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*J Immunol* 2012; 188:5935-5943; Prepublished online 18 May 2012; doi: 10.4049/jimmunol.1102116

http://www.jimmunol.org/content/188/12/5935

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**Supplementary Material**

http://www.jimmunol.org/content/suppl/2012/05/21/jimmunol.1102116.DC1

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Pten Loss in CD4 T Cells Enhances Their Helper Function but Does Not Lead to Autoimmunity or Lymphoma

Dalya R. Soond,* Fabien Garçon,* Daniel T. Patton,* Julia Rolf,* Martin Turner,* Cheryl Scudamore,† Oliver A. Garden,‡ and Klaus Okkenhaug*  

PTEN, one of the most commonly mutated or lost tumor suppressors in human cancers, antagonizes signaling by the PI3K pathway. Mice with thymocyte-specific deletion of Pten rapidly develop peripheral lymphomas and autoimmunity, which may be caused by failed negative selection of thymocytes or from dysregulation of postthymic T cells. We induced conditional deletion of Pten from CD4 Th cells using a Cre knocked into the *Tnfrsf4* (OX40) locus to generate OX40<sup>Cre/+Pten<sup>-/-</sup> mice. Pten-deficient Th cells proliferated more and produced greater concentrations of cytokines. The OX40<sup>Cre/+Pten<sup>-/-</sup> mice had a general increase in the number of lymphocytes in the lymph nodes, but not in the spleen. When transferred into wild-type (WT) mice, Pten-deficient Th cells enhanced anti-*Listeria* responses and the clearance of tumors under conditions in which WT T cells had no effect. Moreover, inflammatory responses were exaggerated and resolved later in OX40<sup>Cre/+Pten<sup>-/-</sup> mice than in WT mice. However, in contrast with models of thymocyte-specific Pten deletion, lymphomas and autoimmunity were not observed, even in older OX40<sup>Cre/+Pten<sup>-/-</sup> mice. Hence loss of Pten enhances Th cell function without obvious deleterious effects.  


D4 T cells support the coordinated activation of other leukocytes during immune responses. For instance, CD4 T cells secrete inflammatory cytokines during contact hypersensitivity (CHS) reactions, augment tumor surveillance, help B cells during the germinal center reactions, and license dendritic cells to express high levels of MHC and costimulatory ligands (1–4). Full activation of naive CD4 T cells requires persistent stimulation of the TCR and CD28 over a period of ~24 h. During this time, the T cells interact with APCs and integrate signals needed to increase metabolism, grow in size, and upregulate cytokine, chemokine, and costimulatory receptors (5). After initial activation, CD4 T cells begin to divide in response to cytokines and express additional costimulatory receptors such as ICOS and OX40. They then differentiate into different Th cell lineages that secrete cytokines such as IFN-γ, IL-4, or IL-17. Eventually, most CD4 Th cells die through apoptosis, but some survive as CD4 memory Th cells (6). Similarly, after initial activation, naive CD8 T cells differentiate to become CTLs, and the ones that survive the cycle of expansion and contraction become CD8 memory T cells (7).  

The TCR and many costimulatory and cytokine receptors activate PI3Ks (8). The class I PI3Ks (p110α, p110β, p110γ, and p110δ) use PtdIns(4,5)P<sub>2</sub> as their preferred substrate to generate the second-messenger molecule PtdIns(3,4,5)P<sub>3</sub>, which helps activate PIP domain-containing proteins such as Akt. Akt phosphorylates Foxo transcription factors and proteins associated with mTOR complex activation (9, 10). PI3K activity is also required for optimal Erk phosphorylation in T cells (11). By these and other mechanisms, PI3Ks contribute to the proliferation, growth, survival, cytokine production, trafficking, and homeostasis of CD4 T cells (8–16). In T cells, roles for p110δ and p110β have not been reported, but the p110γ isoform is activated by G protein-coupled receptors and regulates basal motility in the lymph node (LN), chemotaxis of effector T cells to sites of inflammation, and the survival of memory T cells (17–21). p110γ can also regulate chemotaxis in human T cells (22). p110δ is the functionally dominant isoform downstream of TCR, ICOS, and the IL-2R, and controls Ag-specific events such as differentiation (11, 12, 23–25). PI3K activity remains high for several days after CD4 T cell activation (26), and using acute inhibition with an isoform-selective inhibitor, we demonstrated that p110δ activity is required beyond the first 24 h after TCR activation to regulate cytokine production (16). We also demonstrated the p110δ is a major regulator of cytokine production in human T cells from healthy, atopic and arthritic individuals (16). Given the importance of Th cytokine production in supporting protective and pathological immune responses, it is important not only to understand how the PI3K pathway is activated, but also how it is sustained or curtailed.  

