CD8+ T cells respond to signals via the T cell receptor (TCR), costimulatory molecules, and immunoregulatory cytokines by developing into diverse populations of effector and memory cells. The relative strength of phosphoinositide 3-kinase (PI3K) signaling early in the T cell response can dramatically influence downstream effector and memory T cell differentiation. We show that initial PI3K signaling during T cell activation results in up-regulation of the signaling scaffold B cell adaptor for PI3K (BCAP), which further potentiates PI3K signaling and promotes the accumulation of CD8+ T cells with a terminally differentiated effector T cell phenotype. Accordingly, BCAP-deficient CD8+ T cells have attenuated clonal expansion and altered effector and memory T cell development following infection with Listeria monocytogenes. Thus, induction of BCAP serves as a positive feedback circuit to enhance PI3K signaling in activated CD8+ T cells, thereby acting as a molecular checkpoint regulating effector and memory T cell development.

Introduction

Infection with intracellular pathogens provokes strong CD8+ cytotoxic T cell responses that help eliminate the pathogen and provide protection from reinfection. This is accomplished through the robust proliferative expansion of pathogen-specific CD8+ T cells such that they increase from a small number of naive precursors to represent up to 30–40% of total CD8+ T cells at the peak of the response. Following pathogen clearance, ~90% of these effector cells undergo programed cell death, and the remaining cells form a stable, long-lived memory cell pool. Importantly, pioneering work over the last decade has demonstrated that this CD8+ T cell response is not uniform but instead is composed of several distinct populations of effector and memory cells that fulfill specialized roles in pathogen elimination and protective immunity (Cui and Kaech, 2010). These include distinct effector cell populations such as terminal effector cells (TECs) and memory precursor effector cells (MPECs) that differ in their effector potential and their contribution to the long-lived memory cell pool. Additionally, specialized memory cell populations have been described, such as effector memory T cells (TEM), which recirculate through nonlymphoid tissues, have limited proliferative potential, and display immediate effector function upon re-stimulation, and central memory T cells (TCM), which recirculate through secondary lymphoid organs and retain extensive proliferative capacity, thereby acting as a reservoir of antigen-specific cells that can be rapidly expanded upon secondary infection. Additionally, recently characterized tissue-resident memory T cells do not recirculate in the blood and lymph but instead establish long-term residency in nonlymphoid tissues where they provide a potent first line of defense against reinfection (Gebhardt and Mackay, 2012).

Although the signals that regulate the phenotypic and functional diversity of effector and memory CD8+ T cells are complex, it is clear that the extent of signaling from the TCR, costimulatory receptors, and immunomodulatory cytokines can dramatically impact CD8+ effector and memory T cell generation (Chang et al., 2014; Kim and Harty, 2014). For instance, during acute infection, strong IL-12 signaling in responding CD8+ T cells favors the development of TECs and TEM cells at the expense of MPECs and TCM cells (Joshi et al., 2007). Similarly, TEC and TEM differentiation is also favored by strong TCR and costimulatory receptor signaling, whereas weaker signals favor TCM cell formation (Chang et al., 2014). Downstream of these external signals, several transcription factor networks help control effector and memory CD8+ T cell differentiation (Gray et al., 2014). These include the t-box transcription factors T-bet and eomesodermin (Eomes); E proteins and their inhibitors Id2 and Id3; and other transcription factors such as Foxo1, Zeb-2, TCF-1, and Blimp-1. Collectively, these data highlight the concept that extrinsic signals from the immune environment direct the differentiation of CD8+ effector and memory T cell populations through induction of distinct transcriptional regulators that control cell phenotype and function. However, the molecular basis by which activated CD8+ cells...
integrate and interpret these signals during effector and memory differentiation is still poorly understood.

During CD8+ T cell activation, engagement of the TCR and costimulatory and cytokine receptors results in the activation of multiple signaling pathways, which ultimately lead to the changes in gene expression and cell behavior associated with different effector and memory T cell differentiation. Of these, activation of the phosphoinositide 3-kinase (PI3K) signaling cascade has dramatic consequences on T cell responses by influencing cell proliferation and survival, metabolic reprogramming, cellular migration and tissue tropism, acquisition of effector function, and generation of long-lived memory cells (Han et al., 2012; Kim and Suresh, 2013). Class I PI3Ks catalyze the phosphorylation of P(4,5)P2 to generate the second messenger P(3,4,5)P3, which subsequently acts to recruit proteins containing pleckstrin homology domains to the plasma membrane and initiates signaling cascades that proceed through the Akt/mTor and phospholipase Cy/Ca2+/RAS pathways. PI3K signaling during T cell activation is essential for the proper clonal expansion of antigen-specific T cells (Shi et al., 1997). Additionally, strong PI3K-dependent Akt activation favors the generation of short-lived TECs at the expense of the longer-lived MPECs (Rao et al., 2010, 2012; Kim and Suresh, 2013), and manipulation of PI3K signaling can be used to alter the balance of effector and memory T cell generation (Macintyre et al., 2011; Kim et al., 2012). The influence of PI3K signaling on T cell differentiation is due in part to the ability of Akt to regulate expression of cytokine and chemokine receptors such as the IL-7R and CCR7 via phosphorylation and inhibition of the key transcriptional regulator Foxo1 (Fabre et al., 2008; Haxhinasto et al., 2008). Additionally, PI3K signaling promotes cell proliferation and metabolic reprogramming of cells based on Akt-mediated phosphorylation of glycosyn thase kinase 3β (GSK3β), and by activation of mTOR signaling complexes TORC1 and TORC2. Importantly, recent data have highlighted the concept that PI3K signaling in lymphocytes should not be viewed as a binary on/off switch but instead acts more as a rheostat in which graded amounts of PI3K signaling result in vastly different cellular responses (So and Fruman, 2012). This highlights the need to understand how PI3K activity is controlled by multiple inputs during T cell activation in order to pharmacologically promote or inhibit different types of T cell responses.

Despite the dramatic impact of PI3K signaling on T cell responses, the mechanisms by which PI3K is activated during T cell activation and differentiation are still poorly defined. Of the class I PI3Ks, p110δ appears to play the dominant role in T cell activation/differentiation (So and Fruman, 2012). In resting T cells, p110δ exists in a catalytically inactive state and is bound by the regulatory p85 subunit. The p85/p110δ complex is recruited to sites of active signaling via interaction between SH2 domains in p85 and phosphorylated tyrosines contained within YxxM motifs in signaling adaptors. This leads to a conformational change in p85, resulting in increased catalytic activity of the associated p110δ. In T cells, the costimulatory receptors CD28 and ICOS both contain YxxM motifs in their cytoplasmic tails, and, thereby, both can activate PI3K signaling (Arimura et al., 2002; Parry et al., 2003). Surprisingly, however, mutation of the YxxM motif in either CD28 or ICOS had a minimal impact on the clonal expansion of antigen-specific T cells in vivo (Gigoux et al., 2009; Pagán et al., 2012). Additionally, signaling through the IL-2 receptor can activate PI3K via phosphorylation of the adaptor protein shc (Gu et al., 2000). Finally, cross-linking of the TCR-associated CD3 complex also activates PI3K, although the mechanisms underlying this activation are poorly defined. The signaling adaptor TRIM was identified as a YxxM-containing protein that associates with the CD3 complex, is phosphorylated by Src-family kinases upon TCR engagement, and can bind p85 and potentiate PI3K activation in Jurkat T cells (Bruyns et al., 1998). However, TRIM-deficient mice showed normal T cell development and function in vivo, and Akt phosphorylation following α-CD3 cross-linking was actually enhanced in the absence of TRIM (Kölsch et al., 2006). Other adaptors implicated in PI3K activation following TCR/CD3 stimulation include SLP-76, LAT, and TIM-3 (Lee et al., 2011; Shim et al., 2011). Additionally, activation of PI3K by ZAP-70 or Src-family kinases has been proposed (Pleiman et al., 1994; Moon et al., 2005), as has direct activation of p110δ by Ras (Rodriguez-Viciana et al., 1994), but the importance of these mechanisms of CD3-mediated PI3K activation for T cell proliferation and differentiation in vivo is not clear.

