Protein Kinase C-θ Is an Early Survival Factor Required for Differentiation of Effector CD8$^+$ T Cells[1]

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CD8$^+$ T cells are crucial for host defense against invading pathogens and malignancies. However, relatively little is known about intracellular signaling events that control the genetic program of their activation and differentiation. Using CD8$^+$ T cells from TCR-transgenic mice crossed to protein kinase C-θ (PKCθ)-deficient mice, we report that PKCθ is not required for Ag-induced CD8$^+$ T cell proliferation, but is important for T cell survival and differentiation into functional, cytokine-producing CTLs. Ag-stimulated PKC$\theta^{-/-}$ T cells underwent accelerated apoptosis associated with deregulated expression of Bcl-2 family proteins and displayed reduced activation of ERKs and JNKs. Some defects in the function of PKC$\theta^{-/-}$ T cells (poor survival and reduced Bcl-2 and Bcl-x$_L$ expression, CTL activity, and IFN-γ expression) were partially or fully restored by coculture with wild-type T cells or by addition of exogenous IL-2, whereas others (increased Bim$_{EL}$ expression and TNF-α production) were not. These findings indicate that PKCθ, although not essential for initial Ag-induced proliferation, nevertheless plays an important role in promoting and extending T cell survival, thereby enabling the complete genetic program of effector CD8$^+$ differentiation. The requirement for PKCθ in different types of T cell-dependent responses may, therefore, depend on the overall strength of signaling by the TCR and costimulatory receptors and may reflect, in addition to its previously established role in activation, an important, hitherto unappreciated, role in T cell survival. *The Journal of Immunology,* **2005, 175: 5126–5134.**

The CD8$^+$ T cell is crucial for host defense against pathogens and malignancies (1). Recent studies have shed light on the genetic program that controls the activation of CD8$^+$ T cells, their differentiation into effector and memory T cells, and their survival (2). Upon recognition of cognate Ag, naive CD8$^+$ T cells undergo a rapid clonal expansion that is tightly coupled to their activation and differentiation into effector CTL (1). CTLs mediate their effector functions by the secretion of cytokines, mainly IFN-γ and TNF-α, and cytotoxic effector molecules such as perforin and granzymes (3–5). After their activation and proliferation, the majority of CTLs ($\geq$90%) die during a contraction phase, whereas the remaining cells differentiate into memory T cells (6, 7). The magnitude and persistence of the memory CD8$^+$ T cells determine the efficiency of the recall response against invading pathogens, a critical issue for the design of effective vaccines (8).

Protein kinase C-θ (PKCθ),[3] a member of the Ca^{2+}-independent, novel PKC subfamily, which is expressed in a relatively selective manner in T cells, has emerged in recent years as a key enzyme involved in T cell activation. PKCθ is recruited to the central supramolecular activation cluster of the immunological synapse after the encounter of Ag-specific T cells with APCs (9, 10). The central role of PKCθ in T cell activation is well established by findings that PKCθ-deficient peripheral T cells display impaired in vitro proliferation and cytokine production in response to TCR/CD28 costimulation, defects associated with deficient activation of AP-1, NF-κB, and NFAT (11, 12).

Given the importance of CD8$^+$ T cells in protective immunity to pathogens and our limited understanding of the intracellular signaling pathways that control the fate of this T cell compartment, we addressed the role of PKCθ in CD8$^+$ T cell activation vs survival using a well-characterized system of Ag-specific T cells, i.e., OT-I TCR-transgenic (TCR-Tg) mice, whose CD8$^+$ T cells express an MHC class I-restricted OVA-specific TCR. Crossing these mice to PKCθ-deficient (PKC$\theta^{-/-}$) mice (11) allowed us to analyze the role of PKCθ both in vitro and in vivo. We found that PKCθ was not required for Ag-induced CD8$^+$ T cell proliferation, but was critical for their survival and differentiation into effector T cells. Thus, Ag-stimulated, PKCθ-deficient CD8$^+$ T cells failed to accumulate to the same extent as their normal counterparts and underwent enhanced cell death, which was associated with down-regulation of antiapoptotic proteins (Bcl-2 and Bcl-x$_L$), increased expression of the proapoptotic protein Bim$_{EL}$, and markedly impaired activation of the MAPKs ERK1/2 and JNK1/2 and differentiation into cytokine-producing CTLs. Furthermore, some of these impaired events were rescued by coculturing PKC$\theta^{-/-}$ T cells with wild-type (WT) T cells, whereas others were not. These findings reveal a novel role for PKCθ in CD8$^+$ effector cell survival and differentiation, via pathways that are either cytokine dependent or independent, and have potential implications for the development of memory CD8$^+$ cells.

Materials and Methods

Mice

PKC$\theta^{-/-}$ mice on a mixed 129/sVJ $\times$ C57BL/6 background (F_5 backcrossing to C57BL/6) were obtained from Dr. D. Littman (New York University School of Medicine, New York, NY) (11) and were backcrossed to C57BL/6J mice for three additional generations (F_8) in our animal facility.

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[3] Abbreviations used in this paper: PKCθ, protein kinase C-θ; ICCS, intracellular cytokine staining; PI, propidium iodide; Tg, transgenic; WT, wild type; qVD, q-Val-Asp; BAD, Bcl-associated death promoter.

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OT-I TCR-Tg mice were obtained from W. R. Health (Walter and Eliza Hall Institute, Victoria, Australia). These mice were crossed with PKC$\theta^{-/-}$ mice in our animal facility. C57BL/6J, B6.C-H2^{bm1}/ByJ (H-2K^{bm1}), B6.SJL-Ptprc^{a}/BoAiTac (B6.SJL), and B6.PL-Thy1^{a}/Cy (B6.PL) mice were purchased from The Jackson Laboratory. All mice were maintained under specific pathogen-free conditions in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International.

Abs and reagents

Anti-CD3 (2C11) and anti-CD28 (37.51) mAbs were purified from culture supernatants of the corresponding hybridomas. The following Abs were used for immunoblotting: anti-Bim (14A8; Chemicon International); anti-Bcl-2 (7; BD Pharmingen); anti-PKCθ (residues 21–217; Transduction Laboratories); and anti-Bcl-x_L, anti-IκBα, anti-p44/42 ERK (ERK1/2) and phospho-p44/42-specific (Thr202/Tyr204), anti-JNK1/2 and phospho-JNK1/2-specific (Thr183/Tyr185), anti-p38 and phospho-p38-specific (Tyr182), anti-Akt and phospho-Akt-specific (Ser473; all from Cell Signaling Technology. Abs for cell staining were obtained from BD Pharmingen and included PE-conjugated anti-Vα2 (B20.1), FITC-conjugated anti-Vβ5.1/2 (MR9-4), PerCP-Cy5.5-conjugated anti CD8α (Ly-2), PE-conjugated anti-CD25 (3C7), allophycocyanin-conjugated anti-CD90.2 (Thy-1.2), R-PE-conjugated anti-CD45.1 (Ly-5.1), anti-CD69 (H1.2F3), or anti-CD44 (IM7.8.1). PE-conjugated Abs to IL-2 (JES6-5H4), IFN-γ (XMG1.2), or TNF-α (MP6-XT22), used for intracellular cytokine staining (ICCS), were also obtained from BD Pharmingen. CFSE was purchased from Renovar. OVA was purchased from Worthington Biochemical. The general caspase inhibitor, q-Val-Asp (qVD) was purchased from Enzyme Systems. U0126 and SP600125 were purchased from Calbiochem. Annexin V-allophycocyanin and propidium iodide (PI) were obtained from BD Pharmingen and Sigma-Aldrich, respectively. CFA H37 Ra was purchased from Difco Laboratories.