Pten dephosphorylates PtdIns(3,4,5)P<sub>3</sub> to produce PtdIns(4,5)P<sub>2</sub>, thus terminating PI3K signaling. PTEN is frequently lost or mutated in human cancers, including 20% of T cell acute lymphoblastic leukemias (27). PTEN expression is also lost in commonly used human T cell lines such as Jurkat, which may have confounded some studies where these cells are used to study T cell signaling (28, 29). Conditional deletion of *Pten* in thymocytes led to defective negative selection, progressive lymphoproliferation, autoimmunity, and CD4 T cell lymphomas (30–32). Similar effects were observed in mice that overexpressed micro-RNAs that target Pten (33). After activation, peripheral Pten<sup>−/−</sup> T cells proliferated more, resisted apoptosis, and failed to contract after superantigen stimulation (30, 34). Pten<sup>−/−</sup> T cells also could be
activated in the absence of CD28 costimulation and resisted CTLA4-Ig–induced anergy induction, and thus Pten was sug-
gested to impose a threshold for activation during initial TCR
sensing (32). Although Pten expression levels may modulate im-
mmediate responses downstream of the TCR in thymocytes and
naïve T cells, the role of Pten during an ongoing immune re-
sponse is not well understood.

To address the role of Pten in mature CD4 Th cells, we condi-
tionally deleted the Pten gene in Th cells using a Cre-recombinase
gene knocked into Tnfrsf4 (the gene that encodes OX40) (35).
OX40 is transiently expressed ~24 h after activation in the ma-
ajority of CD4 T cells, but rarely in CD8 T cells (36). OX40 is also
constitutively expressed on regulatory T cells (Tregs) (36). We
found that deletion of Pten after TCR stimulation regulated the
magnitude and duration of T cell responses, but not apoptosis or
contraction. Furthermore, contrary to when Pten was deleted in
thymocytes, lymphoma did not develop when the deletion oc-
curred in activated Th cells. Instead, overproduction of cytokines
in these mice leads to altered homeostasis of the lymphocyte
compartment and enhanced inflammatory, antibacterial, and an-
titumor responses.

Materials and Methods

Mice
All mice were maintained under specific-pathogen-free conditions.
All experiments were performed in accordance with U.K. Home Office regu-
lations. OT2 (37), Rag1−/− (38), Rag2−/− (39), OX40Cre (35), Pten−/− (40),
and R26EGFP (41) and R26mRFP (42) mice were described previously.

Reagents
Unless otherwise stated, all chemicals were from Sigma-Aldrich. IC87114
(IC) was synthesized as previously described (16) and anti-CD3 (2C11)
was purified in-house. IL-2 was synthesized by GlaxoSmithKline. Cells
were cultured in RPMI 1640 supplemented with 100 U/ml penicillin/
streptomycin, 2 mM l-glutamine, 10 mM HEPES, 20 µM 2-ME, and 5% FCS at
5% CO2.

Abs, flow cytometry, and Western blot
All Abs were from eBioscience except for Fas and CD4-V500 (BD); Pten,
p-Akt, and p-Erk (Cell Signal Technologies); and anti-rabbit Ig Alexa 647
(Invitrogen). To stain for transcription factors, Pten or intracellular cytokine,
we used Foxp3 staining buffer set or IC Fixation Buffer Kits (both eBio-
sience), respectively. CD4 T cells were purified with a Naive CD4 T cell
Kit (Miltenyi) and activated with 1 µg/ml anti-CD3 on irradiated APCs
to detect signaling molecules. Every 24 h, an aliquot of cells was treated
with Fix Buffer I and Permeabilization Buffer 3 (BD), according to manu-
facturer’s instruction, and cells were stained at the end of the time course.
Apoptotic cells were detected with 7-aminoactinomycin D (Annexin V
(BD), according to manufacturer’s instructions. Flow cytometry data were
acquired with FACSCalibur or LSRII instruments (BD) and analyzed with
FlowJo (Tree Star). Cells were sorted using a FACSARia (BD) machine.
Cell counts were performed either using a CASYCounter or FlowCount
Fluorospheres (Beckman Coulter).

