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Signal Integration by Akt Regulates CD8 T Cell Effector and Memory Differentiation

Eui Ho Kim,* Jeremy A. Sullivan,* Erin H. Plisch,* Melba Marie Tejera,* Anna Jatzek,* Kwan Yong Choi,† and M. Suresh*

During a T cell response, the effector CTL pool contains two cellular subsets: short-lived effectors (SLECs), a majority of which are destined for apoptosis, and the memory precursor effector cells, which differentiate into memory cells. Understanding the mechanisms that govern the differentiation of memory CD8 T cells is of fundamental importance in the development of effective CD8 T cell-based vaccines. The strength and nature of TCR signaling, along with signals delivered by cytokines like IL-2 and IL-12, influence differentiation of SLECs and memory precursor effector cells. A central question is, how are signals emanating from multiple receptors integrated and interpreted to define the fate of effector CTLs? Using genetic and pharmacological tools, we have identified Akt as a signal integrator that links distinct facets of CTL differentiation to the specific signaling pathways of FOXO, mTOR, and Wnt/β-catenin. Sustained Akt activation triggered by convergent extracellular signals evokes a transcription program that enhances effector functions, drives differentiation of terminal effectors, and diminishes the CTLs’ potential to survive and differentiate into memory cells. Whereas sustained Akt activation severely impaired CD8 T cell memory and protective immunity, in vivo inhibition of Akt rescued SLECs from deletion and increased the number of memory CD8 T cells. Thus, the cumulative strength of convergent signals from signaling molecules such as TCR, costimulatory molecules, and cytokine receptors governs the magnitude of Akt activation, which in turn controls the generation of long-lived memory cells. These findings suggest that therapeutic modulation of Akt might be a strategy to augment vaccine-induced immunity. The Journal of Immunology, 2012, 188: 4305–4314.

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this article, we posit that Akt integrates convergent signals from the TCR and receptors for cytokines like IL-2 and IL-12, and the magnitude of Akt activation dictates the differentiation of CTLs into terminal effectors or memory cells. These findings have significant implications for the development of vaccines and the treatment of T cell-dependent immune disorders.

Materials and Methods

Mice and viral infection

C57BL/6 (B6) mice were purchased from the National Cancer Institute or The Jackson Laboratory. Akt transgenic (tg) mice that express constitutively active Akt in T cells were a kind gift from Dr. Hotchkiss (Washington University, St. Louis, MO) (18). P14/Ly5.1 mice that express the tg TCR specific for the Dβ-restricted GP33 epitope of lymphocytic choriomeningitis virus (LCMV) (provided by Dr. Murali-Krishna, University of Washington, Seattle, WA) were crossed with Akt tg mice to derive the P14/Akt tg mouse. The IL-7R tg mice were kindly provided by Dr. Stoger (National Institutes of Health, Bethesda, MD) (19), which were crossed with P14/Akt mice to generate the P14IL-7R or P14/Akt/IL-7R tg mice. Mice were infected i.p. with 2 x 10^6 PFU LCMV Armstrong strain to induce an acute infection. Infectious LCMV was quantified by a plaque assay on Vero cells, as described previously (20). Recombinant vaccinia virus expressing the LCMV glycoprotein (VV-GP) (kindly provided by Dr. Whitton, Scripps Research Institute) (21) was injected i.p. at a dose of 2 x 10^6 PFU. Experiments were conducted in accordance with the approved protocols of the institutional animal care committee.

Adoptive transfer of P14 CD8 T cells

P14/Ly5.1 CD8 T cells were purified from spleen of P14/wild-type (WT), P14/Akt, P14/IL-7R, or P14/Akt/IL-7R mice using the MACS protocol (Miltenyi Biotec). Purified P14 CD8 T cells (10^6 cells) were adoptively transferred into congenic Ly5.2 B6 mice, as before (11, 22). At 1 d after cell transfer, recipient mice were infected with LCMV Armstrong.

