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Cutting Edge: IL-12 Inversely Regulates T-bet and Eomesodermin Expression during Pathogen-Induced CD8⁺ T Cell Differentiation

Naofumi Takemoto,* Andrew M. Intlekofer,* John T. Northrup,* E. John Wherry,† and Steven L. Reiner²*¹

Cytokines are critical determinants for specification of lineage-defining transcription factors of CD4⁺ T cell subsets. Little is known, however, about how cytokines regulate expression of T-bet and eomesodermin (Eomes) in effector and memory CD8⁺ T cells. We now report that IL-12, a signature of cell-mediated immunity, represses Eomes while positively regulating T-bet in effector CD8⁺ T cells during infection with Listeria monocytogenes. After resolution of infection and abatement of IL-12 signaling, Eomes expression rises whereas T-bet expression declines in memory CD8⁺ T cells. Eomes becomes derepressed in effector cells by ablation of IL-12 signaling. In the absence of IL-12, the dynamics of clonal expansion and contraction are also perturbed. Together, these results reveal how a pathogen-associated signal, such as IL-12, could act as a switch, regulating appropriate clonal growth and decline while, in parallel, shaping a unique pattern of fate-determining transcription factors. The Journal of Immunology, 2006, 177: 7515–7519.

Both CD8⁺ T cells and NK cells play a critical role in host defense against infection with intracellular bacteria and viruses. The T-box transcription factors T-bet and Eomes appear to cooperate in regulating differentiation, function, and homeostasis of cytotoxic cells (1–8). During the immune response to viral infection, Eomes and T-bet expression are dynamically regulated (8). Eomes expression progressively increases from the effector to memory phases, after clearance of the pathogen. In contrast, T-bet expression is maximal during acute infection and declines after resolution (8).

The signals that regulate induction and repression of T-bet and Eomes during a CD8⁺ T cell-mediated immune response have not yet been defined. In CD4⁺ T cells, a paradigm has emerged indicating that key fate-determining transcription factors, including T-bet, can be heavily regulated by the cytokine environment. T-bet expression is primarily regulated in a positive manner by IFN-γ signaling in Th cells, whereas IL-12 signaling acts in a selective or instructive manner to “lock-in” T-bet signaling in Th1 cells (2, 9, 10). In contrast, IL-12, a signature of intracellular infection, promotes Th1 polarization by repressing GATA-3, the critical transcription factor for Th2 differentiation (11, 12). IL-4 negatively regulates T-bet expression and Th1 differentiation while promoting GATA-3 induction and Th2 differentiation (2, 11, 12).

We examined the effect of IL-12 on Eomes and T-bet expression during the immune response to Listeria monocytogenes. IL-12 repressed Eomes expression in Ag-specific CD8⁺ T cells during infection. In contrast, maximal induction of T-bet in CD8⁺ T cells required IL-12 signaling. We propose that IL-12 is a key signal determining the dominance of T-bet or Eomes expression at discrete stages of the immune response.

Materials and Methods

Mice and cell culture

All animal work was in accordance with the guidelines of the University of Pennsylvania (Philadelphia, PA). C57BL/6 and Il2rα−/− (B6.129Cg-Ili2rαtm1Eqj) mice were obtained from The Jackson Laboratory. P14 (B6.129Cg-TcrLCMV327Sd2/Idvs) mice expressing transgenic TCR specific for lymphocytic choriomeningitis virus (LCMV)³-derived peptide GP33–41 were used for adoptive transfer. Tbx21−/− (B6.129Cg-Tbx21tm1.Yang) mice were generated by targeted deletion of exons 2–6, and were phenotypically identical to published Tbx21−/− (B6.129S6-Tbx21tm1(Fsun)) mice (3). Mice were infected with L. monocytogenes expressing GP33–41 (LMgp33) with either 1000 or 5000 CFU. P14 cells and D⁻/GP33⁺ CD8⁺ T cells were sorted based on Thy1.1 expression and using D⁻/GP33 tetramer, respectively, with ≥95% purity. Polyclonal CD8⁺ T cell stimulation using Ab ligation, and P14 T cell activation using peptide and T cell-depleted splenocytes as APC were done as previously described (8), FACS analyses, chromatin immunoprecipitation (ChIP) assay, and real-time PCR were performed as previously described (8).

Results and Discussion

Limited induction of Eomes in the effector CD8⁺ T cell response to L. monocytogenes

To elucidate the mechanism that regulates Eomes expression in vivo, we first examined Eomes expression over the course of L. monocytogenes infection. At 7 days after infection, effector...
CD8<sup>+</sup> T cells did not show substantial Eomes induction relative to naive cells (Fig. 1A). Eomes expression did, however, increase in Ag-specific memory cells (6 wk postinfection). T-bet expression reached its maximal induction at the effector phase and partially declined in the memory phase (Fig. 1A). The limited induction of Eomes during effector differentiation to <i>L. monocytogenes</i> infection was unexpected, because Eomes is induced in effector CD8<sup>+</sup> T cells during LCMV and vaccinia virus infection (8).