Cell purification, proliferation, and cytokine assays
CD4 T cells were purified, stimulated, and proliferation and cytokine pro-
duction measured as described previously (12, 16). To measure cyto-
kines by intracellular FACS or by Mouse Cytokine Array Panel A (R&D
Systems), we stimulated cells with 1 µg/ml PDB and 5 nM ionomycin for
5 h and 10 µg/ml brefeldin A was added for the final 2 h. For the Mouse
Cytokine Array Panel A, lysates then were made from 5 × 10⁶ cells and
cytokines detected according to manufacturer’s instructions. Pixel density
was analyzed using Aida Image Analysis software.

Treg suppression assay
Suppression assays with anti-CD3–coated APCs were performed as de-
scribed previously (43). Suppression was calculated as the amount of
proliferation when Tregs were present compared with when they were
absent: % suppression = (cpm responders with Tregs/cpm responders
alone) * 100.

Autoantibody detection
Autoantibodies were detected using a Hep2 ANA kit (The Binding Site).
Positive control serum from an MRL mouse was a gift from L. Martensson-
Bopp (University of Gothenburg, Sweden).

Transwell assays
OT2 Pten−/− and OT2 OX40Cre−/−Pten−/− cells were activated in vitro with OVA
peptide for 3 d, then live CD4 T cells were purified. Next, CD4 T cells were
cultured at various rates with CD45.1+ naïve wild-type (WT) splenocytes,
either in direct contact or separated by 0.4-µm Transwell inserts (Fisher).
After 3 d, the numbers of CD45.1+ CD4, CD8, and B220 cells were calculated.

CHS assays
CHS assays were done as described previously (16). In brief, trimet-
rochlorobenzene (TNB)-sensitized mice were rechallenged with TNB and
dosed twice daily with 30 mg/kg IC or 1% methylcellulose vehicle control
for 2 d. Twenty-six days after the first challenge, the same ear was re-
challenged to examine secondary responses. These mice had not received
drug during the primary elicitation. Ear size was measured with a
micrometer (Kroeplin).

Adoptive transfer assays
CD45.1 hosts were injected i.v. with 10⁶ OT2 Pten−/− or OT2 OX40Cre−/−Pten−/−
CD4 T cells and the next day injected s.c. with PBS or 50 µg LPS O26:B6 +
1 mg OVA. Skin draining LNs were harvested on day 3, 6, or 9 after im-
munization.

Listeria monocytogenes assays
CD45 mismatched hosts were injected i.v. with PBS or 1.5 × 10⁶ OT2
Rag2−/− or OT2 OX40Cre−/−Pten−/− cells. The following day, hosts were
injected with 10³ CFU attenuated OVA-secreting Listeria mono-
cytogenes (ActA-LM-OVA) (44). After 3 d, splenocytes were stained for
intracellular cytokines. APC-labeled SHINEKEL-MHC class I tetramer
(Beckman-Coulter) were used to detect OVA-specific CD8 T cells.

Tumor assays
CD45.1+ hosts were injected i.v. with PBS or 2 × 10⁵ CD45.2+ OT2
Rag2−/− or OT2 OX40Cre−/−Pten−/− cells. The following day, 5 × 10⁵ EG7 were injected s.c.
into hosts. Tumors were palpable between days 10 and 11. On day 14 after tumor injection, mice were culled and
tumors were weighed.

Statistics
Statistics were calculated with GraphPad or SSCS software. The following
symbols are used on graphs and tables: *p < 0.01, **p < 0.001.

Results

P15K signaling in OX40Cre−/−Pten−/− T cells is sustained
In OX40Cre mice, Cre is expressed almost exclusively in activated
CD4 T cells and Tregs, and only 2–5% of CD8 T cells (35). Using
reporter mice, we detected Cre activity in 20% of CD4+ T cells
1 d after activation, increasing to 80% after 3 d (Supplemental Fig.
1A). PCR analysis demonstrated nearly complete recombination
of the Pten locus in YFP+, OX40Cre−/−Pten−/− CD4 T cells, whereas no
deletion was observed in YFP− cells. This showed that the YFP
accurately reported Cre activity on the Pten−/− gene (Supplemental Fig.
1B). Flow cytometry showed loss of Pten protein in OX40Cre
Pten−/− CD4+CD25+ and CD4+CD44hi T cells, but not in CD4+
CD44low T cells (Fig. 1A). These results confirm that Pten was de-
leted in Tregs and memory T cells, but not in naïve T cells. When
stimulated with anti-CD3 and APCs, Akt and Erk phosphorylation
were sustained for longer in OX40Cre+Pten−/− CD4 T cells compared
with controls (Fig. 1B). This was consistent with the kinetics ob-
served for Cre expression after activation (Supplemental Fig. 1A).

