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HIGHLIGHTS

Basic

COMMENTARY

Paths to expansion: Differential requirements of IRF4 in CD8$^+$ T-cell expansion driven by antigen and homeostatic cytokines

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Interferon regulatory factor 4 (IRF4) regulates the clonal expansion and metabolic activity of activated T cells, but the precise context and mechanisms of its function in these processes are unclear. In this issue of the *European Journal of Immunology*, Miyakoda et al. [Eur. J. Immunol. 2018. 48: 1319–1328] show that IRF4 is required for activation and expansion of naïve and memory CD8$^+$ T cells driven by T-cell receptor (TCR) signaling, but dispensable for memory CD8$^+$ T-cell maintenance and homeostatic proliferation driven by homeostatic cytokines. The authors show that the function of IRF4 in CD8$^+$ T-cell expansion is partially dependent upon activation of the PI3K/AKT pathway through direct or indirect attenuation of PTEN expression. These data shed light upon the differential intracellular pathways required for naïve and memory T cells to respond to self-antigens and/or homeostatic cytokines, and highlight the potential translational relevance of these findings in the context of immune reconstitution such as following allogeneic stem cell transplantation.

Keywords: IRF4 · Naïve T cell · Memory T cell · Homeostatic proliferation · Clonal expansion

See accompanying article by Miyakoda et al.

Naïve CD8$^+$ T cells integrate multiple signals derived from the extracellular environment including contact of the T cell receptor (TCR) with peptide:MHC (pMHC) complexes on the surface of antigen-presenting cells, costimulation induced by the binding of costimulatory molecules to their cognate ligands, and cytokines. Of these, TCR signaling plays an important initiating role in the activation and expansion of naïve CD8$^+$ T cells during primary and secondary responses to cognate antigens. However, homeostatic cytokines such as IL-7 and IL-15 can also promote CD8$^+$ T-cell activation and expansion (Fig. 1). Among memory T cells, homeostatic cytokines can drive expansion or maintenance in a TCR-independent fashion [1]. Among naïve CD8$^+$ T cells, homeostatic expansion requires tonic signals from the TCR in addition to homeostatic cytokine signaling. Thus, while T-cell activation and expansion can be a consequence of both TCR and homeostatic cytokine signaling, it is important to determine whether

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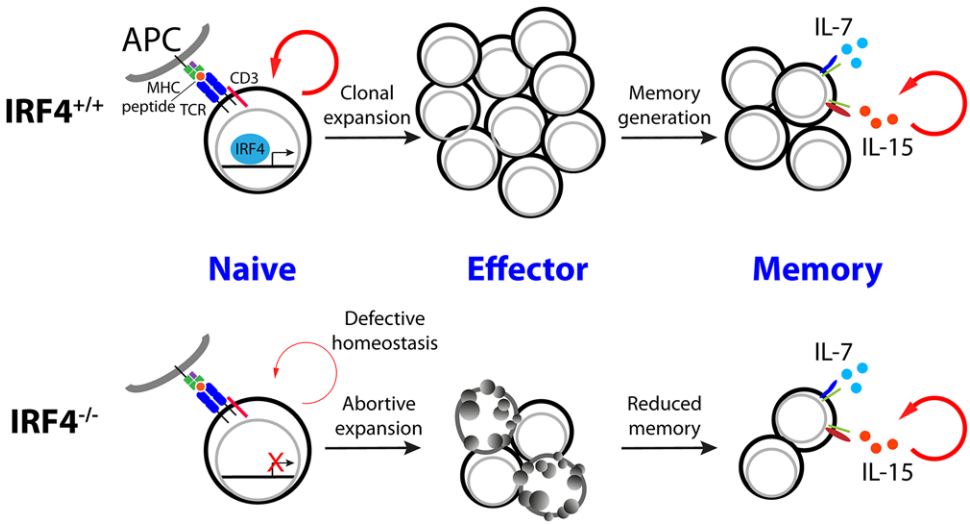

Figure 1. Differential requirement of IRF4 for antigen-driven and homeostatic expansion of CD8$^+$ T cells. Antigen-driven responses of naïve CD8$^+$ T cells result in the induction of the AP-1/IRF4 supercomplex, which is required for activation and clonal expansion (upper panel). Loss of IRF4 results in abortive clonal expansion (lower panel). However, a few IRF4$^{-/-}$ cells survive and develop into long-lived memory cells, although their number is decreased compared to IRF4$^{+/+}$ cells. Homeostatic expansion (circular arrow) of memory T cells is antigen-independent, thus not requiring IRF4. By contrast, naïve CD8$^+$ T cells partially require IRF4 for their homeostatic proliferation. Not shown: memory T cells require IRF4 for optimal recall responses induced by cognate antigen.

shared or distinct molecular pathways regulate these distinct phenomena.

TCR ligation results in a cascade of signaling events leading to activation of signal-dependent transcription factor complexes, including Nuclear Factor κB (NF-κB), Activator Protein 1 (AP-1) and Nuclear Factor of Activated T-cells (NFAT), which are pivotal in driving the gene expression changes that underlie clonal expansion and effector cell differentiation [2]. Recent findings have indicated an important role of the transcription factor Interferon Regulatory Factor 4 (IRF4) in TCR-driven gene expression. In CD8$^+$ T cells, IRF4 is induced by TCR stimulation and regulates the expression of genes involved in T-cell expansion, effector differentiation and metabolism. IRF4 is important for T-cell function, as IRF4-deficient cells undergo abortive expansion and differentiate less efficiently into effector cells [3]. IRF4 binds DNA poorly in its monomeric form and in T cells IRF4 forms complexes with the AP-1 family members BATF and JUN at AP-1 IRF composite elements (AICE) within regulatory DNA [4, 5]. Thus, involvement in AP-1 signaling provides IRF4 with a critical function in regulating T-cell receptor-driven gene expression. IRF4 is involved CD8$^+$ T-cell activation and expansion, but whether it controls these events in response to both TCR and homeostatic cytokine signaling has been unclear.

