Cutting Edge: Generation of Memory Precursors and Functional Memory CD8⁺ T Cells Depends on T Cell Factor-1 and Lymphoid Enhancer-Binding Factor-1

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Cutting Edge: Generation of Memory Precursors and Functional Memory CD8+ T Cells Depends on T Cell Factor-1 and Lymphoid Enhancer-Binding Factor-1

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T cell factor (TCF)-1 and lymphoid enhancer-binding factor (LEF)-1 transcription factors have redundant roles in promoting thymocyte maturation. TCF-1 has been recently shown to critically regulate memory CD8+ T cell differentiation and persistence. The complete spectra of regulatory roles for TCF-1 and LEF-1 in CD8+ T cell responses are yet unknown. We conditionally targeted LEF-1, and by combination with germine deletion of TCF-1, we found that loss of both factors completely abrogated the generation of KLRG1loIL-7Rα+ memory precursors in effector CD8+ T cell populations in response to Listeria monocytogenes infection. Whereas CD8+ effectors deficient for TCF-1 and LEF-1 retained the capacity to express IFN-γ, granzyme B, and perforin, they were defective in TNF-α production. In the memory phase, the Ag-specific CD8+ T cells lacking TCF-1 and LEF-1 exhibited an effector phenotype and were severely impaired in secondary expansion upon rechallenge. Thus, TCF-1 and LEF-1 cooperatively regulate generation of memory precursors and protective memory CD8+ T cells. The Journal of Immunology, 2012, 189: 2722–2726.

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Flow cytometry and cell sorting

Cell surface staining, intracellular staining for cytokines, intranuclear staining for comesoderm (Eomes) and T-bet, and OVA325–334 (SIINFEKL)-MHC class I tetramer staining were performed as previously described (14, 19). The flow data were analyzed using FlowJo software (Tree Star). Tetramer-stained CD8+ effector or memory T cells from the spleens were sorted on a FACSAria.

Chromatin immunoprecipitation and quantitative RT-PCR

A LEF-1 Ab (C18A7; Cell Signaling Technology) was used in chromatin immunoprecipitation on CD8+ T cells as described (14). RNA extraction, reverse transcription, and quantitative PCR were performed as described (14).

Results and Discussion

TCF-1 and/or LEF-1 deficiency diminished expansion of effector CD8+ T cells

TCF-1 and LEF-1 have redundant roles during T cell development, with TCF-1 exhibiting a more dominant role (9, 10). Whereas LEF-1 deficiency alone did not have detectable impact on thymocyte maturation, deletion of LEF-1 in Tcf7−/− mice exacerbated T cell developmental defects (12). By adoptive transfer of wild-type or Tcf7−/− OT-1 CD8+ T cells followed by infection with actA+ LM-OVA, we previously demonstrated that TCF-1 deficiency reduced effector CD8+ T cell expansion by ~50% (14). We confirmed this finding when we directly infected Tcf7−/− mice without adoptive transfer and detected the endogenous OVA-specific effector CD8+ T cells (Fig. 1A, 1B). To address possible compensatory roles between TCF-1 and LEF-1 in regulating CD8+ responses, we investigated the impact of deficiency in both factors.

To avoid perinatal lethality, we conditionally targeted the Left allele in a single cell, LeflFL/FL and Gzmb-Cre +Rosa26-EGFP+ mice (hereafter designated as FL/FL and Gzmb-Cre +Rosa26-EGFP+ mice for a consistent comparison. On day 7 postinfection, OVA-specific IFN-γ production or detected for TNF-α function of effector CD8+ T cells and generation of memory precursors. (A) Kinetics of CD8+ T cell responses in PBLs. Mice were infected with actA+ LM-OVA, and OVA-specific CD8+ T cells in the PBLs were detected by peptide-stimulated IFN-γ production. The frequency of IFN-γ+ GFP+ cells among CD8+ T cells was shown. Data are representative of three independent experiments (n ≥ 4 for each time point). (B) Numbers of OVA-specific CD8+ effectors in the spleen, as determined on day 7 postinfection. Characterization of CD8+ effectors in the absence of TCF-1 and/or LEF-1. On day 7 postinfection, OVA-specific IFN-γ+ GFP+ CD8+ T cells were detected in the spleen and fractionated based on KLRG1 and IL-7Rα expression or detected for TNF-α and granzyme B induction. The gating is based on isotype control staining, and the frequency of each subset is shown in representative contour plots from three independent experiments (n ≥ 6). (D) Cumulative frequency of KLRG1+ and IL-7Rα memory precursors in IFN-γ+ GFP+ CD8+ effector T cells. (E) Perforin expression in CD8+ effectors. Tetramer+GFP+ CD8+ effector T cells were sorted from the spleens on day 7 postinfection, and Prf1 expression was measured by quantitative RT-PCR. The primers are 5′-GATGTGAACCCTAGGCCAGA-3′ and 5′-GGTTTTTGTACCGGCGAAA-3′. Data in (B), (D), and (E) are means ± SD from three to four independent experiments (n ≥ 6). **p < 0.01, ***p < 0.001 by Student t test.