Flow cytometry and cell surface staining

Mononuclear cells from spleen, liver, or lymph nodes were prepared using standard techniques. MHC class I tetramers, specific for the LCMV epitopes NP396-404 (NP396) and GP33-41 (GP33), were prepared and used as described previously (23). Briefly, cells were stained with anti-CD8 and MHC class I tetramers and/or anti-CD45.1 (Ly5.1). In some experiments, cells were costained with anti-CD44, anti-CD62L, anti-CD43, anti-CD69, anti-LFA-1, and anti-CD127. All Abs were purchased from BD Biosciences or eBioscience except the anti-KLRG1 Ab (Southern Biotech). Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (Tree Star).

Intracellular staining

For intracellular cytokine staining, splenocytes were stimulated ex vivo with LCMV epitope peptides in the presence of brefeldin A for 5 h. After culture, cells were stained for cell surface molecules and intracellular IFN-γ, TNF-α, and IL-2, using a Cytofix/Cytoperm intracellular staining kit (BD Biosciences). To stain for intracellular Bcl-2 and granzyme B, splenocytes were stained for cell surface molecules and subsequently permeabilized and stained for intracellular proteins using an Anti-Mouse Bcl-2 set (BD Biosciences) and anti-granzyme B Ab (Invitrogen).

Phospho-specific staining

Splenocytes were stained with anti-CD8 and anti-CD45.1 in conjunction with MHC class I tetramers. Following surface staining, cells were fixed, lysed, and washed using the PhosFlow Kit (BD Biosciences). After blocking with 10% goat serum in 2% BSA/PBS, cells were stained for intracellular phospho-specific proteins, including p-Akt (Ser^308), p-FOXO1/O3 (Thr^24), p-mTOR (Ser^2448), and Tcf1 at a 1:50 dilution of the Ab. As negative controls, cells were stained with phospho-specific Abs preincubated with the specific blocking peptide. Following incubation with primary Abs, cells were washed and incubated with an anti-rabbit Ab (Invitrogen). All phospho-specific Abs were purchased from Cell Signaling Technology. Staining for T-bet and Eomes was done as described above, and Abs were purchased from eBioscience.

In vitro activation and Western blot analysis

Splenocytes from B6 mice were enriched for CD8 T cells using the MACS protocol (Miltenyi Biotec). For in vitro activation, purified cells were stimulated for 2 d with plate-bound anti-CD3 (300 ng/ml) and anti-CD28 (100 ng/ml) Abs (Southern Biotech) in the presence or absence of IL-12 (10 ng/ml; R&D Systems). After activation, CD8 T cells were harvested and resuspended in a radioimmunoprecipitation assay buffer. An equal amount (40 μg/sample) of total protein was subjected to Western blot analysis. The HRP-conjugated Abs were detected by amplification with West-Zol (Intron Biotechnology) reagents and visualized by exposure to x-ray film. β-actin was used as an internal control for normalization.

Microarray and quantitative RT-PCR

Splenocytes from uninfected P14/WT or P14/Akt mice were stained with anti-CD8 and anti-Ly5.1, and CD8 T cells were sorted using a FACSaria II instrument (BD Biosciences). Purified Ly5.1 CD8 T cells from P14/WT or P14/Akt mice were transferred into B6 mice, which were subsequently infected with LCMV Armstrong. At day 8 postinfection, splenocytes were stained with anti-CD8, anti-Ly5.1, anti-KLRG1, and anti-CD127. Following staining, SLECs and MPECs were sorted using the FACSaria II instrument; the purity of the sorted cells was >95%. Total RNA was extracted from sorted cells by TRizol Reagent and was subsequently cleaned up using an RNeasy kit (Qiagen). RNA was reverse transcribed, and the cDNA was analyzed using the Affymetrix GeneChip Mouse Gene 1.0 ST Array at the University of Wisconsin Gene Expression Center. Statistically significant differences in gene expression were identified with Multiple Experiment Viewer 4.1 and Microarray Suite 6. The data discussed in this publication have been deposited in National Center for Biotechnology Information Gene Expression Omnibus and are accessible through Gene Expression Omnibus Series accession number GSE36168 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36168). For quantitative RT-PCR, cDNA was synthesized from the isolated RNA, using SuperScript III (Invitrogen). Equivalent amounts of cDNA (as determined by 18S RNA measurements by quantitative PCR) were amplified in 35 cycles of PCR with SYBR Green, using intron-spanning primers designed for each gene. Applied Biosystems 7300 Real-Time PCR System was used for this analysis.