T cell isolation, labeling, and culture

Primary CD8^{+} T cells were isolated from WT or PKC$\theta^{-/-}$ mice expressing or not expressing the OT-I TCR-Tg and were purified to >92% purity by magnetic bead isolation using a cell separation negative selection mixture kit (StemCell Technologies) according to the manufacturer's protocol. OT-I T cells were identified by FACS analysis as CD8^{+}, Vα2^{+}, and Vβ5^{+} cells. Primary CD4^{+} T cells were enriched by negative selection using a MACS system with rat anti-mouse CD8 plus B220 Abs (BD Pharmingen), followed by incubation with goat anti-rat Ig-coated magnetic beads (Miltenyi Biotec). The cells were washed twice and resuspended in IMDM medium supplemented with 10% FBS for culture or in PBS for i.v. injection into recipient mice. The engineered fibroblast APC line MEC.B7.SigOVA, which expresses B7-1 and the OVA$_{257–264}$ peptide (SIINFEKL) covalently linked to H-2K^{b}, has been described previously (13). For in vitro priming, MEC.B7.SigOVA cells were seeded at 1.2×10^{5} cells/well in 24-well plates and cultured overnight. The next day, wells were washed twice with medium, and CFSE-labeled OT-I cells (0.5×10^{6}) were added in 2 ml of medium. After 20 h of culture, the cells were removed by gentle pipetting and transferred to empty wells. On different days thereafter, the cells were collected, restimulated with OVA$_{257–264}$ peptide (10 μg/ml) for the indicated times, and analyzed as described in *Results*. Primary CD4^{+} and CD8^{+} T cells were left unstimulated or were activated with anti-CD3 (10 μg/ml) plus anti-CD28 (5 μg/ml) mAbs, followed by cross-linking with a goat anti-hamster Ig Ab (15 μg/ml) for the indicated times. In some experiments, CFSE-labeled WT and unlabeled PKC$\theta^{-/-}$ OT-I T cells were cocultured at a 1:1 ratio with the same total cell number (0.5×10^{6}) as in control cultures containing only WT or PKC$\theta^{-/-}$ cells. After the indicated time, the two populations were separated by FACS sorting and analyzed immediately as indicated or were cultured for an additional day in the presence of supernatant from the coculture and analyzed for survival on different days.

Retroviral transduction

The murine stem cell virus 2.2 vectors encoding Bcl-x_L-GFP or Bcl-2-GFP were provided by Dr. M. Croft (La Jolla Institute for Allergy and Immunology, San Diego, CA) and have been described previously (14). Platinum-E packaging cells (15) (0.5×10^{6}) in 3 ml of DMEM plus 10% FBS were plated in 60-mm culture dishes. After overnight incubation, the cells were transfected with 3 μg of retroviral plasmid DNA using FuGene 6 transfection reagent (Roche). After 20 h, the medium was replaced with DMEM plus FBS. Cultures were maintained for 24 h, and the retroviral supernatant was harvested, supplemented with 5 μg of polybrene, and used to transduce OT-I cells that had been preactivated on MEC.B7.SigOVA for 20 h. The cells were spun for 1 h (2000 rpm, 32°C) and then incubated for 6 h at 32°C and for 17 h at 37°C. This procedure was repeated the following day. The expression of GFP was determined by flow cytometry.

In vivo priming

OVA-specific CTLs were generated as previously described (13). Briefly, OT-I (CD45.1^{-},CD90.2^{+}) cells (2–5 $\times 10^{6}$) were labeled with CFSE and injected i.v. into B6.PL (CD90.2^{-}) recipient mice along with the same number of B6.SJL (CD45.1^{+},CD90.2^{+}) splenocytes, which served as an internal control. One day later, the mice were immunized either by s.c. injection of OVA (50 μg) in CFA (1/1, v/v) into the base of the tail or by cross-primed immunization, in which spleen cells were isolated from H-2K^{bm1} mice, irradiated (1500 rad), and incubated for 30 min at 37°C in the presence or the absence of 10 μg/ml OVA. The cells were washed, and 1×10^{7} cells were injected i.v. into B6.PL recipient mice. Three days after challenge, spleen and lymph node cells were isolated for FACS analysis. The transferred OT-I T cells were identified as CD8^{+},CD90.2^{+},CD45.1^{-} cells.

Flow cytometry

Cells were stained by incubating them in cold PBS containing 0.5% BSA, 0.05% sodium azide, and the relevant labeled Abs. For ICCS, cells were stimulated for 5 h with OVA$_{257–264}$ peptide (5 μg/ml) in the presence of brefeldin A (BD Pharmingen), and ICCS was performed using a Cytofix/Cytoperm Kit (BD Pharmingen) according to the manufacturer's protocol. To assess apoptosis, annexin V- and PI-labeled cells were analyzed with a FACSCalibur (BD Biosciences) and Cell Quest software.

Cytotoxicity assay

The in vitro cytotoxicity JAM assay was performed as previously described (16). Briefly, 0.5×10^{6} naive OT-I (WT or PKC$\theta^{-/-}$) T cells/well were stimulated with MEC.B7.SigOVA cells. After 3 days, live cells were isolated by a Lympholyte-M (Cedarlane Laboratories) gradient separation, and different numbers of effector cells were seeded in triplicate in 96-well, round-bottom plates. EL-4 target cells (1×10^{4}) were added to each well in the presence or the absence of OVA$_{257–264}$ peptide (0.1 μg/ml) and were pulsed for 5 h with [^{3}H]thymidine (1 μCi). Spontaneous ^{3}H retention was determined by adding medium instead of effector cells. After 4 h of culture, cells were collected on glass-fiber filters, and the ^{3}H label retained in live EL-4 cells was measured in a beta plate counter. The percentage of specific killing was calculated as follows: (spontaneous cpm − experimental cpm)/spontaneous cpm $\times$ 100.

Immunoblotting

Cells were lysed in 30 μl of lysis buffer containing 20 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, 0.1 mM EDTA, 100 mM NH_4SO_4, 30 mM β-glycerol phosphate, 1 mM Na_3VO_4, 0.75% Brij 58, and 10 μg/ml each of aprotinin and leupeptin. Equal amounts of proteins (25 μg) were separated on SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and then exposed to the appropriate Abs. Blots were developed using an ECL kit (Amersham Biosciences).

Results

Intact Ag-induced proliferation, but impaired survival, of PKC$\theta^{-/-}$CD8^{+} T cells

To examine the proliferative capacity of PKCθ-deficient CD8^{+} T cells, we initially assessed their OVA-induced proliferation in vivo. WT or PKC$\theta^{-/-}$ OT-I T cells labeled with CFSE were adoptively transferred to B6.PL (Thy1.1) recipients together with an equal number of splenocytes from B6.SJL (CD45.1^{+}) mice, which served as a reference population for normalizing the recovery of the transferred OT-I cells. Recipient mice were then immunized either with OVA-loaded H-2K^{bm1} spleen cells as a source of APCs (17) or with OVA plus CFA, and the proliferation of OT-I cells from recipient spleen and draining lymph nodes was analyzed after 3 days by CFSE dilution. Surprisingly, PKC$\theta^{-/-}$CD8^{+} T cells underwent roughly the same number of cell divisions (approximately seven) as their normal, WT counterparts after either cross-primed immunization (Fig. 1*A*) or the stronger, OVA/CFA immunization (Fig. 1*B*). However, the area of the CFSE peaks of the PKC$\theta^{-/-}$ T cells, which reflects the number of cells in each division cycle, was consistently and significantly smaller than that of

