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Brief Communication

Mcl-1 regulates effector and memory CD8 T-cell differentiation during acute viral infection

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ABSTRACT

Mcl-1, an anti-apoptotic member of Bcl-2 family maintains cell viability during clonal expansion of CD8 T cells, but the cell intrinsic role of Mcl-1 in contraction of effectors or the number of memory CD8 T cells is unknown. Mcl-1 levels decline during the contraction phase but rebound to high levels in memory CD8 T cells. Therefore, by overexpressing Mcl-1 in CD8 T cells we asked whether limiting levels of Mcl-1 promote contraction of effectors and constrain CD8 T-cell memory. Mcl-1 overexpression failed to affect CD8 T-cell expansion, contraction or the magnitude of CD8 T-cell memory. Strikingly, high Mcl-1 levels enhanced mTOR phosphorylation and augmented the differentiation of terminal effector cells and effector memory CD8 T cells to the detriment of poly-cytokine-producing central memory CD8 T cells. Taken together, these findings provided unexpected insights into the role of Mcl-1 in the differentiation of effector and memory CD8 T cells.

Introduction

Following vaccination or infections, antigen receptor signaling in concert with appropriate inflammatory and costimulatory signals trigger antigen-specific naïve CD8 T cells to expand and differentiate into effector cells in secondary lymphoid organs (Jameson and Masopust, 2009; Kaech and Cui, 2012; Zhang and Bevan, 2011). Subsequently, effector CD8 T cells traffic to the peripheral tissues and control the infection by MHC I-restricted cell-mediated cytotoxicity and/or by producing cytokines such as IFN-γ and TNF-α (Sprent and Surh, 2002; Zhang and Bevan, 2011). During the contraction phase, ~90% of the effector CD8 T cells undergo apoptosis and the remainder of the expanded CD8 T cells differentiate into effector or central memory CD8 T cells. The self-renewing population of memory CD8 T cells provides rapid immunity to re-infection but such protective immunity depends upon the number, anatomical localization and functional attributes of memory CD8 T cells (Jameson and Masopust, 2009; Kaech and Cui, 2012; Zhang and Bevan, 2011). The number of memory CD8 T cells is a function of the extent of expansion and contraction of effector CD8 T cells. Hence, there is high level of interest in determining the mechanisms that govern expansion and contraction of CD8 T cells during an immune response. The number of antigen-specific CD8 T cells at a given time during the T cell response is controlled by the relative rates of cellular proliferation and/or apoptosis. While it is clear that death receptor-mediated apoptosis can govern the death of activated T cells in vitro (Brunner et al., 1995; Dhein et al., 1995; Ju et al., 1995), cell-intrinsic pathway of mitochondria-mediated apoptosis is considered as the major mechanism of the death of effector T cells in vivo (Hildeman et al., 2002; Prlic and Bevan, 2008; Weant et al., 2008).

The Bcl-2 family proteins are critical regulators of mitochondria-mediated apoptosis, and consist of pro-apoptotic members (Bim, Bad, Puma, Noxa, Bid, Bax, Bak and Bok) and anti-apoptotic members (Bcl-2, Bcl-xL, Mcl-1 and A1) (Chipuk et al., 2010; Czabotar et al., 2014). These Bcl-2 family proteins are known to interact with each other with varying affinities (Chen et al., 2005), and the balance of the pro and anti-apoptotic proteins in the cell determines the cell fate. For example, Bim binds to all anti-apoptotic members with similar affinity, while Noxa binds to Mcl-1 and A1 selectively (Chen et al., 2005; Kurtulus et al., 2010). It is well established that the pro-apoptotic Bim is required for contraction of effector CD8 T cells and Bcl-2 is known as an antagonist to Bim (Prlic and Bevan, 2008; Wojciechowski et al., 2006, 2007). However, unlike Bim deficiency, overexpression of Bcl-2 fails to rescue effector CD8 T

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cells from apoptosis in vivo (Petschner et al., 1998). These data suggested that high level of Bcl-2 alone is insufficient to overcome the pro-apoptotic effects of Bim in effector CD8 T cells during the contraction phase of the CD8 T-cell response.