As its name implies, the B cell adaptor for PI3K (BCAP) protein was originally identified as a B cell–expressed protein that can recruit and activate PI3K signaling when phosphorylated on its four YxxM motif tyrosines after BCR ligation (Okada et al., 2000). Additionally, BCAP is highly expressed in several myeloid cell populations, where it regulates PI3K activation downstream of Toll-like receptor signaling (Ni et al., 2012; Troutman et al., 2012). BCAP is a relatively large ~91-kd protein, and in addition to its YxxM sequences, it contains numerous potential protein–protein interaction domains, including an N-terminal DBB domain, an ankyrin repeat region, a coiled coil domain, and several C-terminal proline-rich regions. However, BCAP does not contain any obvious enzymatic domains. Therefore, BCAP has the potential to act as a signaling adaptor that can integrate different inputs and facilitate and coordinate activation of multiple downstream signaling pathways including PI3K. Although not expressed in naive T cells (Yamazaki et al., 2002), we found that BCAP is rapidly up-regulated in CD8+ T cells upon activation in a PI3K-dependent manner. In the absence of BCAP, activated CD8+ T cells showed impaired CD3-dependent PI3K activation and blunted clonal expansion associated with altered effector and memory T cell differentiation in vivo. Thus, we have identified BCAP as a critical signaling hub in T cells that is up-regulated upon initial activation and helps coordinate CD8+ T cell responses through activation of PI3K signaling.

Results

BCAP is up-regulated in activated T cells

BCAP is constitutively expressed in B cells, NK cells, and myeloid cells but has not previously been found in T cells (Yamazaki et al., 2002; MacFarlane et al., 2008; Ni et al., 2012). However, analysis of publically available data from the Immgen Consortium showed that BCAP (gene name Plik3ap1) is rapidly up-regulated in OVA-specific CD8+ T cells responding to infection with recombinant Listeria monocytogenes (LM) OVA or vesicular sto-

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matitis virus–OVA (Best et al., 2013). Indeed, following LM–OVA infection, increased Pik3ap1 expression was detected as early as 12–24 h after infection, reached a peak by 6 d after infection, and was maintained in long-lived memory cells to at least 100 d after infection (Fig. 1 A). By intracellular staining with a BCAP–specific monoclonal antibody, we confirmed that although expression could not be detected in naive CD8+ T cells directly ex vivo, BCAP was detectably expressed within 1 d of stimulation with plate-bound anti-CD3/anti-CD28 in vitro and further up-regulated by day 2 (Fig. 1 B). Moreover, analysis of BCAP expression in CFSE-labeled CD8+ T cells 1 d after stimulation revealed that BCAP could be detected in activated CD25+ cells even before initiation of cell division and thus is poised to influence early events in the clonal expansion and functional differentiation of CD8+ T cells (Fig. 1 C). Similarly, activated CD4+ T cells also up-regulated BCAP, and expression was higher when cells were cultured in Th1-polarizing conditions vs. Th2-polarizing conditions (Fig. 1 D). We also observed BCAP expression in human effector/memory CD8+ T cells, particularly in CD45RA−CCR7− TEm cells and in terminally differentiated CD45RA−CCR7− TemRA cells (Fig. 1 E).

Similar to what has been observed in macrophages and B cells, Western blot analysis of activated CD8+ T cells showed two dominant BCAP isoforms, a full-length ~97-kD isoform and a short ~64-kD isoform that lacks the N-terminal domain (Fig. 2 A). Additionally, as in activated B cells, BCAP was tyrosine phosphorylated in activated CD8+ T cells, and coimmunoprecipitation showed association with the p85 regulatory subunit of PI3K (Fig. 2 B and C). Thus, rapid induction of BCAP in activated CD8+ T cells may influence PI3K activation/signaling during T cell clonal expansion and effector/memory T cell differentiation.

**BCAP expression in CD8+ T cells is modulated by PI3K signaling, T-bet, and IRF4**

To identify factors that regulate BCAP induction upon T cell activation, we mined publicly available epigenetic and chromatin immunoprecipitation (ChIP) sequencing data to assess changes in chromatin structure and transcription factor binding that occur at the Pik3kap1 locus during T cell activation. Consistent with rapid BCAP up-regulation, CD8+ T cell activation and differentiation into effector cells were associated with opening of the Pik3ap1 locus at several sites identified by ATAC-seq analysis, and these sites were further decorated with H3K27Ac histone modifications, indicative of active enhancers (Fig. 3 A). This was particularly evident in the large intron between exons 2 and 3 of the Pik3kap1 gene. Interestingly, in naive CD8+ T cells the transcription factor Foxo1 is bound to multiple sites in the Pik3kap1 locus, and these overlap with several of the ATAC-seq peaks identified in this population. PI3K signaling in CD8+ T cell results in the Akt-mediated phosphorylation of Foxo1, leading to its nuclear exclusion and changes in expression of Foxo1-regulated genes. Thus, we hypothesized that induction of BCAP depends on PI3K-dependent inactivation of Foxo1, and that BCAP can therefore act in a positive feedback loop to amplify PI3K signaling during T cell activation. Indeed, we found that blocking PI3K signaling using the pan class I PI3K inhibitor ZSTK474 potently inhibited BCAP induction during CD8+ T cell activation in vitro, while having only minimal effects on cell proliferation or expression of other activation markers such as CD69 (Fig. 3 B). Additionally, RNA sequencing (RNA-seq) analysis of activated CD8+ T cells expressing a constitutively activated allele of Foxo1 showed significantly diminished up-regulation of the Pik3ap1 mRNA compared with control WT cells (Fig. 3 C). Elevated expression of BCAP in Th1 vs. Th2 polarized cells (Fig. 1 D) suggests that in addition to Foxo1, lineage-specific factors help control the level of BCAP expression in effector T cells. Differentiation of both TH1 cells and effector CD8+ T cells relies on expression of the transcription factor T-bet, and ChIP-seq analyses identified several active enhancers in the Pik3ap1 locus that were bound by T-bet in TH1 cells (Fig. 3 A; GEO accession no. GSE33802). Similarly, the transcription factor Irf4, which helps control effector and memory T cell differentiation, is also bound at the Pik3ap1 locus in activated T cells (GEO accession no. GSE49930), and consistent with a role for these transcription factors in promoting BCAP expression, activated T cells lacking either of these proteins showed impaired Pik3ap1 induction in RNA-seq analysis (Fig. 3 D; GEO accession nos. GSE68056 and GSE49929). Taken together, these data suggest that optimal induction of BCAP expression during T cell activation occurs in a multistep process that requires both the PI3K-dependent inhibition of Foxo1 and the cooperative functions of the effector transcription factors T-bet and Irf4.