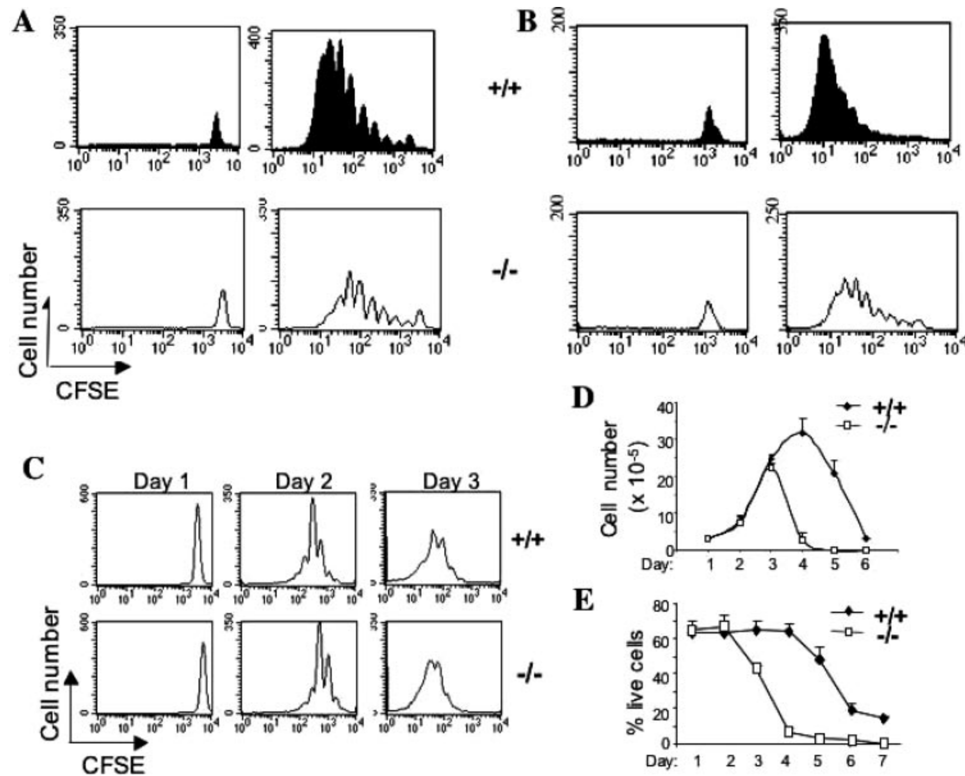

FIGURE 1. Intact proliferation but impaired accumulation of Ag-stimulated PKC$\theta^{-/-}$ OT-I cells. *A* and *B*, $CD8^+$ OT-I cells were isolated from spleen and lymph nodes of WT or PKC$\theta^{-/-}$ OT-I Tg mice and labeled with CFSE. Cells (5×10^6) were injected i.v. into naive B6.PL mice. As an internal control, B6.SJL ($CD45.1^+$) splenocytes were coinjected at a 1:1 ratio. One day later, the recipients were challenged with OVA-coated (stimulated; *right panels*) or uncoated (unstimulated; *left panels*) H-2K^{bm1} spleen cells (2×10^7; *A*) or with OVA plus CFA (*B*). Three days later, splenocytes (*A*) or draining inguinal and para-aortic lymph nodes (*B*) were harvested, and the proliferation of OT-I cells was analyzed by FACScan analysis of CFSE dilution after gating on live $CD8^+$,$CD90.2^+$ $CD45.1^-$ (OT-I) cells. Filled and empty histograms correspond to WT or PKC$\theta^{-/-}$ OT-I cells, respectively. *C–E*, CFSE-labeled cells isolated from WT or PKC$\theta^{-/-}$ OT-I mice were stimulated for 20 h in culture wells seeded with OVA-presenting MEC.B7.SigOVA, then transferred to empty wells. *C*, CFSE dilution of the cells was determined 1, 2, or 3 days after in vitro stimulation by FACScan analysis. Histograms represent gating on live $CD8^+V\alpha2^+$ (OT-I) cells. Results are representative of three experiments. *D*, Live cells, as determined by trypan blue exclusion, were counted on the indicated days. Results are displayed as the mean ± SE of six experiments. *E*, Cell survival was determined on the indicated days by staining with annexin V and PI. Results are displayed as the mean ± SE of eight experiments.

the WT T cells. The reduced accumulation of PKC$\theta^{-/-}$$CD8^+$ T cells was confirmed by direct cell counts (data not shown). In the absence of OVA stimulation, there was no significant difference in the number of OT-I T cells recovered from WT vs PKC$\theta^{-/-}$ mice. These findings suggest that the survival and, hence, the accumulation of PKC$\theta^{-/-}$$CD8^+$ OT-I T cells might be impaired. Similar differences were observed when analyzing WT vs PKC$\theta^{-/-}$$CD4^+$ T cells from TCR-Tg OT-II mice (data not shown).

We also analyzed the proliferation and accumulation of PKC$\theta^{-/-}$ OT-I T cells in vitro as previously described (13, 18). Proliferation was first observed 2 days after transfer to empty wells (Fig. 1, *C* and *D*). Both WT and PKC$\theta^{-/-}$ OT-I cells underwent the same number (five or six) of cell divisions 2–3 days after stimulation and expanded to a similar extent between days 1 and 3 (Fig. 1*D*). Live cell counts by trypan blue exclusion revealed a dramatic reduction in the number of PKC$\theta^{-/-}$ cells on day 4 of culture, and most of the PKC$\theta^{-/-}$ T cell loss occurred between days 3 and 4 (Fig. 1*D*). In contrast, WT T cells continued to accumulate until day 4, and their number declined thereafter. Direct assessment of apoptotic cell death by annexin V and PI staining revealed a marked decline in the number of surviving PKC$\theta^{-/-}$ OT-I cells between days 2 and 4, at which time <10% of the cells survived (Fig. 1*E*). In contrast, the first decrease in survival of WT T cells was observed only after 5 days of culture. Pretreatment of PKC$\theta^{-/-}$ T cells with qVD, a general caspase inhibitor, effectively blocked the accelerated death of PKC$\theta^{-/-}$ OT-I T cells (data not shown), confirming that a caspase-mediated apoptotic process is responsible for the defective survival of PKC$\theta^{-/-}$ cells. Thus, the similarity of the results obtained by in vivo and in vitro analyses of labeled OT-I T cells and the direct demonstration that PKC$\theta^{-/-}$ T cells undergo accelerated death indicate that PKCθ is required for promoting the survival of $CD8^+$ T cells, but not for their initial proliferation.