Unlike Bcl-2, which is highly expressed in naïve and memory T cells and rapidly downregulated after T cell activation, Mcl-1 expression is strongly induced after TCR stimulation (Dunkle et al., 2011; Opferman et al., 2003). Therefore, Mcl-1 is believed to maintain T cell viability during antigen-driven proliferation, especially when Bcl-2 levels are extremely low. Consistent with this idea, Hildeman's group recently reported that Mcl-1 deficiency impaired the accumulation of effector T cells following acute viral infection, a likely sequel to increased mitochondria-mediated apoptosis (Tripathi et al., 2013). Further, Gui et al. (2015) reported that transgenic expression of Mcl-1 in bone marrow-derived cells enhanced the number of memory CD8 T cells following infection of mice with vaccinia virus. Reports also indicate that Mcl-1 expression is induced by IL-7 and IL-15 in T cells, and it might share the function of antagonizing Bim with Bcl-2 (Dunkle et al., 2011). However, the CD8 T cell-intrinsic role of Mcl-1 in regulating contraction and development of memory and whether Mcl-1 overexpression can override Bim-dependent apoptosis of effector CD8 T cells remain unknown.

T cell activation leads to profound alterations in cellular energy metabolism characterized by increased glucose uptake and glycolysis to support the enhanced bioenergetics needs of the proliferating cell (Pearce et al., 2013; Pollizzi and Powell, 2014). Exquisite control of cellular energy metabolism is not only essential for optimal expansion and effector functions it is also linked to the differentiation of memory CD8 T cells (Finlay and Cantrell, 2011; Finlay, 2012; Pearce et al., 2013; Prlic and Bevan, 2009; van der Windt et al., 2012). In this context, Mcl-1 is a unique member of the Bcl-2 family because, independent of its canonical function to enhance cell survival, it regulates mitochondrial physiology and cellular energy metabolism (Perciavalle and Opferman, 2013). Notably, loss of Mcl-1 leads to reduced oxidative phosphorylation and cellular ATP levels (Perciavalle et al., 2012) in cells such as fibroblasts and hepatocytes. However, it remains to be determined whether Mcl-1 regulates mitochondrial function, energy homeostasis and the differentiation of effector and memory CD8 T cells by cell-intrinsic mechanisms.

Mcl-1 deletion invariably results in apoptosis of naïve T cells (Opferman et al., 2003), which precludes experiments to assess the cell survival-independent roles of Mcl-1 in T cells. To overcome this constraint in studying the role of Mcl-1 in CD8 T-cell immunity, we have assessed the effects of Mcl-1 overexpression on the dynamics of the CD8 T-cell response in vivo (Zhou et al., 1998). This approach allowed us to delineate the cell-intrinsic role of Mcl-1 in regulating homeostasis and differentiation of effector and memory CD8 T cells. Surprisingly, we find that overexpression of Mcl-1 had a minimal impact on the expansion or the contraction of effector CD8 T cells and the magnitude of CD8 T-cell memory. However, elevated levels of Mcl-1 promoted the differentiation of short-lived effector cells (SLECs) at the expense of the memory precursor effector cells (MPECs) and skewed the development of effector memory CD8 T cells to the detriment of central memory CD8 T cells and their polyfunctionality. These results ascribe a previously unidentified role for Mcl-1 in regulating the differentiation of effector and memory CD8 T cells, which might have implications in vaccine development.

Results

Mcl-1 expression in naïve, activated, effector and memory CD8 T cells

First, we examined the expression of Mcl-1 in naïve ($CD44^{LO}$) and activated/memory ($CD44^{HI}$) CD8 T cells from uninfected B6 mice by flow cytometry. Interestingly, $CD44^{HI}$ CD8 T cells showed higher levels of Mcl-1 protein, as compared to those in naïve CD8 T cells (Supplementary Fig. 1A). We also investigated whether activation of CD8 T cells induced the expression of Mcl-1. Consistent with a previous report (Dzhagalov et al., 2008), in vitro stimulation with anti-CD3 and anti-CD28 antibodies rapidly increased (3-fold) Mcl-1 protein expression within 24 h, as compared to un-stimulated CD8 T cells; Mcl-1 protein levels further increased in the next 24 h. (Supplementary Fig. 1B). Together, these data demonstrated that Mcl-1 is robustly expressed in naïve CD8 T cells, but their activation or differentiation into memory further enhanced Mcl-1 levels in CD8 T cells.