Pten regulates lymphocyte homeostasis
Pten deletion led to enhanced activation of CD4 T cells as evi-
denced by increased proportion of cells expressing high levels of
CD44 and lower levels of CD62L, and CD45RB in LNs, spleen, and blood of OX40CrePtenf mice (Fig. 1C). However, unlike mice with Pten deletion in thymocytes (30), autoantibodies were not detected in OX40CrePtenf mice even at an advanced age (Supplemental Fig. 2). Furthermore, histology of 22 organs from 19-wk-old OX40CrePtenf mice (n = 5) and controls (n = 5) showed no signs of inflammation or autoimmunity. More than 80% of mice with a germline heterozygous deletion in Pten form lymphomas by 24 wk of age, and 100% of mice with a thymocyte deletion of Pten develop lymphomas by 12 wk of age (30, 45, 46). These lymphomas derive from the thymus (47–49), but this did not exclude the possibility of lymphomas also arising in mice with postthymic deletion of Pten. However, none of 31 OX40CrePtenf mice culled between 25 and 37 wk of age showed evidence of lymphomas. In addition, none of five mice aged over 52 wk had lymphomas. Therefore, the pathology associated with Pten dele-

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
tion in all T cells was not evident when using OX40Cre. Although OX40CrePten mice did not develop lymphomas, their LNs, but not spleens, were enlarged from 5 wk after birth, with 2- to 3-fold more T and B cells in the LNs compared with littersmates. In contrast, lymphocyte numbers in the spleen and blood were similar (Fig. 1D).

Because OX40cre is expressed by Tregs in the thymus (35) and because Pten can affect Treg expansion in vitro (50), we enumerated Tregs in OX40CrePten mice. There were similar numbers of Tregs in the thymus of OX40CrePten mice, but more in the LNs (Supplemental Fig. 3A–D). Consistent with previous results (50, 51), there were no significant differences in suppression of WT responders by OX40CrePten Tregs or in the ability of Tregs of either genotype to suppress OX40CrePten responders (Supplemental Fig. 3E). Hence deficiencies in Tregs or susceptibility of Th cells to suppression are unlikely to have caused LN hyperplasia in OX40CrePten mice.

Lymphocyte expansion is controlled in trans by Th cells

We next considered whether Pten-deficient Th cells altered homeostasis of Pten-sufficient bystander lymphocytes. To test this hypothesis, we generated mixed bone marrow chimeras in lethally irradiated Rag2−/− mice, in which 50% of the donor bone marrow derived from CD45.1+ WT SJL mice and 50% derived from either CD45.2+ OX40CrePten or CD45.2+ WT mice. Eight weeks after reconstitution, the number of lymphocytes in spleens of hosts receiving WT:SJL or OX40CrePten:SJL bone marrow was similar (Fig. 2A). By contrast, there was 3- to 4-fold more B and T cells in LNs of mice receiving SJL:OX40CrePten bone marrow than controls (Fig. 2B). When the ratio of CD45.1 to CD45.2 cells was analyzed, however, we found both donors contributed equally to cells repopulating the LNs and spleens, suggesting that OX40Cre Pten CD4 T cells support general accumulation of LN cells independently of their Pten status.

To test whether a soluble factor was needed to expand lymphocytes, Pten or OT2 OX40CrePten CD4 T cells were activated using the OVA-derived peptide recognized by the OT2 TCR. The activated OT2 T cells were then purified and cocultured with unfractioated WT lymphocytes. After 3 d, the number of WT lymphocytes was counted (Fig. 2C). OT2 OX40CrePten CD4 T cells maintained WT lymphocytes better than controls when effectors were in direct contact with responders. This difference between genotypes was unaffected by inclusion of a Transwell filter, demonstrating that expansion of responders was due to secretion of a soluble factor by OX40CrePten effector cells.

