities of IFN-γR−/− OT-I CD62LhiBcl-2hiEomeshi T MPs generated in response to weak TCR ligands (Fig. 1B) are preserved under nonlymphopenic conditions (Fig. 2H) and correlate positively with tumor growth control (Fig. 2G).

Reduced mTOR signaling facilitates the formation of IFN-γR−/− T MPs.

Signaling via mTOR limits CD8+ T MPs' T development (18, 19). Furthermore, cell culture experiments with human cell lines and mouse embryonic fibroblasts demonstrated that IFN-γR signaling promotes mTOR function in a PI3K- and protein kinase B/AKT-dependent fashion (22–24). However, it remained unclear whether the modulation of mTOR by IFN-γ is also operative in CD8+ T cells and whether it is influenced by TCR signaling. To test this possibility, WT and IFN-γR−/− OT-I cells were stimulated with V4, and the degree of mTOR activity was quantified by flow cytometry. As shown in Fig. 3A, the levels of p-mTOR were blocked by rapamycin (Fig. 3C). Conversely, tamoxifen induced reduced mTOR signaling, which could be blocked by rapamycin (Fig. 3C). Conversely, tamoxifen induced
the downmodulation of CD62L (Fig. 3D). This effect was inhibited by rapamycin, thus excluding mTOR-independent effects of myrAKTER activation.

Together, the data presented so far suggest that the blockade of early IFN-γR signaling in CD8+ T cells responding to weak TCR signals limits mTOR-dependent signaling, subsequently favoring the generation of CD62L\(^{hi}\)Bcl-2\(^{hi}\)Eomes\(^{hi}\)TMPs. This interpretation was further supported by experiments blocking the function of mTOR or autocrine IFN-γ activity (Supplemental Figs. 1, 2). In contrast, strong TCR signals do not appear to be affected by IFN-γR signaling. This might be due to the fact that mTOR activity correlates positively with TCR signal strength (26). Hence, a strong TCR signal would be sufficient to drive mTOR-dependent effects, independent of IFN-γR signaling.

mTOR-related and -unrelated signaling events contribute to the IFN-γ-dependent regulation of Eomes and T-bet in CD8+ T cells primed with weak TCR ligands

The data presented in Fig. 3 and Supplemental Fig. 2 suggest that CD8+ T cell–derived IFN-γ modulates mTOR-dependent signaling in an autocrine fashion. However, it remains unclear whether rapamycin blocked TCR-driven IFN-γ production or IFN-γR signal transduction.

To study IFN-γ production, we first stimulated WT OT-I cells encoding an eYFP reporter transgene under control of the ifn-γ promoter with V4 and N4 in the presence or absence of rapamycin. As shown in Fig. 4A, ifn-γ promoter activity was strongly suppressed by rapamycin, correlating with a strong reduction in IFN-γ protein amounts in culture supernatants (Fig. 4B). Hence, rapamycin treatment might favor the differentiation of low-affinity T\(_{MP}\)s by two different mechanisms: the inhibition of IFN-γ production and direct suppression of IFN-γR signal transduction.

To further study the relative impact of both effects, we established an experimental system that enabled us to induce IFN-γR signaling at any given time point, independent of endogenous IFN-γ production. For this purpose, we made use of IFN-γR\(^{-/-}\)OT-I cells, which cannot produce IFN-γ but are still sensitive to it.

First, we verified that Eomes and T-bet regulation in IFN-γR\(^{-/-}\)OT-I cells was comparable to that in WT and IFN-γR\(^{-/-}\)OT-I cells. As shown in Fig. 4C, V4-stimulated, but not N4-stimulated, IFN-γR\(^{-/-}\)OT-I cells reduced Eomes expression in response to rIFN-γ. However, T-bet was upregulated after rIFN-γ treatment, irrespective of TCR signal strength (Fig. 4D). Hence, IFN-γ controlled Eomes and T-bet levels in IFN-γR\(^{-/-}\)OT-I cells, further confirming the data obtained with WT and IFN-γR\(^{-/-}\)OT-I cells.

Next, we tested the relative importance of mTOR for the IFN-γR-dependent regulation of the two transcription factors. Notably, rapamycin treatment of V4-stimulated IFN-γR\(^{-/-}\)OT-I cells promoted Eomes expression and repressed T-bet expression (Fig. 4E, 4H). Thus, weak TCR signals modulate the expression of both transcription factors in an mTOR-dependent, but IFN-γR-independent, fashion. However, the addition of rIFN-γ partially reversed these effects of rapamycin (Fig. 4F, 4I), further supporting that mTOR-independent signals contribute to the IFN-γR-mediated regulation of Eomes and T-bet. IFN-γR signaling leads to STAT1-dependent gene transcription and PI3K/AKT-dependent activation of mTOR and subsequent mRNA translation (22–24, 27). The activation of both signaling cascades is JAK1 and JAK2 dependent. Consequently, the inhibition of JAK1/2 activity fully prevented the IFN-γR–mediated regulation of Eomes and T-bet. Hence, the addition of rIFN-γ partially reversed these effects of rapamycin (Fig. 4F, 4I), further supporting that mTOR-independent signals contribute to the IFN-γR–mediated regulation of Eomes and T-bet in V4-stimulated IFN-γR\(^{-/-}\)OT-I cells (Fig. 4G, 4J). Together, these results demonstrate that mTOR-dependent and -independent signaling events contribute to the IFN-γR–mediated regulation of Eomes and T-bet in conjunction with weak TCR signals. The relative contributions of different IFN-γR signaling modules to the TCR-dependent modulation of Eomes and T-bet, as well as the subsequent CD8+ T\(_{TM}\)/T\(_{M}\) differentiation, remain to be elucidated.