Pharmacologic inhibitors and IL-2 treatment

Rapamycin (LC Laboratories) was diluted in sterile PBS and injected i.p. daily on days 1–35 after LCMV infection at a dose of 75 μg/kg body weight. A-443654, a selective inhibitor of Akt, was a kind gift from V.L. Giranda (Abbott Laboratories) (24). The Akt inhibitor A-443654 was diluted in 0.2% hydroxypropyl methylcellulose and administered s.c. twice per day for 8 d at a dose of 15 mg/kg/d. Mice were injected with 15,000 IU human rIL-2 (TECIN/Roche; provided by the National Cancer Institute) or PBS i.p. twice per day from days 0 to 7 after LCMV infection.

Statistical analysis

Experimental data were analyzed using commercially available statistical software (SigmaPlot). Groups were compared by Student t test, and significance was defined at p < 0.05.

Results

Dynamics of Akt phosphorylation in vivo in virus-specific CD8 T cells

First, we examined the in vivo dynamics of Akt phosphorylation in Ag-specific CD8 T cells during an acute LCMV infection. Akt phosphorylation (Thr^308) in LCMV-specific TCR tg P14 CD8 T cells peaked at days 5 postinfection, and subsided thereafter (Fig. 1A), which mirrored the kinetics for LCMV level in the serum (data not shown). These data, along with a previous report (15), suggest that the Akt phosphorylation can be triggered by antigenic stimulation in vivo.

Seminal studies have demonstrated that cytokines such as IL-2 and IL-12 promote the differentiation of SLECs by inducing the transcription factor T-bet (4, 8, 11, 25). Ahmed’s group (11) showed that CD8 T cells expressing high levels of CD25 receive sustained IL-2 signals, express high levels of T-bet, and differentiate into SLECs. Therefore, we evaluated the relationship between CD25 expression and Akt phosphorylation in Ag-specific CD8 cells during an acute LMCV infection. CD8 T cells with high CD25 expression clearly displayed higher levels of phosphorylation on Akt and its potential substrate mTOR, as well as increased levels of T-bet, as opposed to CD25<sup>lo</sup> CD8 T cells (Fig. 1B).
Moreover, continued IL-2 signaling induced by exogenous IL-2 treatment sustained the activation of Akt and mTOR, enhanced the expression of T-bet, and reduced the percentages of MPECs among P14 CD8 T cells. These data indicated that CA-Akt impaired the generation of memory CD8 T cells impaired generation of CD8 T cell memory and protective recall responses without affecting the primary expansion or effector functions.

Next, we investigated whether CA-Akt impaired the generation of memory CD8 T cells by disrupting the differentiation of MPECs. Data in Fig. 2F show that MPECs were barely detected among P14/Akt effector CD8 T cells at days 8 and 17 postinfection, as a consequence of a severe defect in the expression of the IL-7R (CD127). In addition, P14/Akt effector CD8 T cells displayed reduced IL-2 and CD122 expression, compared with those in P14/WT CD8 T cells (Fig. 2G). Thus, diminished IL-7- and/or IL-15-dependent survival signals might have contributed to the exaggerated loss of P14/Akt CD8 T cells (27).

**Sustained Akt activation dramatically alters the transcriptome of virus-specific CD8 T cells**

To decipher the transcriptional basis of CA-Akt actions, we compared the transcriptomes of P14/WT and P14/Akt naive and effector CD8 T cells. The transcriptomes of naive P14/WT and P14/Akt CD8 T cells were largely similar. However, marked differences in the expression of several key molecules were noted in P14/Akt effector CD8 T cells, suggesting that CA-Akt promoted expression of effector molecules but repressed pro-survival genes in effector CD8 T cells. The balance between the transcription factors T-bet and Eomes might control the fate of effector CD8 T cells: terminal differentiation versus memory (8, 28, 29). Fig. 3C illustrates the stepwise increase in expression levels of T-bet in P14/WT MPECs, P14/WT SLECs, and P14/Akt SLECs. Conversely, P14/WT MPECs, P14/WT SLECs, and P14/Akt SLECs displayed an incremental decline in the levels of Eomes (Fig. 3C). Note the striking increase in the T-bet/Eomes ratio upon activation by fas ligand.