Pten regulates cytokine production by activated CD4 T cells

In an effort to determine which cytokines might be responsible for the hyperplasia, LN cells were stimulated with PDB and ionomycin to stimulate cytokine production. Next, lysates made from equal numbers of cells were used to probe a cytokine array testing 40 cytokines and chemokines, and the signal from OX40CrePten cells was divided by the signal from control cells (Supplemental Fig. 4A). This chemiluminescent array is a relative measure of cytokine production, where a small difference in pixel density between genotypes can indicate a large difference in the absolute amount of cytokine. Although we could not identify a single factor responsible for hyperplasia, we found

![FIGURE 2. OX40CrePten CD4 T cells support expansion of lymphocytes. Lethally irradiated Rag2−/− mice were reconstituted with a 50:50 mix of CD45.1+ WT SJL bone marrow and either CD45.2+ WT (n = 7) or OX40CrePten (n = 9) bone marrow. (A) Spleen and (B) LN were analyzed 8 wk later for the number of lymphocytes and the relative contribution (ratio) of CD45.1 and CD45.2 bone marrow. Student t test was used to calculate p values. (C) Activated CD4 cells from CD45.2+ OT2 Pten (n = 3) and OT2 OX40CrePten (n = 3) mice were cocultured at various ratios with CD45.1+ splenocytes while in direct contact (top panels) or while separated by a Transwell filter (bottom panels). After 3 d, the number of CD45.1+ lymphocytes was calculated. Data show mean ± SD. Three-way ANOVA was used to calculate statistical significance in number of responder cells recovered. OT2 OX40CrePten versus WT OT2 without Transwell: CD4 and CD8 T cells *0.01 < p ≤ 0.05; B cells: ***p ≤ 0.001. Transwell versus Transwell: no significant difference in either OT2 OX40CrePten or WT OT2. Results of significant Bonferroni posttests for each titration point are indicated on the graph. **0.001 < p ≤ 0.01.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.1700455)
a limited range of factors were overproduced in OX40\(^{CrePten}\) T cells, and these included cytokines made by different Th subsets and those that could be induced indirectly by Th cells, such as the chemokine CXCL9 (also known as monokine induced by IFN-\(\gamma\)). Therefore, we postulate that an environment rich in multiple Th cell-dependent cytokines resulted in LN hyperplasia.

OT2\(^+/+\)OX40\(^{CrePten}\) showed enhanced proliferation and produced greater amounts of IL-2, IL-4, and IFN-\(\gamma\) when stimulated with peptide (Supplemental Fig. 4B–E). The enhanced proliferation and cytokine production could be blocked by inhibitors against p110\(d\), Akt, and Erk added 24 h after activation (Supplemental Fig. 4B–E). The results from Supplemental Fig. 4 could be affected by the increased number of CD4\(^+\) T cells with an activated phenotype (32). We therefore purified naive CD62L\(^+\) and Ag-experienced CD62L\(^-\) CD4 \(T\) cells from OX40\(^{CrePten}\) mice. Both populations showed a 2- to 3-fold increase in proliferation and cytokine production when stimulated with anti-CD3 (Fig. 3A–D). High concentrations of the pan-PI3K inhibitor LY294002 or the p110\(d\)-selective inhibitor IC reduced proliferation and cytokine production in OX40\(^{CrePten}\) and WT cells to near-background levels. At lower concentrations of IC, OX40\(^{CrePten}\) T cells were less sensitive than WT T cells, suggesting that the activity of PI3K isoforms other than p110\(d\) are also normally restrained by Pten (Fig. 3A–D). To examine Th cell differentiation, CD4 T cells were activated in vitro in the presence of IL-12 to produce IFN-\(\gamma\)-secreting Th1 cells. A greater proportion of OT2 OX40\(^{CrePten}\) T cells had divided and produced IFN-\(\gamma\) than controls (Fig. 3E–G). In addition, IFN-\(\gamma\) secretion from Pten\(^f\) T cells into WT hosts. After immunization with LPS and OVA peptide, there were significantly more OT2 OX40\(^{CrePten}\) donor cells than controls after 6 d of activation. However, 9 d after activation, both donor cell types had returned to baseline levels (Fig. 3H).