**BCAP potentiates PI3K signaling in response to CD3 engagement in activated T cells**

During B cell activation, BCAP serves as a critical adaptor protein that links engagement of the BCR to activation of PI3K (Okada et al., 2000). In T cells, the molecular pathways linking the T cell receptor to activation of PI3K are poorly defined, and thus, we hypothesized that BCAP may function analogously in activated T cells to amplify PI3K signaling downstream of antigen receptor engagement. To test this, we assessed PI3K activation by measuring Akt and S6 phosphorylation in activated CD8+ T cells from WT or BCAP-deficient mice following restimulation with plate-bound anti-CD3. Consistent with BCAP expression only in activated T cells, there was no difference in the extent of PI3K-dependent S6 phosphorylation in anti-CD3–stimulated naive CD8+ T cells from WT and BCAP-deficient mice (now shown). By contrast, in activated CD8+ T cells, phosphorylation of Akt and S6 in response to anti-CD3 stimulation was significantly decreased in BCAP-deficient cells (Fig. 4, A and B). Importantly, no defect was observed in IL-2–mediated Akt–S6 phosphorylation in BCAP-deficient cells, indicating that loss of BCAP does not lead to a general defect in PI3K signaling (not shown).

To determine if BCAP modulates signaling during CD8+ T cell activation in vivo, we cotransferred OVA-specific TCR transgenic OT-1 cells from WT (CD45.1+) or BCAP-deficient (CD45.2+) OT-1 mice into intact CD45.1+/.2+ recipients and infected these mice 24 h later with recombinant LM–OVA. To further facilitate identification of the transferred cells and to correlate cell signaling events with different stages of cell division, the donor OT-1 cells were also CFSE labeled before transfer. At 54 h after infection, cells were harvested from the spleens of the recipient animals, and S6 phosphorylation in each donor T cell population was assessed by flow cytometry directly ex vivo (without any further in vitro restimulation). At this time point, proliferation was ob-
served in both WT and BCAP−/− OT-I cells, indicating that both populations had undergone antigen-driven activation. However, in each recipient mouse, the fraction of cells with high levels of S6 phosphorylation was greater in WT cells, demonstrating that loss of BCAP impairs antigen-dependent signaling in CD8+ T cells in vivo (Fig. 4, C and D).
BCAP modulates effector and memory T cell differentiation

The magnitude of PI3K signaling during CD8+ T cell activation is a critical factor that modulates their clonal expansion and differentiation into specific effector and memory cell populations. Importantly, differences in signals delivered in the initial phases of T cell activation can result in dramatic alterations in T cell phenotype and function in both the effector and memory phases of the response (Yang et al., 2011). Therefore, to analyze the cell intrinsic function of BCAP throughout the course of the CD8+ T cell response to infection, we cotransferred WT and BCAP-deficient OT-I+ cells into congenically marked recipients and subsequently infected with LM-OVA as described above. We then assessed the clonal expansion, tissue localization, phenotype, and function of each donor population at various times after infection. Examining cells at the peak of the effector response on day 7 after infection, we found that accumulation of BCAP-deficient OT-I cells was reduced approximately three- to fivefold in the spleen compared with WT OT-I cells (Fig. 5, A and B). More strikingly, formation of long-lived memory cells is severely impaired in the absence of BCAP, and contraction of BCAP+/- OT-I cells between days 7 and 42 after infection was ~10-fold greater than that observed for WT cells. Examining OT-I cells accumulating in the
lungs as a representative nonlymphoid tissue, this phenotype was even more dramatic, with an ~100-fold difference in the number of WT and BCAP-deficient OT-I cells present at this site at day 42 after infection.

Effector CD8+ T cells can be divided based on differential expression of the markers KLRG1 and CD127 into distinct populations with varying effector function, proliferative potential, and ability to form long-lived memory cells. Most extreme among these are the KLRG1+CD127lo TECs, which are potent effector cells but have little potential for long-term memory formation, and KLRG1−CD127+ MPECs, which are relatively poor effector cells but highly proliferative and give rise to long-lived memory cells capable of robust clonal expansion and effector differentiation upon secondary infection (Joshi et al., 2007). Additionally, KLRG1+CD127+ double-positive effector cells (DPECs) and KLRG1−CD127− early effector cells (EECs) have been described, but their functional significance and contributions to pathogen clearance and memory cell formation are not well defined. Consistent with the notion that strong PI3K signaling favors differentiation of TECs (Rao et al., 2010), at day 7 after infection we observed a significant reduction in the frequency of KLRG1+CD127− cells among BCAP-deficient OT-I cells compared with WT OT-I cells in both spleen and lungs (Fig. 5, C and D; and not shown). Similarly, the frequency of EECs was reduced among BCAP-deficient OT-I cells, whereas the proportion of DPECs was significantly increased in the BCAP-deficient population, and no difference in the frequency of MPECs was observed. Consistent with decreased TEC formation, BCAP-deficient OT-I cells also showed diminished...
Figure 5. **Loss of BCAP alters CD8+ T cell responses in vivo.** (A) CD8+ T cells from WT (CD45.1+) or Pik3ap1−/− (CD45.2+) OT-I mice were cotransferred into CD45.1+CD45.2+ recipient animals, which were subsequently infected with LM-OVA. Flow cytometry analysis of CD45.1 and CD45.2 expression by gated CD8+CD44hi effector/memory T cells from the spleens and lungs of the recipient animals at the indicated times after infection. Data are representative of eight mice per time point that were analyzed in two independent experiments. (B) Graphical analysis indicating the number of WT and Pik3ap1−/− CD8+ T cells (mean ± SD) recovered from the spleens and lungs of recipient mice at the indicated times after infection. n = 4 mice analyzed per group in a single experiment, representative of two individual experiments. (C) Flow cytometry analysis of KLRG1 and CD127 expression by gated WT and Pi3kap1−/− OT-I T cells as indicated in spleens of recipient mice at day 7 after infection. Data are representative of eight mice per genotype that were analyzed in two independent experiments. (D) Graphical analysis of the frequency of WT and Pi3kap1−/− splenic OT-I T cells (mean ± SD) with the indicated phenotype at day 7 after infection. n = 4 mice analyzed in a single experiment, representative of two individual experiments. Overall statistical significance is determined by two-way paired ANOVA, and multiple comparisons were performed using Sidak’s multiple comparisons test. *, P ≤ 0.05. (E) Flow cytometry analysis of IFN-γ and IL-2 production by gated splenic WT and Pi3kap1−/− OT-I T cells with or without SIINFEKL peptide restimulation as indicated. Data are representative of eight mice that were analyzed...
production of the proinflammatory cytokines IFN-γ and IL-2 following in vitro restimulation with the antigenic SIINFEKL peptide (Fig. 5, E and F).