**FIGURE 1.** In vivo dynamics of Akt phosphorylation in Ag-specific CD8 T cells. (A) Dynamic alterations in Akt phosphorylation (Thr308) measured by phosho-specific staining. Naive P14 (Ly5.1) cells were adoptively transferred into B6 mice (Ly5.2), which were subsequently infected with the Armstrong strain of LCMV. The FACS histograms showing p-Akt staining in (A) are gated on Ly5.1+P14 CD8 T cells from mice infected with LCMV Armstrong. Control represents staining with p-Akt Ab that was preblocked with the specific antigenic peptide. (B) Naive P14 CD8 T cells were transferred into congenic B6 mice and infected with LCMV Armstrong. Cohorts of mice were treated with IL-2 or the vehicle (PBS) daily between days 0 and 7 postinfection. At day 8 postinfection, p-Akt, p-mTOR, and T-bet levels in splenic P14 CD8 T cells were quantified as in (A). The histograms in (C) are gated on P14 CD8 T cells; data in (D) show the percentages of MPECs among P14 CD8 T cells in spleen (mean ± SD). (E) CD8 T cells purified from spleen of naive B6 mice were activated with anti-CD3 and anti-CD28 for 48 h in the presence or absence of IL-12. Shown are immunoblots of p-Akt (Ser473) and p-GSK3β (Ser9) directly ex vivo. Data are representative of two independent experiments, and the numbers are calculated change in mean fluorescence intensities. (C and D) Naive P14 CD8 T cells were transferred into congenic B6 mice and infected with LCMV Armstrong. Cohorts of mice were treated with IL-2 or the vehicle (PBS) daily between days 0 and 7 postinfection. At day 8 postinfection, p-Akt, p-mTOR, and T-bet levels in splenic P14 CD8 T cells were quantified as in (A). The histograms in (C) are gated on P14 CD8 T cells; data in (D) show the percentages of MPECs among P14 CD8 T cells in spleen (mean ± SD). (E) CD8 T cells purified from spleen of naive B6 mice were activated with anti-CD3 and anti-CD28 for 48 h in the presence or absence of IL-12. Shown are immunoblots of p-Akt (Ser473) and p-GSK3β (Ser9). Representative immunoblots from three independent experiments are presented; *p < 0.05.
The expression of Blimp-1, another regulator of T cell differentiation (30), was not different in P14/Akt SLECs (Fig. 3C). Alterations in gene expression induced by CA-Akt were confirmed by real-time PCR and/or flow cytometry (Fig. 3D–F). Taken together, these data suggested that CA-Akt likely drove terminal differentiation of P14 CD8 T cells and apoptosis by disrupting the balance between T-bet and Eomes, and repressing the expression of survival factors (CD127, CD122, and Bcl-2).

Sustained Akt phosphorylation inactivates FOXO transcription factors, reduces IL-7R expression, and impairs the survival of MPECs