To determine the impact of TCF-1 and LEF-1 deficiency on CD8+ T cell responses, we tracked OVA-specific IFN-γ+ GFP+ CD8+ T cells in both PBLs and spleens. In the PBLs,
whereas TCF-1 and/or LEF-1 deficiency affected the magnitude of CD8+ effector expansion to a different extent, the peak expansion in all genotypes occurred on day 7 postinfection (Fig. 1A). Consistent with our previous findings (14), TCF-1 deficiency reduced frequency of CD8+ effectors in the PBLs as well as their numbers in the spleen by >50% (Fig. 1A, 1B). Although ablation of LEF-1 only minimally affected the frequency of CD8+ effectors in the PBLs (Fig. 1A), it significantly reduced the number of effector CD8+ T cells in the spleen (Fig. 1B). These results suggest that LEF-1 is also required for optimal expansion of CD8+ effectors, and this is in contrast to the observation that LEF-1 is dispensable for normal T cell development (12). In contrast, deletion of both TCF-1 and LEF-1 substantially reduced the frequency of OVA-specific CD8+ effectors detected in PBLs (Fig. 1A). In the spleen, albeit there was a trend of further reduction of CD8+ effectors in dKO mice compared with Left−/− or Tcf7−/− mice, the additive effect of TCF-1 and LEF-1 double deficiency was not evident (Fig. 1B). We also used SIINFEKL-MHC class I tetramer to detect OVA-specific CD8+ effectors and confirmed those findings using IFN-γ-based functional measurements (Supplemental Fig. 1C). These data collectively suggest that TCF-1 and LEF-1 transcription factors are necessary for optimal expansion of effector CD8+ T cells, and that deletion of both factors did not completely abrogate CD8+ effector differentiation.

**Loss of TCF-1 and LEF-1 abrogated generation of memory precursors**

In addition to quantitative measurements, we next performed in-depth phenotypic and functional analysis of effector CD8+ T cells lacking TCF-1 and/or LEF-1. When fractionated based on KLRG1 and IL-7Rα expression, the KLRG1loIL-7Rα+ memory precursors were diminished by either LEF-1 or TCF-1 deficiency (Fig. 1C, 1D). Strikingly, the memory precursors were almost completely abrogated in dKO mice (Fig. 1C, 1D), indicating that the generation of memory precursors depends on TCF-1 and LEF-1. Additionally, although loss of either TCF-1 or LEF-1 alone exhibited little effect, the double deficiency substantially diminished production of TNF-α in CD8+ effectors (Fig. 1C). In contrast, TCF-1 and/or LEF-1 deficiency did not compromise the ability of CD8+ effectors in acquiring the cytolytic effector molecules such as granzyme B (Fig. 1C). In fact, the expression of perforin (encoded by Prf1) was most evidently increased in dKO CD8+ effectors (Fig. 1E).

**TCF-1 and LEF-1 deficiency impaired maturation and functions of memory CD8+ T cells**

After the peak response, a fraction of the CD8+ effector T cells survives the contraction and transition into memory phase. On day 35 postinfection, the numbers of OVA-specific memory CD8+ T cells were reduced in the Tcf7−/− spleens, as detected by OVA peptide-stimulated IFN-γ production or MHC class I tetramer (Fig. 2A, Supplementary Fig. 1E-G). Double deletion of TCF-1 and LEF-1 further reduced the numbers of memory CD8+ T cells, albeit the reduction did not reach statistical significance compared with TCF-1 single deficiency (Fig. 2A, Supplementary Fig. 1G). It is noteworthy that loss of both TCF-1 and LEF-1 abrogated generation of KLRG1loIL-7Rα+ memory precursors in the effector clonal expansion phase (Fig. 1C, 1D). Although the memory precursors are considered to have increased potential to give rise to memory CD8+ T cells, in the context of TCF-1 and LEF-1 double deficiency, the dKO OVA-specific CD8+ T cells detected at the early memory phase are most likely derived from the KLRG1loIL-7Rα+ effector CD8+ T cells. In line with this notion, the dKO memory CD8+ T cells exhibited almost exclusively a KLRG1+ effector phenotype (Fig. 2B, 2C). Although not affected in granzyme B expression, the dKO memory CD8+ T cells manifested decreased production of TNF-α and failed to produce IL-2 (Fig. 2D). We also sorted tetramer+GFP+CD8+ memory T cells to assess Left1 excision and found that the cells with complete deletion of Left1 transcripts were reduced to ~60% at the memory stage, compared
with ∼80% excision in effectors (compare Supplemental Fig. 1H with Supplemental Fig. 1D). This suggests that the cells that escaped Left1 excision may have had growth/survival advantage during effector-to-memory transition, and hence the defects of memory CD8+ T cells in Left1−/− and dKO mice may have been underestimated in this experimental system.