Memory CD8+ T cells can also be divided into multiple functionally distinct populations that work in concert to protect from reinfection (Cui and Kaech, 2010). TCM cells recirculate through secondary lymphoid organs and act as a reservoir of antigen-specific cells that can be rapidly expanded upon secondary infection, whereas TEM cells recirculate through nonlymphoid tissues and have limited proliferative potential but display immediate effector function upon restimulation. Additionally, a subset of TEM cells retains effector cell characteristics such as high KLRG1 expression and low expression of CD62L and CXCR3 and CD27, and these effector-like TEM cells provide potent protective immunity (Olson et al., 2013). To determine how BCAP influences the development and persistence of these different memory cell populations, we examined CD62L and KLRG1 expression by WT and BCAP-deficient OT-I T cells in the spleens of mice at different times after infection with LM-OVA. As expected, at day 7 after infection, very few CD62L+ cells were seen in either population, and the vast majority of cells are KLRG1+ effector T cells. However, as early as day 14 after infection, KLRG1 expression was significantly lower among BCAP-deficient OT-I cells, and by day 21 after infection this was accompanied by a significant increase in the frequency of CD62L+ TCM cells (Fig. 6, A and B). Nevertheless, the absolute number of BCAP-deficient OT-I cells in each subset was substantially decreased relative to WT by day 21 after infection (Fig. 6 C). Consistent with impaired differentiation of effector-like TEM cells in the absence of BCAP, at days 21 and 42 the remaining BCAP-deficient KLRG1+ memory cells showed increased expression of the markers CD27 and CXCR3 and therefore display a less terminally differentiated phenotype relative to their WT counterparts (Fig. 6 D and data not shown). Thus, loss of BCAP-dependent signaling results in altered memory cell differentiation and maintenance. However, BCAP-deficient memory cells did not display any significant defects in TNF-α, IFN-γ, or IL-2 production at any of the memory time points examined (data not shown).

The differentiation and function of different CD8+ effector and memory T cell populations is driven by their expression of distinct combinations of key transcription factors, and among these the balance of the two T box transcription factors T-bet and Eomes plays a central role. Although partially redundant, T-bet and Eomes also have specialized functions. Whereas T-bet promotes the differentiation and function of CD8+ TECs, high levels of Eomes are associated with memory cell development and particularly with the differentiation of CD62L+ TCM cells (Banerjee et al., 2010). Therefore, we also assessed T-bet and Eomes expression by WT and BCAP-deficient OT-I T cells at different times after infection (Fig. 7). At day 7 after infection, WT and BCAP-deficient cells expressed equivalent levels of T-bet; however, levels of Eomes were higher in cells lacking BCAP. Moreover, consistent with their increased TCM phenotype, at day 21 after infection, Eomes expression was further increased in BCAP-deficient OT-I cells, which also displayed a small but significant decrease in expression of T-bet. Taken together, these data demonstrate that BCAP has a key role in the differentiation and function of effector and memory CD8+ T cell responses and that this is due in part to altered Eomes and T-bet expression.

**BCAP potentiates effector differentiation to low affinity TCR ligands**

Due to its ability to potentiate PI3K signaling downstream of TCR engagement, we hypothesized that loss of BCAP would have even more dramatic consequences on T cell activation and effector differentiation upon stimulation with low-affinity TCR ligands. To test this, we cotransferred naive WT and BCAP-deficient OT-I cells into congenically marked recipients as above and infected them with a series of LM strains expressing OVA variants with decreasing affinities for the OT-I TCR based on substitution of the N4 residue of the SIINFEKL peptide (Zehn et al., 2009). We then assessed the activation, clonal expansion, and effector differentiation of responding WT and BCAP-deficient OT-I cells. Infection with all of the LM-OVA variants led to activation of both WT and BCAP-deficient OT-I cells as assessed by CD44 up-regulation, and decreasing ligand affinity did not significantly impact the relative clonal expansion of the WT and BCAP-deficient OT-I cells (not shown). However, BCAP-deficient cells responding to the lowest-affinity T4 and V4 OVA-variants showed significantly increased proportions of KLRG1+CD62L+ cells at day 7 after infection (Fig. 8, A and B). Thus, by enhancing PI3K signaling, BCAP potentiates effector T cell differentiation in response to low-affinity ligands.

**Discussion**

The ability of CD8+ T cells to undergo robust clonal expansion and differentiation into specialized populations of effector and memory cells underlies adaptive immune responses to a wide range of intracellular pathogens. However, the signals that direct these processes and the mechanisms by which they are interpreted by responding CD8+ T cells remain incomplete. Here, we have demonstrated that the signaling adaptor BCAP is rapidly up-regulated in responding CD8+ T cells and that this molecule acts as a key regulator of CD8+ T cell activation and effector/memory differentiation.

Although BCAP is constitutively expressed in naive B cells, NK cells, and multiple myeloid cell populations, we and others have not detected expression in naive T cells (Okada et al., 2000; Yamazaki et al., 2002). However, BCAP was rapidly up-regulated upon stimulation both in vitro and in vivo, and through analysis of transcription factor binding to the Pik3ap1 locus in publicly available ChIP-seq data, we identified several signaling and transcriptional pathways that appear to regulate BCAP expression in responding T cells. Because up-regulation of BCAP