The FOXO family of transcription factors, which drive the expression of CD62L, CCR7, and IL-7R genes in T cells (31), is a direct substrate of Akt. Phosphorylation of FOXOs by Akt results in their inactivation by cytoplasmic relocalization and consequent reduction in the expression of their target genes. Indeed, impaired expression of IL-7R on P14/Akt effector CD8 T cells was associated with CA-Akt–induced phosphorylation of FOXO1/03 (Fig. 4A). Because expression of IL-7Rα is necessary for generating long-lived memory CD8 T cells (32–34), we tested whether enforced expression of IL-7Rα would rescue P14/Akt effector CD8 T cells from exaggerated contraction. P14/Akt mice were crossed with IL-7R tg mice to derive the triple tg P14/Akt/IL-7R mice. The responses of P14/WT, P14/IL-7R, P14/Akt, and P14/Akt/IL-7R CD8 T cells to an acute LCMV infection are shown in Fig. 4B. As reported before, the expansion and contraction of P14/WT and P14/IL-7R CD8 T cells were similar (34). P14/Akt CD8 T cells displayed exaggerated contraction, but notably, the extent of contraction of P14/Akt/IL-7R CD8 T cells (∼120-fold) between days 8 and 36 postinfection was substantially reduced, compared with that of P14/Akt CD8 T cells (∼1900-fold). Strikingly, tg expression of IL-7R was clearly more effective in mitigating the loss of KLRG1low (MPECs) P14/Akt cells than of P14/Akt KLRG1high (SLECs) cells (Fig. 4C, 4D). In addition to the prominent rescue of KLRG1low P14/Akt CD8 T cells, IL-7R expression enhanced Ag-induced IL-2 production.
These data suggested that impaired expression of IL-7R, a likely consequence of FOXO inactivation (31), might underlie the loss of KLRG1LO P14/Akt CD8 T cells. Surprisingly, the increased survival of P14/Akt/IL-7R CD8 T cells was not associated with restoration of Bcl-2 expression to the levels observed in P14/WT cells (Fig. 4F). This result implies that IL-7R signaling might have protected P14/Akt CD8 T cells from apoptosis through a Bcl-2–independent mechanism.

Sustained Akt activation phosphorylates mTOR and exaggerates the loss of SLECs

Another important substrate for Akt is the tuberous sclerosis complex, which regulates the activity of mTOR complex 1. mTOR complex 1 signaling is known to promote differentiation of CD8 T cells into SLECs by a mechanism involving T-bet (8, 13), and in vivo inhibition of mTOR by rapamycin treatment enhances the number of memory CD8 T cells (13); however, it is unclear whether Akt regulates CTL differentiation in vivo via mTOR. Compared with effector P14/WT CD8 T cells, P14/Akt effector CD8 T cells clearly displayed increased phosphorylation (Ser2448) of mTOR (Fig. 5A), along with elevated levels of T-bet (Fig. 3F).

To test whether CA-Akt–induced activation of mTOR activity contributed to the loss of CD8 T cell memory, we adoptively transferred P14/WT or P14/Akt CD8 T cells into congenic B6 mice and treated these mice with rapamycin daily between days 1 and 35 after LCMV infection. Rapamycin treatment reduced the contraction of both KLRG1HI and KLRG1LO effector P14/WT CD8 T cells into SLECs (Fig. 5B–D). Of interest, rapamycin reduced the
contraction to a much greater degree in KLRG1HI P14/Akt CD8 T cells than in KLRG1LO P14/Akt CD8 T cells (Fig. 5C, 5D). However, rapamycin treatment did not rescue the IL-2–producing ability of P14/Akt CD8 T cells (Fig. 5E). These findings suggested that CA-Akt–driven mTOR activation accentuated the loss of KLRG1HI effector CD8 T cells, with less remarkable effects on the contraction of KLRG1LO cells. The effects of mTOR inhibition by rapamycin treatment also included downregulation of T-bet levels in P14/Akt effector CD8 T cells. Next, we investigated whether tg expression of IL-7R and mTOR inhibition would have additive or synergistic effects on the rescue of P14/Akt effector CD8 T cells. Of interest, inhibition of mTOR by rapamycin treatment did not further reduce the contraction of KLRG1LO P14/Akt/IL-7R CD8 T cells (Fig. 5F). Nor did tg expression of IL-7R enhance the prosurvival effects of rapamycin on KLRG1HI P14/Akt CD8 T cells (Fig. 5F). These data suggested that CA-Akt–induced impairment of the Wnt/β-catenin signaling pathway aggravated the survival and homeostatic proliferation of memory P14/Akt CD8 T cells.