PKCθ controls the expression of Bcl family proteins

Proteins of the Bcl-2 family regulate the activation of caspase cascades in T cells, including in $CD8^+$ T cells (reviewed in Ref. 19). Bcl-2 and Bcl-x_L act as antiapoptotic proteins, whereas others, including Bax, Bak, and Bim, promote apoptosis. Additionally, the ratio between Bcl-2 and released Bim in activated T cells seems critical in determining the balance between T cell life and death (20). To evaluate the involvement of Bcl-2 family members in the enhanced death of PKC$\theta^{-/-}$ OT-I cells, we assessed the expression levels of several Bcl-2 family proteins in cultured $CD8^+$ T cells after stimulation with MEC.B7.SigOVA cells. In WT $CD8^+$ cells, Ag stimulation strongly up-regulated (beginning on days 1–2 of culture) the expression of both Bcl-2 and Bcl-x_L, which remained elevated until day 4 and gradually declined thereafter (Fig. 2*A*). The expression of Bim$_{EL}$ in WT T cells was low during the first 3 days of culture and increased on day 4 and later, preceding

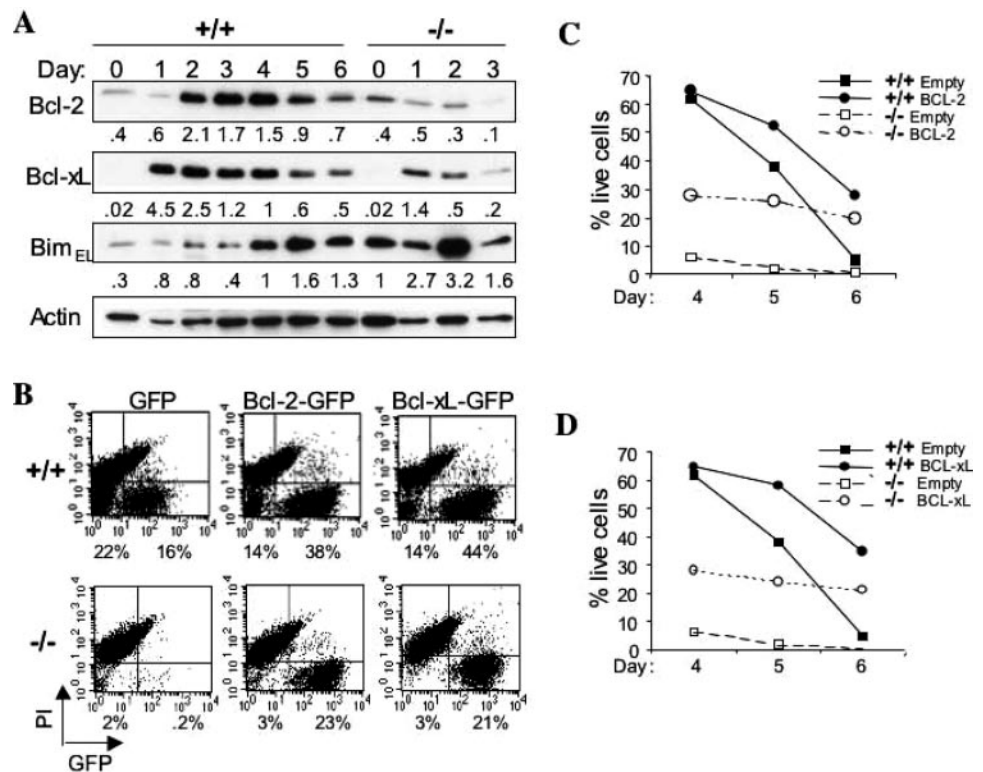

FIGURE 2. Expression of Bcl-2 family proteins and partial rescue of PKC$\theta^{-/-}$ OT-I cell survival by retroviral Bcl-2 or Bcl-x_L transduction. *A*, Cells stimulated and cultured as described in Fig. 1, *C–E*, were lysed, and the expression of the indicated proteins was analyzed by immunoblotting with the corresponding Abs. Relative densities of the protein signals normalized to the actin signal are shown. The data shown are representative of three similar experiments. *B*, PKC$\theta^{-/-}$ (−/−) or WT (+/+) cells were stimulated with MEC.B7.SigOVA cells for 20 h, transferred to empty wells, and infected on days 2 and 3 with retroviruses expressing GFP, GFP-Bcl-2, or GFP-Bcl-x_L. PI/GFP expression profile of the cells on day 5 of culture is shown. Numbers below each panel represent the percentage of live nontransduced (GFP^-,PI^-; *left*) or transduced (GFP^+,PI^-; *right*) cells. *C* and *D*, Time course of surviving (annexin V^-,PI^-) cells transduced with empty vector (GFP) or with Bcl-2 (*C*) or Bcl-x_L (*D*) between 4 and 6 days of culture. The results shown represent one of three similar experiments.

the onset of the contraction phase (see Fig. 1, *D* and *E*), by ~1 day. In contrast, PKC$\theta^{-/-}$ OT-I T cells displayed a greatly reduced Ag-induced expression of Bcl-2 and Bcl-x_L, which declined sharply on day 3 and, conversely, markedly enhanced basal and inducible (day 2) expression of the proapoptotic protein Bim_{EL} (Fig. 2*A*). No differences in the expression of Bax or Bak were observed between WT and PKC$\theta^{-/-}$ cells, and the low expression level of Bim_L (the long isoform of Bim) precluded meaningful assessment of its expression in the two $CD8^+$ populations (data not shown).

Retroviral transduction of Bcl-2 or Bcl-x_L partially rescues the survival of PKC$\theta^{-/-}$ OT-I cells

To examine whether the reduced expression of Bcl-2 and Bcl-x_L by PKC$\theta^{-/-}$$CD8^+$ T cells is causally related to their impaired survival, we transduced MEC.B7.SigOVA-stimulated PKC$\theta^{-/-}$ or PKC$\theta^{+/+}$ OT-I T cells with retrovirus expressing Bcl-2 or Bcl-x_L and a GFP marker protein under the control of a bicistronic promoter. Transduction efficiencies of 30–60% were routinely obtained in both WT and PKC$\theta^{-/-}$ T cells, as assessed by FACS analysis of GFP^+ cells (data not shown). As shown in Fig. 2*B*, ectopic expression of either Bcl-2 or Bcl-x_L partially rescued the survival of PKC$\theta^{-/-}$ OT-I T cells. Thus, although only 0.2% of the empty retrovirus-transduced PKC$\theta^{-/-}$ T cells were alive (i.e., PI negative) 5 days after Ag stimulation, 23 and 21% of the Bcl-2- or Bcl-x_L-transduced (GFP^+) cells, respectively, survived (Fig. 2*B*, *bottom panels*). Bcl-2 or Bcl-x_L transduction also increased the number of surviving WT T cells by 2- to 3-fold on day 5 (*top panels*), a time when apoptosis was already evident in these cells (see Fig. 1*E*). Fig. 2, *C* and *D*, shows the time course of $CD8^+$ T cell survival and recapitulates the survival-promoting effect of transduced Bcl-2 and Bcl-x_L in both PKC$\theta^{-/-}$ and WT cells. This effect was evident even on day 6, when only 0.4 and 5% of the empty retrovirus-transduced PKC$\theta^{-/-}$ or WT T cells, respectively, remained alive. The corresponding survival rates of the Bcl-2-transduced cells at this time were 20 and 28%, and those of the Bcl-x_L-transduced cells were 21 and 35%, respectively. This partial rescue of cells lacking PKCθ by overexpression of Bcl-2 and Bcl-x_L together with the impaired expression of these proteins in the PKC$\theta^{-/-}$ T cells (Fig. 2*A*) suggest that Bcl-2 and Bcl-x_L mediate in part the survival effect of PKCθ, and that the apoptotic death of PKC$\theta^{-/-}$ T cells is partially due to their inability to maintain adequate expression of these proteins as well as to the abnormally high expression of Bim_{EL}. We also analyzed the effect of a Bim_{EL}-specific inhibitory RNA, which reduced expression of the corresponding protein by ~50%, and found that this level of reduction had no detectable effect on the apoptosis of PKC$\theta^{-/-}$ OT-I T cells (data not shown). This lack of effect may be due to insufficient reduction of Bim_{EL} protein expression and/or to the very low expression of Bcl-2 and Bcl-x_L in these cells (Fig. 2*A*).