During a CD8 T cell response, naïve CD8 T cells undergo clonal expansion and differentiation into effector cells. Subsequently, ~90% of the effector cells are lost during the contraction phase. The net number of CD8 T cells during expansion or contraction are linked to the rate of cellular proliferation and/or apoptosis (Hildeman et al., 2007; Kaech and Cui, 2012). Since Bcl-2 family proteins are important regulators of this process (Hildeman et al., 2002; Kaech et al., 2003b; Wojciechowski et al., 2006, 2007), we assessed the expression dynamics of anti-apoptotic Bcl-2 family proteins Bcl-2 and Mcl-1 in virus-specific CD8 T cells during an acute LCMV infection. Consistent with previous reports (Hildeman et al., 2007, 2002), Bcl-2 levels in CD8 T cells plummeted during the activation and expansion phase, but levels rapidly rebounded during the contraction phase (Supplementary Fig. 1C). By contrast, Mcl-1 levels showed only a transient drop at day 5 post infection (PI), rose dramatically between days 5 and 8 PI, and then decreased during the early contraction phase (Supplementary Fig. 1C). During the late contraction and memory phase however, Mcl-1 expression rebounded to high levels. Thus, Bcl-2 and Mcl-1 may exert their anti-apoptotic functions in CD8 T cells at different time points during the T cell response in vivo.

Effect of elevated Mcl-1 expression on clonal expansion of CD8 T cells

Recently, Hildeman's group reported that Mcl-1 is required for the survival and accumulation of effector T cells during the expansion phase of the T cell response to LCMV (Tripathi et al., 2013). Further, it is worth noting that both Bcl-2 and Mcl-1 levels were substantially reduced at day 5 PI (Supplementary Fig. 1C). Therefore, it was of interest to determine whether lower Mcl-1 levels limit the clonal expansion and the magnitude of CD8 T cell memory during LCMV infection. To address this issue, we utilized mice that express human Mcl-1 transgene in the bone marrow compartment. Uninfected Mcl-1 transgenic (tg) mice did not exhibit significant alterations in the frequencies of B cells, CD4 T cells or CD8 T cells in spleen (Supplementary Fig. 2A). Additionally, the relative proportions of various T-cell subsets were largely unaltered in spleen of uninfected Mcl-1 tg mice (Supplementary Fig. 2B). Next, we determined whether forced expression of Mcl-1 in the bone marrow compartment would enhance clonal expansion and/or CD8 T cell memory during an acute LCMV infection. Data in Fig. 1A show that in the Mcl-1 tg mice, LCMV-specific CD8 T cells contained 1.5-2 fold more Mcl-1 protein, as compared to their non-transgenic counterparts during the course of LCMV infection. At day 8 PI, the frequencies of LCMV-specific Mcl-1 tg effector CD8 T cells were comparable to those in WT mice (Fig. 1B). By virtue of increased spleen size and the resulting augmentation of CD8 T cell numbers, the absolute numbers of LCMV-specific effector CD8 T cells were increased by ~2 fold in Mcl-1 tg mice (Fig. 1C). Interestingly, there was a modest but statistically significant ($p < 0.05$) reduction in the apoptosis of Mcl-1 tg CD8 T cells at day 8 PI when the expression of Mcl-1 was highly induced (Fig. 1D).

Previously, Wensveen et al. (2010) showed that Mcl-1/Noxa axis affected the TCR diversity of CD8 T cells by regulating the apoptosis of low avidity CD8 T cells. Therefore, we investigated whether forced expression of Mcl-1 affected the immunodominance hierarchy of LCMV-specific CD8 T cells. Mcl-1 transgene did not significantly affect the responses to either immunodominant (NP396, GP33 and GP276) or subdominant (NP205, GP118 and L2062) epitopes (Fig. 1E). Taken