We previously showed that Eomes is a direct TCF-1 target gene in memory CD8+ T cells (14). Eomes is upregulated in CD8+ effectors and retained at high levels in memory T cells (21). At the effector phase, Eomes was only minimally affected by loss of TCF-1 or LEF-1 alone, but it was evidently reduced in dKO CD8 effectors; in contrast, T-bet expression was not affected by loss of TCF-1 and/or LEF-1 (Supplemental Fig. 2A). Thus, deficiency in both TCF-1 and LEF-1 had an early impact on proper upregulation of Eomes in CD8+ effectors, impairing generation of functional memory CD8+ T cells. At the memory phase, TCF-1 deficiency reduced Eomes expression as expected, and interestingly, loss of LEF-1 alone also modestly reduced Eomes expression (Supplemental Fig. 2B). We previously reported direct binding of TCF-1 to four upstream regulatory regions in the Eomes gene (14). By chromatin immunoprecipitation on CD8+ T cells, we found that LEF-1 occupied the same regulatory sequences (Supplemental Fig. 2C). These data indicate that both TCF-1 and LEF-1 contribute to positive regulation of Eomes during CD8+ T cell response.

**Loss of TCF-1 and LEF-1 compromised recall response of memory CD8+ T cells**

Memory T cells confer enhanced protection upon re-encountering the same Ag. When challenged with virulent LM-OVA, whereas naive mice showed uncontrolled bacteria growth, control immune mice completely cleared L. monocytogenes in the spleen and largely in the liver (Fig. 3A, 3B). In contrast, the bacteria were detected in the spleen of 50% of dKO mice, and in the liver of dKO mice, the bacteria burden was >1 order of magnitude higher than in the control mice (Fig. 3A, 3B). To further investigate the less efficient bacterial clearance in the absence of TCF-1 and LEF-1, we tracked the recall response of memory CD8+ T cells. In PBLs, the secondary expansion of dKO memory CD8+ T cells was greatly diminished, as measured by absolute frequency or relative expansion after normalizing to the starting point (Fig. 3C, 3D). When examined in the spleen, the OVA-specific dKO memory CD8+ T cells were greatly impaired in generating secondary effectors in terms of absolute counts as well as relative expansion (Fig. 3E, 3F). Collectively, most of these defects in dKO CD8+ memory were more severe than those observed in Tcf7−/− memory CD8+ T cells, indicating that TCF-1 and LEF-1 have redundant roles in regulating CD8+ responses, in addition to their well-known roles in cooperatively promoting thymocyte maturation (12).

In summary, using the newly established LEF-1 conditional knockout model, our study revealed unique requirements of TCF-1 and LEF-1 transcription factors in regulating mature CD8+ T cell responses. To our knowledge, this is the first demonstration that deletion of transcription factors (i.e., TCF-1 and its relative LEF-1) in activated T cells completely abrogates the generation of KLRG1loIL-7Rα+ memory precursors. This study further uncovers essential roles of TCF-1 and LEF-1 in TNF-α production and proper upregulation of Eomes in CD8+ effectors. Upon transitioning to the memory phase, the Ag-specific CD8+ T cells lacking both factors exhibit an effector phenotype and fail to effectively acquire functions characteristic of memory T cells, such as rapid secondary expansion and effective control of pathogens. These observations reveal that deficiency in TCF-1/LEF-1 causes the most profound defects in CD8+ T cell responses among all the transcription regulators studied in vivo thus far. Our data collectively indicate that TCF-1 and LEF-1 are necessary for optimal expansion of CD8+ effectors and are indispensable for generation of memory precursors as well as further maturation and acquisition of CD8+ memory functionality. These findings thus identify TCF-1 and LEF-1 as key regulatory nodes in enhancing T cell immunity against infectious agents and malignant cells.
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Disclosures

The authors have no financial conflicts of interest.

References