**IL-12 reciprocally regulates Eomes and T-bet expression in vivo**

One major difference between the immune responses to <i>L. monocytogenes</i> and LCMV is the relative induction of and dependence on IL-12. In response to intracellular bacterial infection, there is substantial induction of IL-12, which, in turn, plays an essential role in host resistance (13, 14). In some viral infections, such as LCMV, IL-12 induction is limited and is dispensable for effective anti-viral immunity (15). We, therefore, wished to test whether robust IL-12 signaling during the acute response to <i>L. monocytogenes</i> could account for the absence of Eomes induction in CD8<sup>+</sup> T cells.

We first examined Eomes mRNA level during <i>L. monocytogenes</i> infection in mice lacking Il12a, the gene encoding the proprietary subunit of IL-12 (p35). Although IL-12 is generally essential for host resistance to <i>L. monocytogenes</i> infection, Il12a<sup>−/−</sup> mice can survive low-doses (up to 1000 CFU) of infection (16). After clearance of a primary low-dose challenge, Il12a<sup>−/−</sup> mice are fully immune to rechallenge with an otherwise lethal dose of bacteria. This experimental system, thus, allowed us to examine effector and memory CD8<sup>+</sup> T cell development against <i>Listeria</i> in the absence of IL-12.

After infection with low-dose <i>L. monocytogenes</i>, P14 cells that developed into effector and memory CD8<sup>+</sup> T cells in Il12a<sup>−/−</sup> recipients exhibited elevated expression of Eomes compared with those that developed in wild-type recipients (Fig. 1B). In addition, low-dose infection caused detectable Eomes induction at the effector phase in wild-type recipients (Fig. 1B), contrasting with the lack of Eomes induction using normal doses of bacteria (Fig. 1A). The elevated expression of Eomes in an IL-12-deficient background was also observed in endogenous Db-GP33<sup>+</sup> CD8<sup>+</sup> T cells at the effector phase of infection (Fig. 1C). Because naive CD8<sup>+</sup> T cells from Il12a<sup>−/−</sup> mice did not show higher Eomes expression compared with wild-type cells, the relative increase in Eomes expression in Il12a<sup>−/−</sup> effector CD8<sup>+</sup> T cells is likely to reflect specific induction in response to infection. Maximal T-bet mRNA and protein induction was diminished in the IL-12-deficient background, both in transferred P14 cells (Fig. 1B) and endogenous Db-GP33<sup>+</sup> CD8<sup>+</sup> T cells (Fig. 1C). The relative difference in Eomes and T-bet expression between Il12a<sup>−/−</sup> and wild-type CD8<sup>+</sup> background was more pronounced at the effector phase than the memory phase, perhaps because IL-12 signaling operates predominantly during the acute phase of infection (17).

Further support that IL-12 negatively regulates Eomes in vivo was obtained from analysis of wild-type mice infected with varying doses of bacteria. We noted that control recipients consistently exhibited Eomes induction in effector P14 cells compared with naive cells using low-dose (1000 CFU) infection (Fig. 1B, left). In contrast, Eomes was not induced in wild-type effector cells with 5000 CFU infection (Fig. 1A, left). This disparity was verified by directly comparing effector cells transferred into recipients that received different doses from the same culture. As anticipated (18), varying the bacterial dose resulted in proportionate induction of IL-12, with low-dose recipients exhibiting significantly reduced splenic IL-12p40 mRNA level at 24 h after infection (Fig. 1D). As noted previously (17), IL-12p35 mRNA was present constitutively in...
CD8+ T cells activated with peptide/APC in vitro (Fig. 2A). By contrast, IL-4 up-regulated Eomes expression in developing effector CD8+ T cells. These differences were accentuated when cells were cultured in the presence of rIL-12 plus anti-IL-4 Ab (Tc1-polarizing conditions) compared with rIL-4 plus anti-IL-12 Ab (Tc2-polarizing conditions). By contrast, T-bet expression, both at the mRNA and protein levels, was positively influenced by rIL-12 (Fig. 2A). The differential effects of IL-12 on Eomes and T-bet expression were also observed in splenic NK cells when directly examined ex vivo (data not shown). Together, these results suggest that the expression of Eomes and T-bet are inversely regulated by IL-12, in vitro and in vivo.

Histone acetylation often correlates with a transcriptionally poised status of a gene (19). We, therefore, examined the histone acetylation status of the Eomes locus by ChIP assay in CD8+ T cells activated under type 1 or type 2 cytokine conditions. To detect potential regulatory regions, we searched for evolutionarily conserved non-coding sequences (CNS), which often correspond to functional cis-elements (20). Comparison of 10 kb of upstream sequences from human and mouse Eomes genes revealed 7 CNS, which we designated CNS1–7 (Fig. 2B). Consistent with the pattern of Eomes expression, type 2 but not type 1 cytokine conditions were associated with increased acetylation of histone H3 (Fig. 2B) and H4 (data not shown) at the proximal Eomes promoter (CNS4-7).