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**Table**

| In two independent experiments. (F) Graphical analysis of the frequency of WT and Pik3ap1+/− splenic OT-I T cells coproducing IFN-γ and IL-2 following SIINFEKL peptide restimulation. Linked points represent values from cotransferred WT and Pik3ap1+/− OT-I T cells recovered from individual recipient mice. n = 4 mice analyzed in a single experiment, representative of two individual experiments. Statistical significance is determined by paired Student’s t test. *P ≤ 0.05.
Figure 6. Altered CD8+ memory T cell development in the absence of BCAP. (A) Flow cytometry analysis of KLRG1 and CD62L expression by splenic WT and Pik3ap1−/− OT-I T cells gated as in Fig. 4A at the indicated times after LM-OVA infection. Data are representative of eight mice per time point that were analyzed in two independent experiments. (B) Graphical analysis of the frequencies of splenic WT and Pik3ap1−/− OT-I T cells with the indicated KLRG1−TEM, KLRG1−TEm, and TCM phenotypes at each of the indicated times after LM-OVA infection. n = 4 mice analyzed per time point in a single experiment, representative of two individual experiments. Overall statistical significance is determined by two-way paired ANOVA, and multiple comparisons were performed using Sidak’s multiple comparisons test. *, P ≤ 0.05. (C) Graphical analysis indicating the number of WT and Pik3ap1−/− CD8+ T cells (mean ± SD) of the indicated phenotypes recovered from the spleens of recipient mice at the indicated times after infection. Data are representative of eight mice per time point that were analyzed in two independent experiments. (D) Graphical analysis of the frequencies of CD27+CXCR3+ cells among gated KLRG1+ or KLRG1− splenic WT and Pik3ap1−/− OT-I T cells 21 d after LM-OVA infection. Linked points represent values from cotransferred WT and Pik3ap1−/− OT-I T cells recovered from individual recipient mice. Statistical significance was determined by paired Student’s t test. Data are representative of eight mice per genotype per time point that were analyzed in two independent experiments. *, P ≤ 0.05.
was blocked by inhibition of class I PI3K signaling, we propose that BCAP induction acts as a mechanism by which PI3K signaling is maintained or amplified following activation. Additionally, RNA-seq analysis of T-bet− or Irf4−deficient CD8⁺ T cells showed impaired induction of Pik3ap1 expression in the absence of these transcription factors. T-bet is required for differentiation and function of effector CD8⁺ T cells, and high expression of T-bet helps direct cells to the KLRG1⁺CD127⁻ TEC fate (Dominguez et al., 2015). Thus, induction of BCAP expression and consequent potentiation of PI3K signaling may be one mechanism by which T-bet contributes to TEC differentiation. Like BCAP, IRF4 is rapidly up-regulated upon T cell activation, and IRF4 acts in conjunction with Batf to drive CD8⁺ T cell expansion and effector differentiation (Man et al., 2013; Yao et al., 2013; Kurachi et al., 2014). However, unlike T-bet expression, we have found that IRF4 induction is independent of PI3K signaling (not shown). Therefore...

Figure 7. Decreased T-bet and increased Eomes expression in BCAP-deficient memory CD8⁺ T cells. (A and B) Flow cytometry analysis of T-bet and Eomes expression by splenic WT and Pik3ap1⁻/⁻ OT-I T cells gated as in Fig. 4 A on days (A) 7 or (B) 21 after LM-OVA infection. Data are representative of eight mice per time point that were analyzed in two independent experiments. (C) Graphical analysis of the geometric mean fluorescence intensity of T-bet and Eomes expression by WT and Pik3ap1⁻/⁻ OT-I T cells at days 7 and 21 after LM-OVA infection as indicated. Linked points represent values from cotransferred WT and Pik3ap1⁻/⁻ OT-I T cells recovered from individual recipient mice. n = 4 mice analyzed per time point in a single experiment, representative of two individual experiments. Statistical significance is determined by paired Student’s t test. *, P ≤ 0.05.

Figure 8. BCAP promotes effector cell differentiation to low-affinity TCR ligands. (A) Flow cytometry analysis of KLRG1 and CD62L expression by splenic WT and Pik3ap1⁻/⁻ OT-I T cells gated as in Fig. 4 A on day 7 after infection with LM-OVA strains carrying the indicated antigenic peptide variants. Data are representative of eight mice that were analyzed in two independent experiments. (B) Graphical analysis of the frequency of KLRG1⁻CD62L⁻ TCM cells among gated splenic WT and Pik3ap1⁻/⁻ OT-I T cells (mean ± SD) on day 7 after infection with the indicated LM-OVA variants. Points represent values from cells recovered from individual recipient mice. n = 4 mice analyzed per group in a single experiment, representative of two individual experiments. Overall statistical significance is determined by two-way paired ANOVA, and multiple comparisons were performed using Sidak’s multiple comparisons test. *, P ≤ 0.05.
Thus, BCAP is poised to impact each of these to interact with antigen-bearing dendritic cells in secondary lymphoid tissues. Importantly, up-regulation of BCAP occurs during which T cells display different behaviors within secondary lymphoid tissues. Notably, compared with other memory cell populations, these cells express relatively low levels of receptors for the cytokines IL-7 and IL-15 that support memory cell survival in vivo, and thus, it is not clear how these effector-like cells are maintained following pathogen clearance. Although in response to high-affinity peptide stimulation, BCAP-deficient T cells showed no impairment in induction of KLRG1, the rapid decline in the abundance of KLRG1+ memory cells in the absence of BCAP demonstrates that BCAP-dependent signaling is important for maintenance of these cells into the memory pool. One intriguing possibility is that rather than competing for homeostatic cytokines, effector-like memory cells are maintained by low-level TCR signaling/maintenance was observed with p100δ-deficient cells. In this regard, it is useful to compare the phenotype of BCAP-deficient T cells with that observed in CD8+ T cells with severe defects in PI3K signaling due to loss of the catalytic p1008 subunit (Gracias et al., 2016). Similar to what we observed with BCAP-deficient cells, OT-I cells lacking p100δ displayed a three- to fourfold decrease in clonal expansion in adoptive hosts following infection with LM-OVA or an OVA-expressing strain of influenza virus. However, in contrast to the further loss of BCAP-deficient memory cells, no decrease in memory cell formation/maintenance was observed with p100δ-deficient cells. Thus, rather than simply controlling the magnitude of PI3K signaling, BCAP may coordinate PI3K with other key signaling pathways engaged by TCR and cytokine and costimulatory receptor engagement to help dictate cell expansion, differentiation, and maintenance. In that regard, cross-talk between PI3K signaling and other key signaling nodes that control the proliferation, development, and survival of effector T cells has been observed, and these include the Wnt/β-catenin, NF-kB, and JAK/STAT pathways (Kim and Suresh, 2013).

Recently, a subset of effector-like KLRG1+CD27- cells was shown to persist into the memory phase following pathogen clearance, and these cells could provide potent protection from reinfection upon transfer to naive hosts (Olson et al., 2013). Importantly, compared with other memory cell populations, these cells express relatively low levels of receptors for the cytokines IL-7 and IL-15 that support memory cell survival in vivo, and thus, it is not clear how these effector-like cells are maintained following pathogen clearance. Although in response to high-affinity peptide stimulation, BCAP-deficient T cells showed no impairment in induction of KLRG1, the rapid decline in the abundance of KLRG1+ memory cells in the absence of BCAP demonstrates that BCAP-dependent signaling is important for maintenance of these cells into the memory pool. One intriguing possibility is that rather than competing for homeostatic cytokines, effector-like memory cells are maintained by low-level TCR signaling in response to self-peptide–MHC complexes, and that this tonic signaling is potentiated/amplified by BCAP-mediated signal coordination. Like KLRG1+CD27- memory cells in mouse, human
CD8+CD45RA+CCR7- T EMRA cells show potent effector function upon restimulation, are poorly proliferative, and have a terminally differentiated phenotype (Geginat et al., 2003). Notably, in our analysis of human CD8+ T cell subsets, T EMRA cells showed the highest level of BCAP expression. Analogously, BCAP expression and function may be therefore be required for the development and maintenance of CD8+ T EMRA cells in humans, an important memory subset with roles in diverse processes including protection from viral infection following vaccination, graft rejection, and delayed healing of bone fractures (Miller et al., 2008; Betjes et al., 2012; Reinke et al., 2013).