FIGURE 3. Pten antagonizes p110\(d\)-regulated proliferation and cytokine production. (A–D) CD4 \(T\) cells from Pten\(^f\) (n = 3) and OX40\(^{CrePten}\) (n = 3) were sorted into (A, C) CD62L\(^+\) and (B, D) CD62L\(^-\) populations, and stimulated with anti-CD3 and either DMSO, 10 \(\mu\)M LY294002, or 10, 1, or 0.1 \(\mu\)M IC (doses represented by triangle). Two days later, (A, B) proliferation and (C, D) IFN-\(\gamma\) production were analyzed. Data show mean ± SEM. Data represent three independent experiments. (E–H) CFSE-labeled OT2 Pten\(^f\) and OT2 OX40\(^{CrePten}\) were stimulated with peptide in Th1-skewed conditions, and cells were stained after 3 d for intracellular IFN-\(\gamma\). Data are representative of three independent experiments. (E) Representative plots. (F) CFSE dilution was used to determine the number of mitoses/generations each cell had gone through. (G) The proportion of IFN-\(\gamma\) cells was determined for each generation. (H) The median fluorescence intensity (MFI) in IFN-\(\gamma\) cells was determined for each generation to quantify the amount of IFN-\(\gamma\) each cell was making.

Pten regulates the magnitude and kinetics of immune responses

To understand whether altered homeostasis caused by Pten-deficient Th cells affected immune responses, we used a CHS model where increased ear thickness can be used as a measure of \(T\) cell-dependent inflammation. Mice were sensitized by application of TNCB on their abdomens and then rechallenged 6 and 32 d later on their ear to elicit primary and secondary hypersensitivity responses, respectively. Mice were dosed orally with either IC or vehicle control during rechallenges (Fig. 4A). The magnitude and duration of the primary response was greater in OX40\(^{CrePten}\) than in WT mice. The enhanced inflammation was partially p110\(d\) dependent because IC reduced ear swelling in WT and OX40\(^{CrePten}\) mice. The magnitude and duration of the secondary responses was also greater in OX40\(^{CrePten}\) than WT mice (Fig. 4B). IC again reduced ear swelling in both groups. We conclude that OX40\(^{CrePten}\) mice experience a greater inflammatory immune response, which nonetheless can be resolved and which can be attenuated by oral administration of p110\(d\) inhibitors.

To follow the activation kinetics of OX40\(^{CrePten}\) T cells in vivo, we adoptively transferred OT2 Pten\(^f\) or OT2 OX40\(^{CrePten}\) CD4 \(T\) cells into WT hosts. After immunization with LPS and OVA protein, there were significantly more OT2 OX40\(^{CrePten}\) donor cells than controls after 6 d of activation. However, 9 d after activation, both donor cell types had returned to baseline levels (Fig. 4D).
This shows that Pten can control the magnitude of T cell response to Ag after initial TCR activation events, yet the lack of Pten expression does not interfere with the contraction of the T cell response. Consistent with this, we found no difference in apoptosis of OT2 OX40CrePten−/− cells compared with controls during the first 72 h after activation in vitro (Fig. 5B). Activated OT2 OX40CrePten−/− had a mild survival advantage in response to anti-Fas–induced death, but behaved similarly to controls in response to cytokine deprivation, anti-CD3 stimulation, and gamma irradiation (Fig. 5C). Therefore, although activation is enhanced, the absence of Pten does not necessarily interfere with apoptotic signaling in OX40CrePten−/− T cells.

To test how OX40CrePten−/− T cells respond to pathogens, we transferred naive OT2 OX40CrePten−/−Rag2−/− or OT2 Rag2−/− control T cells into WT hosts and infected these with attenuated ActA-LM-OVA. The numbers of adoptively transferred OT2 OX40CrePten−/−Rag2−/− cells appeared to be enhanced compared with controls in response to infection, although this difference was not statistically significant (Fig. 6A). However, twice as many of these cells produced IL-2 and IFN-γ compared with controls 3 d postinfection (Fig. 6B, 6C). We also measured the magnitude of the endogenous CD8 T cell response to ActA-LM-OVA using MHC tetramers. More OVA-specific CD8 T cells expanded in infected mice that had received OT2 OX40CrePten−/−Rag2−/− compared with those receiving OT2 Pten−/−Rag2−/− T cells, consistent with published results (52).