Akt regulation of Wnt/β-catenin signaling pathway in CD8 T cells

Because the Wnt/β-catenin signaling pathway plays an important role in promoting the survival and homeostatic proliferation of memory CD8 T cells (12, 35, 36), and Akt is known to regulate this pathway (37), we tested whether sustained Akt signaling also repressed Wnt/β-catenin–induced transcription by quantifying the expression of target genes: Tcf1, Lef1, and myc. The expression of Tcf1, Lef1, and myc in P14/WT effector CD8 T cells was lower than in naive P14/CD8 T cells. Of interest, in P14/WT CD8 T cells, these genes showed reduced expression in SLECs compared with MPECs (Fig. 6A, 6B), which may explain the poor survival and homeostatic proliferation of SLECs. Importantly, the expression of these genes (Fig. 6A, 6B) and Tcf1 protein (Fig. 6C) in P14/Akt SLECs was lower than in P14/WT SLECs. These data suggested that CA-Akt–induced impairment of the Wnt/β-catenin signaling pathway aggravated the survival and homeostatic proliferation of memory P14/Akt CD8 T cells.

In vivo inhibition of Akt enhances EM CD8 T cells

Because sustained Akt activation promoted terminal differentiation of effector cells, we hypothesized that inhibition of Akt activity would impede this process and enhance the number of memory CD8 T cells. To test this hypothesis, we treated LCMV-infected mice with a selective Akt inhibitor, A-443654 (24), during the first 8 d postinfection. A-443654 reduced the phosphorylation of Akt substrates mTOR and FOXO1/O3 in CD8 T cells within 2 h in vitro (Supplemental Fig. 4). In vivo inhibition of Akt by treatment of LCMV-infected mice with A-443654 reduced mTOR phosphorylation and the T-bet/Eomes ratio in P14 effector CD8 T cells (Fig. 7A, 7B). At day 35 postinfection, the numbers of P14

**FIGURE 4.** Rescue of CA-Akt MPECs by tg expression of IL-7R. (A) At day 8 after LCMV Armstrong infection, phosphorylation on FoxO1/O3 (T24/T32) in donor P14/WT or P14/Akt CD8 T cells was quantified by phospho–specific staining. (B–F) CD8 T cells purified from P14/WT, P14/ IL-7R, P14/Akt, or P14/Akt/IL-7R mice were transferred into B6 mice, which were subsequently infected with LCMV. The numbers of total (B), KLRG1HI (C), and KLRG1LO P14 cells were quantified at different days after LCMV infection (D, mean ± SD). (E) The number of donor P14 CD8 T cells that produced both IFN-γ and IL-2 was assessed by intracellular cytokine staining. (F) Bcl-2 expression in donor P14 CD8 T cells.*p < 0.05, a significant difference between the P14/Akt and P14/ Akt/IL-7R groups. Data are representative of two independent experiments with four or five replicates.
CD8 T cells in the spleens of A-443654–treated mice were significantly \( (p = 0.052) \) higher than in the spleens of vehicle-treated mice (Fig. 7C). Notably, Akt inhibition led to a selective and significant increase in the number of P14 CD8 T cells that displayed an SLEC or a CD62LLO EM phenotype (Fig. 7D, 7E). From these data, we infer that Akt inhibition rescued a fraction of the SLECs from terminal differentiation and apoptosis, leading to their survival and subsequent transition into EM cells. However, it should be noted that the number of EM cells is greater than the number of SLECs in both vehicle- and A-443654–treated mice, which suggested that EM cells emerged from SLECs and MPECs. Although it is unlikely that A-443654 completely abolished Akt activity in CD8 T cells, these studies strongly suggested that effective therapeutic blockade of Akt is a viable approach to enhance vaccine-induced CTL memory.

**Discussion**

Although it is well established that effector cells differentiate into long-lived memory CD8 T cells (38–40), deciphering the mechanisms that govern the cell fate decision of effector CD8 T cells (death versus differentiation into memory CD8 T cells) is currently a topic of intense investigation. Several extrinsic factors (e.g., Ag, IL-2, and IL-12) and cellular signaling pathways (e.g., mTOR and Wnt/β-catenin) have been implicated in regulating differentiation of memory CD8 T cells (1, 3, 4, 8, 11, 12, 36, 41). However, we do not fully understand the signaling circuitry involved in orchestrating the differentiation of effector and memory CD8 T cells. Specifically, it is unclear whether diverse extracellular signals trigger coincident distinct biochemical pathways or if the differentiation phenotype develops from a signaling hub that then engages common downstream signaling pathways. A common theme in biochemical events triggered by the TCR, CD28, IL-2R complex, and IL-12R is activation of the PI3K/Akt pathway (15, 42). Understanding the role of the PI3K/Akt pathway in memory CD8 T cell differentiation is the focus of this article.