In this issue of the *European Journal of Immunology*, Miyakoda et al. [6] confirm previous observations that IRF4 is required for the expansion and effector differentiation of naïve and memory CD8$^+$ T cells in response to cognate antigens [3]. However, the authors propose that cytokine-driven maintenance of memory cells following primary infection is independent of IRF4. Thus, the authors propose that IRF4 has an indispensable role in CD8$^+$ T-cell activation and expansion in response to TCR signaling but has a dispensable role in contexts where TCR signaling is less relevant. Consistently, wild-type and IRF4-deficient memory cells expanded similarly upon adoptive transfer into lymphopenic hosts. By contrast, IRF4-deficient naïve CD8$^+$ T cells expanded poorly early following transfer into lymphopenic hosts but 'caught-up' with wild-type cells at later timepoints. To account for this, the authors propose that homeostatic expansion of naïve CD8$^+$ T cells occurs in two phases, an early IRF4-dependent phase during which tonic TCR signaling acts in conjunction with homeostatic cytokines to promote expansion, and a later IRF4-independent and likely TCR-independent phase.

Signaling via the TCR, and co-stimulatory and cytokine receptors activate the PI3K/AKT pathway to drive changes in gene expression and alter the translational and metabolic capacity of cells thereby contributing to the widespread changes required for T-cell activation and expansion [2]. While defective AP-1 driven gene expression would initially appear a plausible explanation for loss of TCR-driven expansion in the absence of IRF4, the authors found that IRF4$^{-/-}$ CD8$^+$ T cells exhibit reduced phosphorylation of AKT, S6 and Foxo1, components of the PI3K/AKT pathway [6]. Defective AKT signaling in IRF4-deficient cells was, at least in part, attributed to observed elevated levels of PTEN, and treatment of IRF4-deficient T cells with the PTEN inhibitor SF1670 partially restored AKT activity following activation *in vitro*, and T-cell proliferation following infection *in vivo*. It is currently unclear why PTEN levels are elevated in the absence of IRF4 and future experiments will have to address whether IRF4 represses *Pten* expression directly by binding to its regulatory elements. While the findings support an involvement of IRF4 in direct or indirect regulation of the PI3K pathway, the presence of only a mild reversion of the IRF4-deficient phenotype with PTEN inhibition indicates that

other functions of IRF4, including direct regulation of TCR-driven gene expression, may contribute substantially to the activation and expansion defect seen among IRF4-deficient cells.

Despite that their number was reduced, long-lived $CD44^{high}$ $CD62L^{high}$ memory T cells could still be detected in $IRF4^{-/-}$ mice, as previously reported [7]. Increased activity of AKT, mTOR and AP-1 is known to promote effector differentiation of $CD8^+$ T cells and antagonize memory cell differentiation [8–10]. This is translationally relevant since blocking AKT and mTOR signaling using specific inhibitors can uncouple proliferation from effector differentiation, thereby favoring the formation of stem-like memory T (T_{SCM}) cells with increased engraftment capacity upon adoptive cell transfer immunotherapy [11–13]. Given the observation of decreased AKT/mTOR activity among IRF4-deficient cells, it is possible that interfering with IRF4 activity may enable therapeutic manipulation of $CD8^+$ T-cell differentiation. In support of this concept is the observation that human $CD8^+$ T_{SCM} cells derived from naïve precursors express lower levels of IRF4 compared to effector cells [14].

The intracellular events responsible for homeostatic proliferation remain poorly understood. The data presented by Miyakoda et al. highlight that IRF4 is required for naïve T cells to rapidly respond to lymphopenia (Fig. 1), although the IRF4-dependent gene expression programs under these conditions remain undetermined. IL-7 and IL-15-dependent gene expression is largely regulated by STAT3 and STAT5 binding to DNA. It will be interesting in the future to compare the gene programs directed by IRF4, STAT3 and STAT5 in naïve and memory cells and to determine whether they have overlapping transcriptional consequences. A better understanding of the biology of naïve and memory T-cell responses to lymphopenia has important implications for diseases where immunodeficiency is a clinical consequence such as allogeneic hematopoietic stem cell transplantation (allo-HSCT). Thanks to recent developments in the prevention of graft-versus-host disease, such as through depletion of alloreactive T cells *in vivo* using post-transplant cyclophosphamide, untouched donor T cells are adoptively-transferred with the graft. In this scenario, both naïve and memory cells reconstitute the lymphopenic host [15, 16]. In particular, the survival of naïve cells is fundamental to ensure a more polyclonal TCR repertoire and a broad immune reconstitution. The results presented by Miyakoda et al. suggest that IRF4 plays a nonredundant role in reconstitution under lymphopenic conditions and suggest that enhancing IRF4-dependent signaling may boost naïve T cell recovery and immune function.

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Abbreviations: **IRF4**: Interferon Regulatory Factor 4 · **NF-κB**: Nuclear Factor κB · **AP-1**: Activator Protein 1 · **NFAT**: Nuclear Factor of Activated T-cells · **AICE**: AP-1 IRF composite elements · **T_{SCM}**: T memory stem cells · **allo-HSCT**: allogeneic hematopoietic stem cell transplantation

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