Discussion

Eomes and T-bet are critical regulators of CD8+ T cell function and differentiation (28, 29). High levels of T-bet favor the differentiation of short-lived CD8+ T\(_{TE}\)s (20). Conversely, low levels of T-bet and high levels of Eomes support the differentiation of CD8+ T\(_{MP}\)s and long-term T\(_{M}\) survival (19, 20). mTOR was identified as an
important signaling module in T cells that senses environmental cues, integrates signals from multiple receptor systems (30, 31), determines Eomes and T-bet levels, and modulates CD8+ T cell differentiation (18, 19). Although there is accumulating evidence that multiple signaling pathways converge at the level of mTOR, the context- and interdependent contribution of different pathways to the differentiation of CD8+ T cells is largely unclear. The reconstitution of mTOR signaling in IFN-γ–mediated repression of Eomes in response to weak TCR signals, (A and B) Splenocytes from OT-I mice encoding eYFP under control of the ifn-γ promoter were cultured for 24 h in the presence of V4 (filled bars) or N4 (open bars) without (black) or with (red) rapamycin. (A) Reporter gene activity was determined by flow cytometry after gating on CD8+ OT-I T cells. (B) IFN-γ in culture supernatants was quantified by ELISA. Data are representative (+ SD) from two independent experiments performed in duplicates. (C–J) IFN-γ−/− OT-I T cells were cultured for 72 h in the presence of the indicated peptides, rIFN-γ, rapamycin (rapa), and/or the JAK1/2-inhibitor ruxolitinib (Jaki), as indicated. Eomes and Eomes and T-bet levels were determined by flow cytometry. Data are representative graphs (filled graphs: naive IFN-γ−/− OT-I cells) and pooled results + SEM (bar graphs) from 10 (C), 13 (D), 8 (E, F, H, and I), or 4 (G and J) independent experiments performed in duplicates. **p < 0.01, ****p < 0.001, *****p < 0.0001. ns, not significant.

Inflammation is an important modulator of CD8+ T cell activation, function, and memory formation (4, 20, 32–34). However, the composition of the inflammatory cytokine milieu differs strongly between pathogens (32, 35, 36). Therefore, we do not want to exclude that the relative importance of TCR–IFN-γR interactions for CD8+ T cell formation varies between experimental systems. It might be context dependent and affected by various environmental factors (e.g., cytokines that directly modulate proximal TCR signaling) (4).

During the course of an immune response, high- and low-affinity CD8+ T cells are activated. However, TCR signal strength determines the fate of recently activated CD8+ T cells. For example, weak TCR signals are associated with the premature arrest of CD8+ T cell expansion and the early onset of contraction (9). As a result, high-affinity CD8+ T cells accumulate and dominate the CD8+ T M pool (9). This competition-based model might be further supported by the intracellular signaling events described in this article. Given that low-affinity CD8+ T cells are primed in the course of an immune response, early IFN-γ signals would help to initiate a differentiation program dominated by high levels of T-bet and low levels of CD62L, Eomes, and Bcl-2 (8). This would favor the generation of short-lived CD8+ T E cells and might further facilitate the accumulation of high-affinity CD8+ T E cells. The IFN-γ–mediated elimination of low-affinity CD8+ T M might facilitate secondary immune responses by high-affinity CD8+ T cells, as well as prevent the unwanted activation of
low-affinity CD8\(^+\) T cells in response to self-peptide–MHC complexes and subsequent autocrine immunity (8). This interpretation is supported by our findings that, compared with WT OT-I cells (Fig. 2G), the survival capabilities of IFN-γR\(^−/−\) OT-I cells (Figs. 1B, 2H) and their cytotoxic functions are more pronounced after priming with low-affinity TCR ligands.

In summary, we show that early IFN-γR signals in CD8\(^+\) T cells modulate T\(_{EM}\)/T\(_{MP}\) differentiation and the size of the resulting T\(_{M}\) pool in a TCR signal strength–dependent fashion. Our findings provide new insights into the regulation of CD8\(^+\) T cell responses by IFN-γ and suggest that TCR affinity determines cytokine responsiveness of CD8\(^+\) T cells.

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Disclosures

The authors have no financial conflicts of interest.

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