CD8+ T cells mediate immunity to a wide range of intracellular pathogens and tumors, and inappropriate CD8+ T cell responses can contribute to development of chronic infections, cancers, and autoimmune disease. Thus, there is an urgent need to continue defining the signals and molecular pathways that regulate expansion, diversification, and maintenance of effector and memory CD8+ T cells in order to harvest the power of CD8+ T cells in vaccination strategies and cellular therapies. Our identification of BCAP as a key regulator of PI3K signaling and effector/memory CD8+ T cell development helps define a new molecular pathway by which these processes are controlled, thus providing new opportunities for therapeutic manipulation of CD8+ T cells in a host of immune-mediated diseases.

Materials and methods

Mice

CS7BL/6 (B6) and CD45.1+ B6 congenic mice were purchased from the Jackson Laboratory and subsequently intercrossed to produce CD45.1+/CD45.2+ mice used as recipients in adoptive transfer experiments. BCAP-deficient mice lacking the Pk3api gene have been described (Yamazaki et al., 2002) and were backcrossed to CS7BL/6 mice for nine generations. OT-I TCR transgenic mice on the B6.CD45.1 background were provided by Dr. Mike Bevan (University of Washington, Seattle, WA) and crossed to BCAP-deficient mice to produce B6.CD45.2 BCAP-deficient OT-1 animals. Mice carrying a stop-floxed allele of the human FOXP1 gene lacking all Akt phosphorylation sites knocked into the Rosa26 locus have been described (Ouyang et al., 2012) and were crossed to mice expressing Cre under the distal-Lck promoter to generate animals in which all T cells express this constitutively active Foxo1 protein. All mice were bred and maintained at the Benaroya Research Institute, and all experiments were approved by the Institutional Animal Care and Use Committee of the Benaroya Research Institute.

Human cells

Human peripheral blood mononuclear cells were obtained from healthy donors participating in the Benaroya Research Institute Immune Mediated Disease Registry. Informed consent was obtained from all subjects according to Institutional Review Board–approved protocols at Benaroya Research Institute.

Cell culture and activation

T cells were activated and cultured in RPMI-1640 medium containing 10% FCS, 50 U/ml Penicillin, 50 μg/ml Streptomycin, 50 μg/ml Gentamycin, 1 nM Sodium Pyruvate, 10 μM Hesper, 2 mM L-glutamine, and 50 μM 2-Mercaptoethanol (complete RPMI). Purified naïve T cells were stimulated in 24-well plates (3–5 x 10^6 cells/well) precoated with the indicated concentrations of anti-CD3 (2C11; Bio-Xcell) and anti-CD28 (37.51; Bio-Xcell) in PBS in complete medium containing 50–100 U/ml recombinant murine IL-2 (eBioscience) for the indicated times. In some experiments, the class I PI3K inhibitor ZSTK474 (Sigma) was added as indicated. For restimulation experiments, cells were stimulated for 2 d with 1 μg/ml plate-bound anti-CD3/anti-CD28 + IL-2, then removed from stimulus and placed in complete media (without IL-2) overnight before restimulation with plate-bound anti-CD3 as indicated. For polarization of CD4+ T cells into TH1 and TH2 cells, total CD4+ T cells were activated with plate-bound anti-CD3 and anti-CD28 in media containing 200 U/ml IL-2 supplemented with 10 ng/ml IL-12 (eBioscience) and 20 μg/ml anti-IL-4 (11B1; Bio-Xcell) for TH1 polarization or 100 ng/ml IL-4 (eBioscience) and 40 μg/ml anti–IFN-γ (XMG1.2; Bio-Xcell) for TH2 polarization.

Flow cytometry and cell sorting

Cells were isolated from the spleen by mashing the tissue between glass slides followed by red blood cell lysis with ACK lysis solution (Gibco). Cell isolation from the lungs was performed as previously described (Sheih et al., 2017). Cell surface staining was performed with the following directly conjugated anti–murine antibodies (all antibodies were from BioLegend unless otherwise specified): anti-CD8 (53–6.7), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-CD45.1 (A20), anti-CD45.2 (104), anti–CD127 (A7R34), and anti–KLRG1 (2F1). For intracellular staining, cells were surface stained and then permeabilized with Fix Perm buffer (eBioscience). Cells were then washed and stained with antibodies against T-bet (4B10) and Eomes (Danilmag; eBioscience). Intracellular staining for BCAP was performed with an unlabeled mouse anti-BCAP antibody (4L8E6, purified from hybridoma) followed by PE- or APC-conjugated anti-mouse IgG1 secondary antibodies (A85-1). For intracellular cytokine staining, splenocytes were stimulated with 1 μM SII peptide in the presence of 10 μg/ml monensin in 1 ml of complete media for 6 h at 37°C, 5% CO2. Following stimulation, cells were harvested, surface stained, permeabilized with BD Fix/Perm, and stained with anti–IFN-γ (XMG1.2) and anti–IL-2 (JES6-5H4). To assess Akt and S6 phosphorylation, cells were stimulated with the indicated concentrations of plate-bound anti-CD3 for 90 min, harvested, and immediately fixed in Cytofix/Cytoperm buffer (BD Bioscience). After incubation for 30 min at room temperature, the cells were washed, resuspended in 400 μl 90% methanol, and incubated on ice for 30 min. After an additional wash, cells were stained for surface and intracellular antigens (including anti–pS6 [S235/236, D57.2.2E; Cell Signaling] and anti–pAkt [S473, D9E; Cell Signaling]) for 45 min at room temperature in the dark. To assess S6 phosphorylation directly ex vivo, spleens were immediately disrupted using glass slides into Cytofix/Cytoperm buffer (BD Bioscience), and staining proceeded as described above. Human cells were surface stained with the following directly conjugated antibodies: anti-CD4 (RPA-T4), anti-CD8 (SK1), anti–CXCR3 (IC6), anti–CD45RA (HI100), and anti–CCR7 (GO43H7). Following surface
staining, cells were permeabilized with FixPerm buffer (eBioscience), washed, and stained with antibodies against T-bet (4B10) and BCAP (4L8E6) as described above. Data were acquired on either LSRII or FACS Canto flow cytometers (BD Bioscience). For cell sorting, CD8+ T cells were enriched from the spleens of WT and BCAP-deficient mice by magnetic selection using anti-CD8 microbeads from Miltenyi, and CD8−CD44− naïve T cells were sorted on a FACSAria high-speed cell sorter (BD Bioscience).

Adoptive transfer and infection
CD8+ T cells from the spleens of B6.CD45.1+ and BCAP-deficient B6.CD45.2+ donor mice were isolated by magnetic selection using anti-CD8 microbeads (Miltenyi). Except as indicated, 5,000 WT and 5,000 BCAP-deficient OT-I T cells were cotransferred into CD45.1+/CD45.2− recipient mice by retro-orbital injection, and the recipient animals were infected the following day by retro-orbital injection of 10^6 CFU of LM-OVA as described previously (Koch et al., 2012). For ex vivo analysis of S6 phosphorylation, infection was as described, except that to facilitate identification of the transferred OT-I T cells, 5 × 10⁶ CFSE-labeled WT and 5 × 10⁶ CFSE-labeled BCAP-deficient OT-I cells were cotransferred into the recipient mice before infection.