PKCθ is required for differentiation of $CD8^+$ effector T cells

To assess the influence of the *PKCθ* mutation on the activation and effector function of OT-I CTLs, we analyzed several parameters characteristic of effector $CD8^+$ cells. Expression of the activation markers CD25 and CD69 was analyzed after stimulation of isolated OT-I cells with MEC.B7.SigOVA cells. No obvious defect in the up-regulation of CD25 and CD69 expression was observed in PKC$\theta^{-/-}$ T cells during the first 2 days following stimulation (Fig. 3*A*; data not shown). However, a dramatic decrease in the expression of these activation markers was observed in PKC$\theta^{-/-}$ deficient T cells on day 3 of culture (Fig. 3*A*). Expression of the common IL-2R and IL-15R β-chain (CD122), which transduces important T cell survival signals (21–23), as well as the γ-chain (CD132) was not reduced either before or 1–3 days after activation in PKC$\theta^{-/-}$ OT-I T cells (data not shown).

Next, we examined the effect of the *PKCθ* mutation on the production of IL-2, TNF-α, and IFN-γ, using two experimental protocols. First, CFSE-labeled OT-I T cells were adoptively transferred into B6.PL (Thy1.1) recipients that were cross-primed 1 day

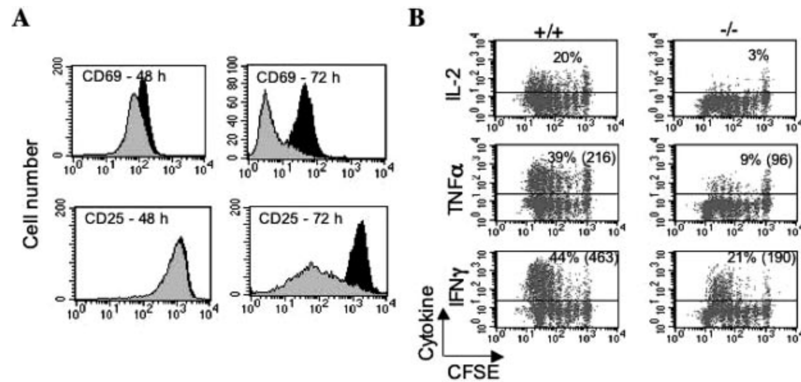

FIGURE 3. Activation and effector differentiation of PKC$\theta^{-/-}$ OT-I T cells. *A*, PKC$\theta^{-/-}$ or WT OT-I cells were stimulated with MEC.B7.SigOVA cells for 20 h, then transferred to empty wells. CD25 and CD69 expression was analyzed by flow cytometry (gating on live CD8$^+$ cells) after 2 or 3 days. Gray and black histograms correspond to PKC$\theta^{-/-}$ or WT cells, respectively. Results are representative of three experiments. *B*, B6.PL mice were injected with CFSE-labeled OT-I cells (5×10^6) from WT or PKC$\theta^{-/-}$ mice and challenged the next day with OVA-coated H-2K^{bm1} spleen cells (2×10^7). Three days later, splenocytes were harvested and restimulated with OVA$_{257–264}$ (SIINFEKL) peptide for 5 h in vitro, and cytokine-producing, gated OT-I T cells were enumerated by ICCS. The isotype control of ICCS staining was <0.1%. The percentage of IL-2-, TNF-α-, and IFN-γ-positive,CFSE$^+$ OT-I cells and the mean fluorescence intensity (MFI; in parentheses) are shown in each panel. ICCS staining of unstimulated control cells was <3%. The results shown represent one of three similar experiments

later by immunization with OVA-loaded H-2K^{bm1} spleen cells. Three days later, splenocytes were collected and restimulated in vitro with OVA$_{257–264}$ peptide for 5 h, and cytokine-producing OT-I T cells were enumerated by ICCS. CFSE labeling allowed us to analyze cell division in parallel. Fig. 3*B* demonstrates that the numbers of IL-2-, TNF-α-, and IFN-γ-producing PKC$\theta^{-/-}$ T cells were reduced by 85, 77, and 52%, respectively, compared with WT T cells. Moreover, there was a significant decrease in the mean fluorescence intensity of TNF-α (56%) and IFN-γ (59%) staining in the PKC$\theta^{-/-}$ T cells, suggesting a reduction in the amount of cytokine produced per cell. Using a different protocol, in which naive OT-I T cells were stimulated in vitro with OVA-presenting cells, we observed a generally similar pattern of differences in cytokine production between WT and PKC$\theta^{-/-}$ OT-I T cells (data not shown).

Coculture with WT T cells does not rescue all impaired functions of PKC$\theta^{-/-}$ T cells

As reported previously (11, 12) and also shown in this study (Fig. 3*B*), T cells lacking PKCθ are greatly impaired in their ability to produce IL-2. Cytokine deprivation can lead to apoptosis of activated T cells and down-regulation of Bcl-2 family antiapoptotic proteins, whereas administration of IL-2 family of cytokines can reverse these effects (24–26). Therefore, it was possible that the defects in survival and differentiation of PKC$\theta^{-/-}$CD8$^+$ T cells are attributable to the lack of IL-2 or other survival-promoting cytokines, which depend on PKCθ, rather than represent a more direct result of the *PKCθ* mutation. To address this possibility, we conducted similar experiments, in which we cocultured PKC$\theta^{-/-}$ T cells with WT T cells (which would provide potentially missing cytokines). Only one of the two cell populations in the coculture was labeled with CFSE to distinguish between the two populations and separate them by cell sorting. Various functional analyses were conducted after 3 days of coculture.

Interestingly, although coculture of PKC$\theta^{-/-}$ T cells with WT T cells markedly increased their survival, we consistently failed to observe complete rescue, such that the survival of cocultured PKC$\theta^{-/-}$ T was still reduced by 30–40% compared with that of their separately cultured or cocultured WT counterparts (Fig. 4*A*). Furthermore, coculture did not significantly reduce the high expression level of Bim$_{EL}$ protein in PKC$\theta^{-/-}$ T cells (Fig. 4*B*). In contrast, coculture restored the expression of both Bcl-2 and Bcl-x$_L$ in the same cells to levels equivalent to those observed in WT T cells (Fig. 4*B*). When we further analyzed effector cell differentiation in similar cocultures, we found that the presence of WT T cells did not restore the impaired TNF-α expression by cocultured PKC$\theta^{-/-}$ T cells, although it completely rescued the ability of these cells to express IFN-γ (Fig. 4*C*). Similarly, the nearly absent OVA-specific CTL activity of the sorted PKC$\theta^{-/-}$ T cells was fully restored by coculture with WT T cells (Fig. 4*D*). We obtained very similar results when instead of culturing PKC$\theta^{-/-}$ OT-I T cells with WT T cells, we supplemented them with exogenous rIL-2 (20–100 U/ml; data not shown). The only consistent difference between these two culture conditions was that in contrast to coculture with WT T cells, IL-2 addition failed to restore Bcl-x$_L$ expression in the PKC$\theta^{-/-}$ OT-I T cells to the level observed in WT T cells (data not shown), suggesting that a cytokine distinct from IL-2 expressed by the cocultured WT T cells or cell-cell contact is responsible for up-regulating Bcl-x$_L$. Therefore, although cytokine (IL-2?) deficiency appears to account for some of the functional deficiencies of PKC$\theta^{-/-}$CD8$^+$ T cells (i.e., reduced Bcl-2 and Bcl-x$_L$ expression, IFN-γ production, and CTL activity), other defects, i.e., the greatly enhanced Bim$_{EL}$ expression and the reduced TNF-α production, seem to reflect more direct effects of the *PKCθ* mutation, including the possibility of impaired use of and response to cytokines (derived from WT T cells) by the PKC$\theta^{-/-}$CD8$^+$ T cells. As for survival, the substantial, but consistently incomplete, restoration of PKC$\theta^{-/-}$ T cell survival by transduced Bcl-2 or Bcl-x$_L$ (Fig. 2, *B–D*) or by coculture with WT T cells (Fig. 4*A*) suggests that PKCθ promotes T cell survival in a more complex manner by regulating both cytokine-dependent and -independent survival pathways (see *Discussion*).