**IL-12-mediated repression of Eomes in the absence of T-bet**

T-bet expression was highest at the acute phase of infection and declined after elimination of bacteria, while Eomes induction was observed most prominently after recovery from primary *L. monocytogenes* infection (Fig. 1A). In addition, T-bet is thought to play a role in enhancing the expression of IL-12Rβ2 in CD4+ T cells (2, 10). Together, these findings raise the possibility that T-bet may contribute to repression of Eomes by regulating IL-12Rβ2 expression in CD8+ T cells. In such a scenario, IL-12 responsiveness, conferred by T-bet, might cause cells exposed to IL-12 to limit their expression of Eomes. *Tbx21*−/− mice are resistant to the normal experimental dose of *L. monocytogenes* infection and eliminate bacteria comparably to wild-type mice (Ref. 21 and data not shown). We found that IL-12Rβ2 expression was substantially but not fully abrogated in *Tbx21*−/− effector CD8+ T cells compared with wild-type effector cells during infection (Fig. 3A). Similar reduction of IL-12Rβ2 expression was also observed in *Tbx21*−/− CD8+ T cells activated in vitro (Fig. 3B).
Despite the apparent role for T-bet in enhancing IL-12Rβ2 expression in CD8+ T cells, we found that this was not likely to be the sole mechanism underlying IL-12-mediated repression of Eomes. Tbx21−/− naive CD8+ T cells exhibited higher basal expression of Eomes than wild-type cells (Fig. 3A), a finding distinct from Il12a−/− naive cells. Following infection, however, Tbx21−/− effector CD8+ T cells did not substantially up-regulate Eomes expression (Fig. 3A). Likewise, type 1 conditions caused substantial repression of Eomes in Tbx21−/− CD8+ T cells activated in vitro (Fig. 3B). IL-12-mediated repression of Eomes, therefore, is not solely reliant on T-bet-mediated regulation of IL-12Rβ2. The observation that Tbx21−/− naive CD8+ T cells exhibit higher Eomes expression than wild-type naive CD8+ T cells may point to a compensatory mechanism to offset T-bet deficiency.

**Diminished expansion and contraction of CD8⁺ T cells in the absence of IL-12**

Eomes is highly expressed in memory CD8⁺ T cells and seems to play a causal role in regulating their homeostasis (8). In addition, Eomes expression in effector cells arising in the absence of IL-12 approximates that of normal memory cells (Fig. 1B). We, therefore, wondered whether the enhanced induction of Eomes in effector cells in the absence of IL-12 (Fig. 1, B and C) would influence the subsequent development of memory cells. In further support of this possibility, Ag-specific effector CD8⁺ T cells that developed in the absence of IL-12 exhibited enhanced expression of IL-7Ra (Fig. 4A), which has been suggested to mark memory precursors during acute infection (22, 23).

When the dynamics of clonal expansion and contraction were evaluated, however, we found that IL-12 was not simply a negative regulator of memory formation, but that it dually promoted expansion and contraction of the clonal burst. During listeriosis, there was diminished expansion of effector cells in IL-12-deficient recipients as judged by the total number of P14 T cells per spleen (Fig. 4B). After resolution of infection, however, there was diminished contraction in the absence of IL-12 as judged by the higher proportion of memory cells relative to the peak of expansion (Fig. 4C). These results suggest that, in the absence of IL-12 signaling, CD8⁺ T cells seem to be less prone to expand and less prone to contract.

At present, we are uncertain whether the observed effects of IL-12 on expansion and contraction are due directly to IL-12 signaling or through another inflammatory mediator that is directly or indirectly regulated by IL-12. Indeed, it has been demonstrated that numerous inflammatory cytokines and effector molecules during acute infection regulate the dynamics of clonal expansion and contraction of CD8⁺ T cells (24–27). Instead of IL-12 signaling, secretion of IFN-γ, which is regulated by IL-12, may play a critical downstream role in mediating clonal expansion and contraction. Treatment with CpG oligonucleotides, for example, reverses defects in clonal contraction caused by antibiotic treatment, but in a manner that is strictly dependent on IFN-γ (25). Effector CD8⁺ T cells from Ifng−/− mice also express elevated levels of IL-7Ra (25), which further suggest that the effects of IL-12 on expansion and contraction may be acting through IFN-γ.

T-bet and Eomes seem to act as functional homologues in several respects (7, 8). Nonetheless, T-bet and Eomes exhibit inverse patterns of expression in effector and memory CD8⁺ T cells, respectively. In addition, our preliminary analyses suggest that some genes associated with effector function and migration appear to be predominantly regulated by T-bet, while some genes that are more likely to control self-renewal and homeostasis are regulated by Eomes (unpublished results). The apparent differences in patterns of T-bet and Eomes expression in different subsets (effector and memory) of CD8⁺ T cells can now be partially explained by the opposing role of IL-12 on their expression. This finding is reminiscent of the opposing role of IL-12 in the balance of type 1 and type 2 subsets of CD4⁺ effector cells. In parallel, IL-12 may regulate expansion and contraction of effector CD8⁺ T cells, perhaps via its regulation of IFN-γ. Agonism and antagonism of the IL-12 signaling pathway may, thus, provide novel strategies to boost or attenuate CD8⁺ T cell-mediated immune responses through parallel effects on transcription factor expression and on inflammatory mediators that influence clonal dynamics.

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**Disclosures**

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**References**