Immunoprecipitation and Western blotting
B cells and CD8+ T cells were isolated from WT and BCAP-deficient mice by magnetic selection using anti-CD19 or anti-CD8 microbeads, respectively (Miltenyi). Splenic B cells were immediately lysed in lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.25% sodium deoxycholate, 1 mM PMSF, 1 mM sodium orthovanadate and protease inhibitor (mammalian protease inhibitor mixture; Sigma), and soybean orthovandate (1 mM; Sigma). CD8+ T cells were lysed in the same buffer following in vitro activation with plate-bound anti-CD3 and anti-CD28 (1 µg/ml each) + 100 U/ml rmIL-2 for 48–72 h as described above. Lysates were either used directly or sequentially incubated with the 4L8E6 anti-BCAP monoclonal antibody and protein A-agarose, and they were then eluted with 1× SDS sample buffer (Invitrogen). Samples were separated by Tris-bis SDS/PAGE gels (Invitrogen); transferred to polyvinylidene difluoride (Millipore) membrane; probed with the 4L8E6 anti-BCAP antibody, the 4G10 anti-pY antibody (Millipore), or a polyclonal anti-PISK p85 antibody (Cell Signaling) as indicated; and detected with the Immobilon chemiluminescence system (Millipore).

Statistical analyses
Comparisons between groups were analyzed using paired Student’s t tests or two-way ANOVA with Sidak’s multiple comparison tests as appropriate and as indicated in the figure legends and were performed using GraphPad Prism Software. Statistical significance was established at the level of P ≤ 0.05.

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References
Arimura, Y., H. Kato, U. Dianzani, T. Okamoto, S. Kamekura, B. Buonfoglio, T. Miyoshi-Akiyama, T. Uchiyama, and J. Yagl. 2002. A co-stimulatory molecule on activated T cells, H4/TCOS, delivers specific signals in T(H) cells and regulates their responses. Int. Immunol. 14:555–566. https://doi.org/10.1093/intimm/dxf022
Banerjee, A., S.M. Gordon, A.M. Intlekofer, M.A. Paley, E.C. Mooney, T. Lindsten, E.J. Wherry, and S.L. Reiner. 2010. Cutting edge: The transcription factor eomesodermin enables CD8+ T cells to compete for the memory cell niche. J. Immunol. 185:4988–4992. https://doi.org/10.4049/jimmunol.1002042
Best, J.A., D.A. Blair, J. Knell, E. Yang, V. Mayya, A. Doedens, M.L. Dustin, and A.W. Goldrath. Immunological Genome Project Consortium. 2013. Transcriptional insights into the CD8(+) T cell response to infection and memory T cell formation. Nat. Immunol. 14:404–412. https://doi.org/10.1038/ni.2536
Betjes, M.G.H., R.W.J. Meijers, E.A. de Wit, W. Weimar, and N.H.R. Litjens. 2012. Terminally differentiated CD8+ Temra cells are associated with the risk for acute kidney allograft rejection. Transplantation. 94:63–69. https://doi.org/10.1097/TP.0b013e31825306ef
Bruyns, E., A. Marie-Cardine, H. Kirchgesner, K. Sagolla, A. Shevchenko, M. Mann, F. Autschbach, A. Bensussan, S. Meuer, and B. Schraven. 1998. T cell receptor (TCR) interacting molecule (TRIM), a novel disulphide-linked dimer associated with the TCR-CD3-zeta complex, recruits intracellular signaling proteins to the plasma membrane. J. Exp. Med. 188:561–575. https://doi.org/10.1084/jem.188.3.561
Castello, A., M. Gaya, J. Tucholski, T. Oellerich, K.-H. Lu, A. Tafuri, T. Pawson, J. Wienands, M. Engelke, and F.D. Batista. 2013. Nck-mediated recruitment of BCAP to the BCR regulates the PI(3)K-Akt pathway in B cells. Nat. Immunol. 14:966–975. https://doi.org/10.1038/ni.2685
Chang, J.T., E.J. Wherry, and A.W. Goldrath. 2014. Molecular regulation of effector and memory T cell differentiation. Nat. Immunol. 15:1104–1115. https://doi.org/10.1038/ni.3031
Cui, W., and S.M. Kaech. 2010. Generation of effector CD8+ T cells and their conversion to memory T cells. Immunol. Rev. 236:151–166. https://doi.org/10.1111/j.1600-065X.2010.00926.x
Domínguez, C.X., R.A. Amexquita, T. Guan, H.D. Marshall, N.S. Joshi, S.H. Kleinstein, and S.M. Kaech. 2015. The transcription factors ZEB2 and T-bet cooperate to program cytotoxic T cell terminal differentiation in response to LCMV viral infection. J. Exp. Med. 212:2041–2056. https://doi.org/10.1084/jem.20150186
Fabre, S., F. Carrette, J. Chen, V. Lang, M. Semichon, C. Denoyelle, V. Lazar, N. Cagnard, A. Dubart-Kpperschmitt, M. Mangeney, et al. 2008. FOXO1 regulates L-Selectin and a network of human T cell homing molecules downstream of phosphatidylinositol 3-kinase. J. Immunol. 181:2980–2989.
Le Flèch, A., Y. Tanaka, N.S. Banlilan, G. Voisinne, G. Altan-Bonnet, Y. Fukui, and R.R. Parry. 2013. Annexin PI3K accumulation controls actin architecture and modulates cytotoxicity at the immunological synapse. J. Exp. Med. 210:2721–2737. https://doi.org/10.1084/jem.201313324

Lin, W.-H.W., W.C. Adams, S.A. Nish, Y.-H. Chen, B. Yen, N.J. Rothman, R. Kratchmarov, T. Okada, U. Klein, and S.L. Reiner. 2015. Asymmetric PI3K signaling driving developmental and regenerative cell fate bifurcation. Cell Reports. 13:2203–2218. https://doi.org/10.1016/j.celrep.2015.10.072

MacLean, A.W., IV, T. Yamazaki, M. Pang, L.J. Sigal, T. Kurosaki, and K.S. Campbell. 2008. Enhanced NK-cell development and function in BCAP-deficient mice. Blood. 112:131–140. https://doi.org/10.1182/blood-2007-08-107847

MacIntyre, A.N., D. Finlay, G. Preston, L.V. Sinclair, C.M. Waugh, P. Tamas, C. Suresh. 2012. Signal integration by Akt regulates CD8 T cell effector and memory. J. Immunol. 188:224–236. https://doi.org/10.1088/1016

Man, K., M. Miassari, W. Shi, A. Xin, D.C. Henstridge, S. Preston, M. Pellegrini, G.T. Belz, G.K. Smyth, M.A. Febbraio, et al. 2013. The transcription factor IRF4 is essential for TCR affinity-mediated metabolic programming and clonal expansion of T cells. Nat. Immunol. 14:1155–1165. https://doi.org/10.1038

Mempel, T.R., S.E. Henrichson, and U.H. Von Andrian. 2004. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature. 427:154–159. https://doi.org/10.1038/nature02238