Impaired ERK and JNK activation in PKC$\theta^{-/-}$CD8$^+$ T cells

To determine whether the impaired effector differentiation of PKC$\theta^{-/-}$ OT-I T cells reflects defects in intracellular signaling pathways, we analyzed the activation of several signaling proteins known to be important for T cell activation and/or survival (Fig. 5). Although OVA$_{257–264}$ peptide restimulation of primed WT OT-I T cells induced clear stimulation of ERK1/2, which peaked after 30 min, only minimal ERK1/2 activation (that could be detected by loading a higher amount of protein or by longer exposure

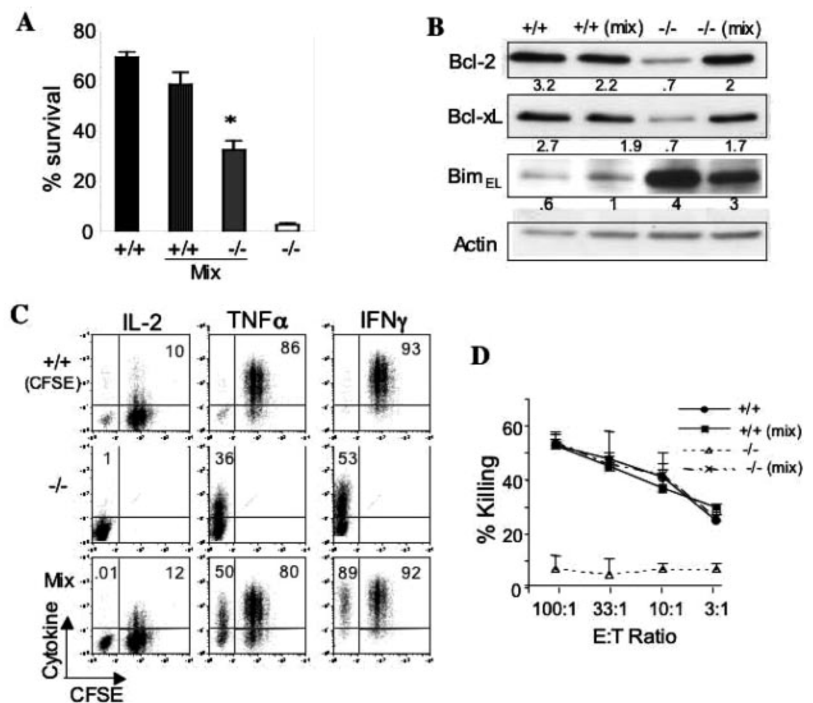

FIGURE 4. Effect of coculture with WT T cells on the survival, Bcl-2 family protein expression, and effector differentiation of PKC$\theta^{-/-}$ OT-I cells. Unlabeled PKC$\theta^{-/-}$ (−/−) and/or CFSE-labeled WT (+/+) cells were stimulated separately or in coculture (mix) with MEC.B7.SigOVA cells for 20 h, transferred to empty wells, and cultured for 3 additional days. Cocultured PKC$\theta^{-/-}$ and WT T cells were separated by FACS sorting. *A*, Survival was assessed in each group as described in Fig. 1*E*. *B*, Cells were lysed, and the expression of the indicated proteins was analyzed by immunoblotting with the corresponding Abs. The relative densities of the protein signals normalized to the actin signal are shown. Data are representative of two similar independent experiments. *C*, The cells were rested for 1 additional day, then restimulated with $OVA_{257–264}$ for 5 h, and cytokine-producing cells were enumerated by ICCS. The percentage of IL-2-, TNF-α-, and IFN-γ-positive,CFSE$^+$ OT-I cells is shown. The isotype control of ICCS staining was <0.1%, and unstimulated control cells were <5% cytokine positive. *D*, Live cells were recovered on day 4 of culture, and specific killing of $OVA_{257–264}$ peptide-loaded EL-4 target cells was determined at the indicated E:T cell ratios.

of the film; data not shown) was detected in the PKC$\theta^{-/-}$ cells (Fig. 5*A*). Similarly, JNK1/2 was markedly activated (peaking at 15 min) in WT T cells, but only minimally in their PKC$\theta^{-/-}$ counterparts. However, we did not observe significant differences in p38 activation between WT and PKC$\theta^{-/-}$ OT-I T cells (data not shown). As an additional control, the activation of Akt in PKC$\theta^{-/-}$ OT-I T cells was intact compared with that in WT T cells. Control experiments confirmed the previously reported (11, 12) impaired NF-κB activation in PKC$\theta^{-/-}$ cells, as indicated by undetectable degradation of IκBα in these cells, as well as the expected absence of PKCθ (Fig. 5*A*). A similar pattern of results manifested by reduced or less persistent activation was obtained when we analyzed MAPK activation in naive, $OVA_{257–264}$-stimulated OT-I cells (data not shown) or when we restimulated the cells 1 day after the initial in vitro stimulation (data not shown). These findings indicate that PKCθ plays an important role in the activation of ERK and JNK during Ag-induced (as opposed to anti-CD3/CD28-induced (11, 12)) activation and differentiation of CD8$^+$ T cells.

Given the reported intact activation of ERK and JNK in naive, unfractionated PKC$\theta^{-/-}$ T cells stimulated by anti-CD3/CD28 Abs (11), we examined whether our different findings can be explained by the use of purified CD8$^+$ T cells and/or specific peptide stimulation. We purified CD4$^+$ and CD8$^+$ T cell populations from WT or PKC$\theta^{-/-}$ mice and stimulated them with cross-linked anti-CD3 plus anti-CD28 Abs. Consistent with the previous findings (11), we observed that this mode of stimulation induced similar activation of ERK and JNK in CD4$^+$ and CD8$^+$ T cells from either WT or PKC$\theta^{-/-}$ mice (data not shown). Thus, we conclude that the use of specific peptide stimulation (as opposed to anti-CD3/CD28 Abs) reveals impaired ERK and JNK activation in the naive CD8$^+$ T cell compartment of PKC$\theta^{-/-}$ mice. Furthermore, the residual ERK and JNK activation observed in PKC$\theta^{-/-}$ OT-I T cells is apparently sufficient to drive their initial proliferation (Fig. 1), but not to sustain it or to maintain the cell survival required for full differentiation into effector T cells.

Effects of ERK and JNK inhibitors on survival and expression of apoptosis-related proteins

To determine whether the impaired ERK and JNK activation in Ag-stimulated, PKC$\theta^{-/-}$ OT-I T cells can account for their defective survival, we studied the effects of selective pharmacological inhibitors of ERK, U0126 (27) or JNK, SP600125 (28), on the survival of WT CD8$^+$ T cells and on the expression of Bcl-2 family proteins. OT-I T cells were stimulated for 20 h with MEC.B7.SigOVA in the absence or the presence of the inhibitors (10 μM), then were transferred to empty wells lacking or containing, respectively, the same inhibitors. Survival and protein expression were analyzed on different days of culture. Control experiments confirmed the specific inhibition of Ag-induced ERK and JNK activation by U0126 (Fig. 5*B*) or SP600125 (data not shown), respectively.