Our initial finding of differential Akt activation in CD25HI and CD25LO CD8 T cells that have disparate cell fates underpinned our hypothesis that the magnitude of Akt activation regulates the differentiation of these CD8 T cell subsets into terminal effectors and MPECs, respectively. In this article, we report that sustained Akt activation causes profound alteration of gene expression in effector CD8 T cells that is reminiscent of terminal differentiation,
consequent to the modulation of biochemical pathways including the FOXO, mTOR, and Wnt/ß-catenin. Conversely, inhibition of Akt activity led to a significant enhancement in the number of memory CD8 T cells. The key point of this article is that diverse extracellular signals converge to activate Akt, and differential Akt activation evokes the heterogeneity and disparate fate of effector cells. These findings have strong implications for the design of CD8 T cell-based vaccines against intracellular pathogens and cancer.

Studies from Ahmed’s group (11) have shown that the duration of IL-2 signaling controls the fate of effector cells during Ag-driven clonal expansion. The signaling basis for the preferential differentiation of CD25HI cells and CD25LO CD8 T cells into terminal effectors and memory CD8 T cells in vivo remains unknown. In this article, we link heterogeneity in CD25 expression to differential activation of Akt and the functional states of its downstream substrates mTOR and FOXO1/3 in vivo. In addition, we find that prolonged exposure to IL-2 sustains activation of Akt/mTOR and reduces the number of MPECs, presumably by promoting differentiation of SLEC. Taken together, these findings strengthen our hypothesis that sustained Akt activation promotes the differentiation of terminal effectors during a CD8 T cell response.

Recently, it was reported that Akt activation is required for development of CTL effector functions (16). Further, it was shown that inhibition of Akt activity in vitro not only diminished effector functions but also induced, in Ag-activated CD8 T cells, a phenotype reminiscent of memory CD8 T cells (16). Although these studies provided important insights, the role of Akt in the differentiation of effector and memory CD8 T cells was not formally tested in vivo. To mimic sustained Akt activation in vivo in CD8 T cells, we used CD8 T cells that express CA-Akt. Although sustained Akt activation in CD8 T cells did not affect clonal expansion or development of effector functions, we did observe that CA-Akt effector CD8 T cells have an abbreviated life span and fail to differentiate into long-lived memory cells. Kaech’s group (43) has reported similar findings, but the underlying mechanisms were not fully elucidated. The failure to form memory CD8 T cells as a consequence of CA-Akt was associated with profound reduction in IL-7R, CD122, and Bcl-2 levels in effector CD8 T cells. The expression of IL-7R could rescue a majority of the CA-Akt MPEC, but not all SLEC, from deletion, which suggests that sustained Akt activation compromises the survival of MPECs by ablating IL-7R expression. The dramatic loss of IL-7R expression induced in CA-Akt CD8 T cells is likely a consequence of impaired FOXO1-dependent transcription (31, 44). Surprisingly, restoration of IL-7R signaling did not rescue Bcl-2 expression in CA-Akt CD8 T cells, which suggests that downregulation of Bcl-2 could not be attributed to loss of IL-7R per se and might be a consequence of diminished levels of CD122 and/or STAT5 signaling triggered by exposure to cytokines like IL-15 (9, 43, 45). An important functional attribute of MPECs is the ability to produce IL-2 (7), and CA-Akt diminished the IL-2–producing ability of effector CD8 T cells. The enforced expression of IL-7R substantially rescued the IL-2–producing ability of CA-Akt MPECs, which implies that IL-7 promotes IL-2 production or the survival of IL-2–producing CD8 T cells.