Miller, J.D., R.G. van der Most, R.S. Akondy, J.T. Glidewell, S. Albott, D. Masopust, K. Murali-Krishna, P.L. Mahar, E. Edupuganti, S. Lalor, et al. 2008. Human effector and memory CD8+ T cell responses to smallpox and yellow fever vaccines. Proc. Natl. Acad. Sci. USA. 105:710–715. https://doi.org/10.1073/pnas.0709254

Moon, K.D., C.B. Post, D.L. Durden, Q. Zhou, P. De, M.L. Harrison, and R.L. Geahlen. 2005. Molecular basis for a direct interaction between the Syk protein-tyrosine kinase and phosphoinositide 3-kinase. J. Biol. Chem. 280:1543–1551. https://doi.org/10.1074/jbc.M407805200

Ni, M., A.W. MacFarlane IV, M. Toft, C.A. Lowell, K.S. Campbell, and J.A. Hamaerman. 2012. B-cell adaptor for PI3K (BCAP) negatively regulates Toll-like receptor signaling through activation of PI3K. Proc. Natl. Acad. Sci. USA. 109:267–272. https://doi.org/10.1073/pnas.111957108

Okada, T., A. Maeda, A. Iwamatsu, K. Gotoh, and T. Kurosaki. 2000. BCAP: The protein kinase B controls cell proliferation and differentiation. Biol. Chem. 381:1250–1260. https://doi.org/10.1016/S0034-0598(00)00295-7

Olonso, J.A., C. McDonald-Hyman, S.C. Jameson, and S.E. Hamilton. 2013. Ef- fector-like CD8+ T cells in the memory population mediate potent protective immunity. Immunity. 38:1250–1260. https://doi.org/10.1016/j.immuni.2013.05.009

Ouyang, W., W. Liao, C.T. Luo, N. Yin, M. Huse, M.V. Kim, M. Peng, P. Chan, Q. Ma, Y. Mo, et al. 2012. Novel Foxo1-dependent transcriptional programs control T(reg) cell function. Nature. 491:554–559. https://doi.org/10.1038

Págn, A.J., M. Pepper, H.H. Chu, J.M. Green, and M.K. Jenkins. 2012. CD28 promotes CD8+ T cell clonal expansion during infection independently of its YMNN and PY AP motifs. J. Immunol. 189:2909–2917. https://doi.org/10.1016/j.immuni.2013.05.009

Parry, R.V., C.A. Rumbley, L.H. Vandenberghe, C.H. June, and J.L. Riley. 2003. CD28 and inducible costimulatory protein Bc1 homology 2 binding domain- mains show distinct regulation of phosphoinositide 3-kinase, Bel-1, and IL-2 expression in primary human CD4 T lymphocytes. J. Immunol. 171:166–174.

Pleiman, C.M., W.M. Hertz, and J.C. Cambier. 1994. Activation of phosphatidylinositol-3’ kinase by Src-family kinase SH3 binding to the p85 subunit. Science. 263:1609–1612. https://doi.org/10.1126/science.8128248

Rao, R.R., Q. Li, K. Odunsi, and P.A. Shrikant. 2010. The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. Immunity. 32:67–78. https://doi.org/10.1016/j.immuni.2009.10.010

Rao, R.R., Q. Li, M.R. Gubbel Bupp, and P.A. Shrikant. 2012. Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8+ T cell differentiation. Immunity. 36:374–387. https://doi.org/10.1016/j.immuni.2012.01.015

Reinke, S., S. Geissler, W.R. Taylor, K. Schmidt-Bleek, K. Juelke, V. Schwach- meyer, M. Danhe, T. Hartwig, L. Akyüz, C. Meisel, et al. 2013. Terminally differentiated CD8+ T cells negatively affect bone regeneration in hu- mans. Sci. Transl. Med. 5:177ra36. https://doi.org/10.1126/scitranslmed.3004754

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Rodriguez-Viciana, P., P.H. Warne, R. Dhand, B. Vanhaesebroeck, I. Gout, M.J. Fry, M.D. Waterfield, and J. Downward. 1994. Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature. 370:527–532. https://doi.org/10.1038/370527a0

Sheih, A., W.C. Parks, and S.F. Ziegler. 2017. GM-CSF produced by the airway epithelium is required for sensitization to cockroach allergen. Mucosal Immunol. 10:705–715. https://doi.org/10.1038/mi.2016.90

Shi, J., T. Cinek, K.E. Truitt, and J.B. Imboden. 1997. Wortmannin, a phosphatidylinositol 3-kinase inhibitor, blocks antigen-mediated, but not CD3 monoclonal antibody-induced, activation of murine CD4+ T cells. J. Immunol. 158:4688–4695.

Shim, E.K., S.H. Jung, and J.R. Lee. 2011. Role of two adaptor molecules SLP-76 and LAT in the PI3K signaling pathway in activated T cells. J. Immunol. 186:2926–2935. https://doi.org/10.4049/jimmunol.1001785

So, L., and D.A. Fruman. 2012. PI3K signalling in B- and T-lymphocytes: New developments and therapeutic advances. Biochem. J. 442:465–481. https://doi.org/10.1042/BJ2012092

Troutman, T.D., W. Hu, S. Fulenchek, T. Yamazaki, T. Kurosaki, J.F. Bazan, and C. Pasare. 2012. Role for B-cell adapter for PI3K (BCAP) as a signaling adapter linking Toll-like receptors (TLRs) to serine/threonine kinases PI3K/Akt. Proc. Natl. Acad. Sci. USA. 109:273–278. https://doi.org/10.1073/pnas.1118579109

Wunderlich, L., A. Faragó, J. Downward, and L. Buday. 1999. Association of Nck with tyrosine phosphorylated SLP-76 in activated T lymphocytes. Eur. J. Immunol. 29:1068–1075. https://doi.org/10.1002/(SICI)1521-4141(199904)29:04%3C1068::AID-IMMU1068%3E3.0.CO;2-P

Yamazaki, T., K. Takeda, K. Gotoh, H. Takeshima, S. Akira, and T. Kurosaki. 2002. Essential immunoregulatory role for BCAP in B cell development and function. J. Exp. Med. 195:535–545. https://doi.org/10.1084/jem.20011751

Yang, C.Y., J.A. Best, J. Knell, E. Yang, A.D. Sheridan, H.S. Li, R.R. Rivera, K.C. Lind, L.M. D’Cruz, et al. 2011. The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets. Nat. Immunol. 12:1221–1229. https://doi.org/10.1038/ni.2158

Yao, S., B.F. Buzo, D. Pham, L. Jiang, E.J. Taparowsky, M.H. Kaplan, and J. Sun. 2013. Interferon regulatory factor 4 sustains CD8(+) T cell expansion and effector differentiation. Immunity. 39:833–845. https://doi.org/10.1016/j.immuni.2013.10.007

Zehn, D., S.Y. Lee, and M.J. Bevan. 2009. Complete but curtailed T-cell response to very low-affinity antigen. Nature. 458:211–214. https://doi.org/10.1038/nature07657