Consistent with our previous findings (Fig. 1*E*), PKC$\theta^{-/-}$ OT-I cells underwent accelerated and enhanced apoptosis (Fig. 5*C*). Interestingly, pharmacological inhibition of ERK in WT T cells resulted in a survival curve that largely mimicked that of untreated PKC$\theta^{-/-}$ T cells. The ERK inhibitor also reduced the low (~20%) survival level of PKC$\theta^{-/-}$ T cells to a nearly undetectable level. Inhibition of JNK by SP600125 caused a massive decline in the number of both WT and PKC$\theta^{-/-}$ surviving T cells by day 2, at which time only 20 and 10% of the cells, respectively, survived (Fig. 5*C*). Several interesting effects of the same inhibitors were observed when the expression of Bcl-2, Bcl-x_L, or Bim_{EL} in WT T cells was analyzed (Fig. 5*D*). The ERK inhibitor (U0126) completely reversed the Ag-induced up-regulation of Bcl-2, suppressed the expression of Bcl-x_L on day 3 of culture, and induced a marked increase in Bim_{EL} expression throughout the duration of the culture (Fig. 5*D*, *left*). In contrast, the JNK inhibitor (SP600125) did not affect the expression Bcl-2 or Bcl-x_L, but strongly increased the level of Bim_{EL}, particularly on day 2 (Fig. 5*D*, *right*). The accelerated death of CD8$^+$ T cells induced by ERK or JNK inhibition, the reduced Bcl-2 and Bcl-x_L expression induced by the ERK inhibitor, and,

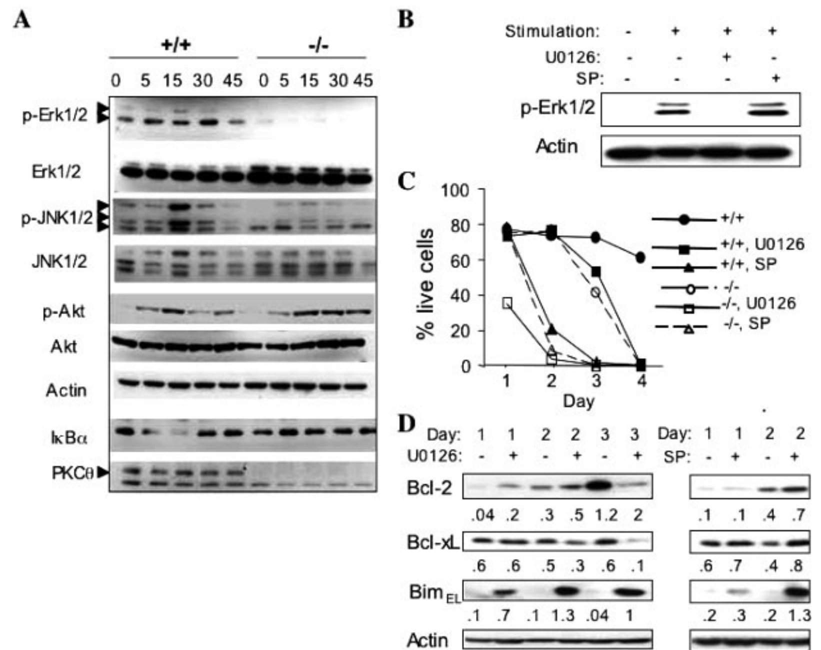

FIGURE 5. Impaired ERK and JNK activation in primed PKC$\theta^{-/-}$ OT-I cells and its implication to cell survival. *A*, Purified OT-I cells preactivated with MEC.B7.SigOVA cells for 20 h and cultured for 2 days in empty wells were restimulated with $OVA_{257-264}$ peptide (10 μg/ml) for the indicated times. Activation of ERK, JNK, and Akt was determined by immunoblotting with the corresponding phospho-specific Abs, and total expression levels of ERK, JNK, Akt, IκBα, PKCθ, and actin were also analyzed. Data are representative of six similar experiments. *B*, Naive WT OT-I cells pretreated without or with 10 μM U0126 or SP600125 (SP) for 1 h were stimulated with MEC.B7.SigOVA cells for 10 min. Aliquots of cell lysates were immunoblotted with phospho-ERK- or actin-specific Abs. *C*, Isolated WT OT-I cells were stimulated as described in *A* without or with U0126 or SP. After 20 h, the cells were transferred to empty wells, and survival was determined on the indicated days. The results shown are representative of three similar experiments. *D*, Expression of the indicated proteins was analyzed by immunoblotting with the corresponding Abs. The relative densities of the protein signal normalized to the actin signal are shown.

conversely, the increased Bim_{EL} expression caused by inhibition of ERK or JNK suggest that ERK and/or JNK are involved in the mechanism(s) by which PKCθ activation regulates the expression of Bcl-2, Bcl-x_L, and Bim_{EL} and promotes T cell survival. We attempted to rescue the impaired survival of PKC$\theta^{-/-}$ T cells by transducing the cells with constitutively active plasmids of MEK (to activate ERK) or a JNK kinase 1-JNK1 fusion protein (an active form of JNK1), but due to low transduction and expression efficiencies of the transduced proteins, we were unable to derive meaningful conclusions.

Discussion

Early functional characterization of fresh PKC$\theta^{-/-}$ T cells, which relied for the most part on assessing their in vitro proliferative responses by thymidine uptake, concluded that PKCθ is required for T cell activation and proliferation (11, 12), leading to the general belief that PKCθ would be globally required for all T cell-dependent immune responses. However, until recently, characterization of in vivo immune responses of PKC$\theta^{-/-}$ mice has been lacking. Very recent studies assessing in vivo responses of PKC$\theta^{-/-}$ mice to various Ags revealed a more complex picture. Thus, certain T cell-dependent immune responses, such as anti-lymphocytic choriomeningitis virus CTL function and anti-vesicular stomatitis virus Ab production (29), Th1-dependent protection against *Leishmania major* infection (30), or Th1-mediated lung inflammation (31), were largely intact, whereas other responses, e.g., peptide-induced CTL responses (29), Th2-mediated allergic lung inflammation (30, 31), or Th2 immunity to *Nippostrongilus* infection (30), were severely impaired. Thus, it is clear that there is a differential requirement for PKCθ for various immune responses.

Given that previous analysis of T cell proliferation did not reveal whether suppressed thymidine uptake by PKC$\theta^{-/-}$ T cells reflected impaired proliferation or enhanced cell death, we directly measured cell survival vs proliferation of these cells by annexin V/PI staining or CFSE dilution, respectively. One novel aspect of our study is that the survival of Ag-stimulated PKC$\theta^{-/-}$CD8$^+$ T cells was grossly impaired due to accelerated and enhanced apoptotic cell death, which was blocked by the caspase inhibitor qVD. In contrast, the initial Ag-induced proliferation of these cells, as assessed by CFSE dilution, was intact. Importantly, this impaired survival does not simply reflect the use of a relatively weak (i.e., peptide) Ag stimulation, because a similarly reduced accumulation of Ag-specific PKC$\theta^{-/-}$ T cells was observed when the mice were immunized with OVA plus CFA. Preliminary results indicate that Ag-specific CD4$^+$ T cells from OT-II mice display similar behavior (data not shown). A second novel aspect is the demonstration of a markedly impaired Ag-induced ERK and JNK (but not p38) activation in PKC$\theta^{-/-}$ T cells, in apparent contrast with previous studies (11, 12). Nevertheless, the residual ERK and JNK activation observed in PKC$\theta^{-/-}$ OT-I T cells is apparently sufficient to drive their initial proliferation, but not to sustain it or to maintain the cell survival required for full differentiation into effector T cells. We believe that the use of specific Ag stimulation, as opposed to anti-CD3/CD28 used in the previous studies, unmasks these defects in ERK and JNK activation. Indeed, when we stimulated purified fresh CD4$^+$ or CD8$^+$ T cells from WT mice with cross-linked anti-CD3 plus anti-CD28 Abs, we observed intact ERK and JNK activation, as reported previously (11, 12). Third, our findings demonstrate that some (but not all) of the signaling and effector differentiation defects of PKC$\theta^{-/-}$ T cells are not due to their cytokine (particularly IL-2) deficiency, because they cannot be rescued by coculture with WT T cells (or by addition of rIL-2). Thus, our results add another level of complexity to the function of PKCθ in mature T cell biology by demonstrating for the first time that PKCθ is an important survival signal for Ag-stimulated CD8$^+$ T cells, which is essential for their differentiation into fully competent cytokine-producing CTLs.