One striking feature of the transcriptome of CA-Akt effector cells is the marked elevation in transcripts for the effector molecules IFN-γ, FasL, and perforin and the transcription factor T-bet.
T-bet is a unique transcription factor that promotes effector functions and possibly terminal differentiation of CTLs (4, 28). However, it is unclear whether development of effector functions is mechanistically linked to terminal differentiation of CTLs. It is quite possible that these two events are coincident because they are both regulated by the same factor, T-bet. Alternatively, switching of energy metabolism pathways due to execution of effector functions might control differentiation of CTLs (29). Because Akt blockade or T-bet deficiency impairs CTL effector functions (16, 28), it is likely that Akt promotes the expression of effector molecules by inducing T-bet, which also might drive differentiation of CTLs into terminal effectors. Currently, we do not know how T-bet accomplishes this differentiation of CTLs into terminal effectors, but possible mechanisms would include regulation of trafficking, metabolism, and survival. Like T-bet, the transcription factor Eomes is also known to promote CTL effector functions, but Eomes appears to oppose the proterminal differentiation effects of T-bet (28, 46). In CA-Akt CTLs, elevated levels of T-bet were associated with lower levels of Eomes, and consequently a marked increase in the T-bet/Eomes ratio, which is believed to favor terminal differentiation over memory (29). Because mTOR inhibition subdued T-bet expression and substantially mitigated the loss of CA-Akt effectors, it is conceivable that inhibited T-bet levels in CA-Akt effectors are linked to hyperactivation of mTOR and exaggerated loss of SLECs. Notably, another hallmark feature of CA-Akt effector cells is the striking reduction in levels of transcripts for Tcf1, Lef1, and myc genes, suggestive of diminished signaling via the Wnt/β-catenin pathway. Because Tcf1 has been reported to be essential for optimal expression of Eomes and CD122, the reduction in expression of these molecules in CA-Akt effector cells may be attributed to diminished Tcf1 levels (12). We propose that deletion of terminal effector cells is a consequence of a combination of mechanisms regulated by Akt activation, including disabling of FOXO/Wnt/β-catenin pathways and enhancement of mTOR activity. This, in turn, would be expected to alter cell survival, trafficking, and metabolism, culminating in the demise of SLECs.

Although our studies with CA-Akt CD8 T cells demonstrate that hyperactivation of Akt exacerbates the loss of effector CTLs, it was equally important to understand the physiological significance of Akt activation for memory development. Inhibition of Akt reduced the T-bet/Eomes ratio and effectively increased the number of memory cells, especially of the EM phenotype. It is possible that treatment with A-443654 mitigated differentiation of CTLs into terminal effectors; instead, they survived and differentiated into EM cells. More potent Akt inhibition might be needed to increase central memory CD8 T cells at the expense of terminal effector cells. Nevertheless, treatment with A-443654 augmented the number of EM cells that may play crucial roles in conferring effective immunity against mucosal pathogens and microbes like HIV (47).

The data presented in this paper suggest a model in which signals from the TCR and cytokine receptors converge on the Akt signaling pathway and the cumulative signal strength determines the amplitude of Akt activation, which in turn governs the differentiation of effector CD8 T cells. This “signal strength” model supports the progressive/decreasing potential model of T cell differentiation (5). We propose that Akt activation at a certain threshold fosters development of effector functions (16) without impeding the differentiation of MPECs and their descendant memory CD8 T cells. However, sustained activation of Akt (e.g., in CD25HIGH cells) drives differentiation of CD8 T cells into terminal effectors at the expense of MPECs by paralyzing a multitude of cell survival mechanisms. Thus, Akt functions as a cellular rheostat controlling distinct facets of the program that governs differentiation of Ag-activated CD8 T cells into terminal effector cells or memory CD8 T cells. In summary, therapeutic modulation of the Akt signaling pathway may be exploited to enhance vaccine-induced protective immunity or treat T cell-dependent inflammatory diseases.

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Disclosures
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References
Akt REGULATES EFFECTOR AND MEMORY CD8 T CELL DIFFERENTIATION