Our findings, demonstrating an important role for PKCθ in CD8$^+$ T cell survival, reveal important clues regarding the relevant mechanism(s) involved in this effect. Previous studies by us and another group (32, 33), which used transformed T cell lines, reported that PKCθ provides a survival signal that protects cells from Fas-induced apoptosis, which was partially mediated by direct phosphorylation of the Bcl-2 family member, Bcl-associated death promoter (BAD). However, we could not detect differences in the phosphorylation level of BAD between WT and PKC$\theta^{-/-}$

primary T cells (data not shown). Rather, the current study revealed that the accelerated death of $CD8^+$ T cells lacking PKCθ most likely cannot be accounted for by Fas-mediated apoptosis, because, first, blocking Fas did not protect PKC$\theta^{-/-}$ from accelerated apoptosis, and, second, WT and PKC$\theta^{-/-}CD8^+$ T cells expressed similar levels of Fas protein (data not shown). Instead, the impaired up-regulation and earlier decline of the antiapoptotic proteins Bcl-2 and Bcl-x_L and, conversely, the increased expression of Bim_{EL} in PKC$\theta^{-/-}CD8^+$ T cells is consistent with the idea that the accelerated death of these cells represents death by neglect, which, in contrast to death receptor-mediated T cell apoptosis, is regulated by members of the Bcl-2 family (19). Indeed, Bim-promoted T cell death is independent of death receptors such as TNFR and Fa11sR (34). Our finding that retroviral transduction of PKC$\theta^{-/-}$ T cells with Bcl-x_L or Bcl-2 enhanced their survival indicates that the lower expression levels of endogenous Bcl-x_L or Bcl-2 in these cells contributed, at least in part, to their accelerated and increased death. However, because Bcl-2 or Bcl-x_L did not completely rescue T cell survival, PKCθ may regulate additional death pathways (e.g., Bim_{EL} expression) besides these proteins.

Our results suggest that activation of ERK and JNK in a PKCθ-dependent pathway is important, at least in part, for the survival of Ag-specific $CD8^+$ T cells. JNK inhibition markedly enhanced the apoptosis of WT $CD8^+$ T cells, consistent with a report that JNK1 deletion accelerates the apoptosis of $CD8^+$ cells during lymphocytic choriomeningitis virus infection (35). The enhanced death of $CD8^+$ T cells treated with the JNK inhibitor was accompanied by up-regulation of Bim_{EL}. In contrast, inhibition of JNK in neurons attenuated Bim_{EL} expression and inhibited apoptosis induced by trophic factor deprivation (36). This disparity most likely reflects opposite roles of the JNK pathway in these different cell types. Additional studies are required to elucidate the regulation of Bim_{EL} by JNK in T cells. Additionally, pharmacological inhibition of ERK accelerated the death of WT $CD8^+$ T cells, reduced the expression of Bcl-2 and Bcl-x_L, and, conversely, increased the level of Bim_{EL}, resulting in a time course of cell death similar to that observed with PKC$\theta^{-/-}CD8^+$ T cells that were not treated with the inhibitor. The effect of the ERK inhibitor revealed by our study is consistent with recent reports that activation of the ERK pathway in fibroblasts promotes Bim_{EL} phosphorylation, which then leads to an increase in its turnover rate (37, 38). Our findings support the idea that JNK and ERK, activated in a PKCθ-dependent manner, negatively regulate the proapoptotic protein Bim_{EL}, thereby promoting T cell survival and differentiation.

Consistent with previous studies (11, 12), we found that Ag-induced IL-2 production by PKC$\theta^{-/-}CD8^+$ T cells was severely reduced (by ~85–90%). However, a less severe reduction was observed in the production of TNF-α (~75%) and IFN-γ (~50%), suggesting that the production of some cytokines is less dependent than that of others on PKCθ. This conclusion is also consistent with the findings that the absence of PKCθ inhibits the production of IL-4 much more severely than that of IFN-γ (30, 31). Given the greatly impaired IL-2 production, the intact initial proliferation of PKC$\theta^{-/-}CD8^+$ T cells is somewhat surprising. However, our results are in line with two recent studies demonstrating that initial Ag-induced T cell cycling and proliferation were IL-2 independent, although IL-2 sustained the late proliferation and expansion of these cells during the contraction phase, presumably by promoting cell survival and contributing to the generation of memory T cells (39, 40). Thus, IL-2 may be more critical at later stages in promoting $CD8^+$ T cell expansion by inhibiting apoptosis via a mechanism that involves both PKCθ and members of the Bcl family.

IL-2 and related cytokines can promote T cell survival and up-regulate the expression of the antiapoptotic proteins, Bcl-2 and Bcl-x_L (24). Therefore, it was formally possible that the observed defects in effector cell differentiation are not a direct outcome of the PKCθ mutation, but, rather, constitute secondary effects that reflect the essential role of PKCθ in IL-2 expression. Importantly, however, our data rule out this trivial explanation. First, although coculture of PKC$\theta^{-/-}$ T cells with WT T cells (or addition of IL-2 to PKC$\theta^{-/-}$ T cells; data not shown) increased their survival, it did not completely rescue the survival defect. Second, although coculture restored the ability of PKC$\theta^{-/-}$ OT-I T cells to produce IFN-γ, it was largely ineffective in restoring deficient TNF-α expression. Therefore, it is highly likely that the pathway(s) through which PKCθ promotes survival and effector T cell differentiation is much more complex and cannot simply be explained by the critical role of PKCθ in the expression of IL-2 or other cytokines. These arguments as well as the observations that activation and translocation of PKCθ to the T cell/APC interface occur within a few minutes after stimulation (10) suggest that PKCθ is an essential early component of a predetermined genetic program that dictates the activation and effector differentiation of Ag-specific $CD8^+$ T cells. Such a requirement for PKCθ at the very early stages of TCR engagement may explain our finding that transduction of PKC$\theta^{-/-}$ T cells with PKCθ-expressing retrovirus 1–2 days after stimulation (necessitated by the need to preactivate the cells) did not rescue the survival and effector differentiation (data not shown).

Given the current idea that memory $CD8^+$ T cells derive from the surviving fraction of fully mature effector cells remaining after the contraction phase, it would be important and interesting to analyze the importance of PKCθ in T cell memory. Such studies are currently in progress. Moreover, the finding that PKCθ is not required for initial T cell proliferation, at least in the $CD8^+$ T cell compartment, suggests that pharmacological inhibition of this enzyme may induce selective immunosuppression and apoptotic elimination of effector T cells (e.g., in autoimmune disease) without interfering with the desired activation of naive T cells against, e.g., pathogens. Together, our findings set the stage for future studies aimed at elucidating the molecular pathways that link PKCθ to T cell survival.

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Disclosures

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