These results raised the possibility that OX40CrePten−/− CD4 T cells could promote cytotoxic immune responses in vivo under conditions where WT CD4 T cells fail to make a difference. To test this possibility further, we inoculated mice with an OVA-expressing thymoma cell line (EG7) and determined the ability of transferred OT2 cells to limit the growth of the tumors. Indeed, mice with adoptively transferred OT2 OX40CrePten−/−Rag2−/− but not OT2 Pten−/−Rag2−/− T cells rejected the tumors (Fig. 6E). We conclude that OX40CrePten−/− CD4 T cells can promote cytotoxic immune responses potentially by activating CD8 T cells under conditions where WT CD4 T cells fail to do so.
FIGURE 6. OX40CrePten+/Th cells can enhance antibacterial and antitumor responses. (A–C) Mice injected with cells from OT2 Rag−/− (n = 5) or OT2-OX40CrePtenRag2−/− (n = 5) mice were immunized with 10^7 CFU ActA-LM-Ova. After 3 d, spleens were analyzed for (A) the number of transferred cells and the proportion that produced (B and C) IL-2 or IFN-γ. (D) Mice injected with PBS (n = 6), OT2 Rag2−/− (n = 6), or OT2 OX40CrePten Rag2−/− (n = 6) were immunized with 10^7 CFU ActA-LM-Ova. The percentage of endogenous OVA-tetramer+ CD8 T cells from blood taken on day 8 postinfection is shown. Repeated-measures ANOVA was used to calculate p values. (E) Mice injected i.v. with OT2 PtenRag2−/− (n = 10), OT2 OX40Cre PtenRag2−/− (n = 10), or PBS (n = 8) were inoculated s.c. with EG7 cells. Excised tumors were weighed on day 14. Data show median values. p values were calculated using Mann–Whitney U tests. Data represent two independent experiments. *0.01 < p ≤ 0.05, **0.001 < p ≤ 0.01, ***p ≤ 0.001.

Discussion
We have shown in this article that Pten is not an essential tumor suppressor in peripheral CD4 Th cells. Instead, Pten plays an important role in regulating lymphocyte homeostasis. When Pten was lost after activation, CD4 Th cells hyperproliferated and produced greater concentrations of cytokines. We postulate that cytokine overproduction turns Pten-deficient Th cells into “superhelpers” that enhance inflammatory, antibacterial, and antitumor responses. However, the enhanced responses could still be resolved and spontaneous disease did not develop, in contrast with pathology in many other mouse knockout models of negative regulatory proteins such as CTLA4 and Cbl (53). In addition, bone marrow chimera and Transwell studies suggest that a soluble factor led to a non-CD4 Th cell autonomous expansion of lymphocytes in LN. Recently, it was shown that cytokines permeate the LN and induce signaling in distant bystander lymphocytes (54). Hence excess cytokines produced by Pten-deficient Th cells might affect other naïve lymphocytes and significantly alter LN homeostasis.

We confirmed in vitro and in a CHS model that Pten acts, at least in part, by antagonizing signaling by the PI3K p110α, which we have recently shown contributes to tonic Ag receptor signaling in B cells (55), or chemokine-dependent p110δ activity (19, 20, 56, 57).

Previous studies have shown that although lymphomas are found in the LN and spleens of Pten-deficient mice, there are actually derived from the thymus (47–49). Mechanistically, this has been linked to c-myc translocation and overexpression (49, 58). Using converse experiments, we show in this study that Pten does not act as a tumor suppressor in mature T cells. In humans, mature T cell lymphomas are rare compared with thymic-derived lymphomas, which may reflect increased genome stability in more mature T cells. Indeed, DNA damage checkpoint regulators were dysregulated in Pten-deficient thymocytes but not peripheral T cells (48). Alternatively, there may be additional backup mechanisms to prevent lymphomagenesis in peripheral T cells. Consistent with this, leukemic oncogenes only caused transformation when ectopically expressed in hematopoietic stem cells and not mature T cells (59).

Pten has previously been shown to deter autoimmunity because when it was deleted from thymocytes, autoantibodies, autoactive T cells, and lymphoid interstitial pneumonia developed (30). Autoantibodies and tissue infiltration still arose when young mice were thymectomized, which the authors suggested was caused by defective activation-induced cell death in peripheral T cells (49). However, autoreactivity in those studies could still have been caused by failed thymocyte negative selection in young animals (30, 32). We detected no evidence of spontaneous autoantibody production, autoimmunity, or inflammation in our model, possibly because there were no defects in Treg suppression or because apoptosis was only mildly affected. We thus conclude that Pten is not an essential repressor of apoptosis or autoimmunity stemming from mature T cells.

Although spontaneous immunopathologies did not develop in OX40CrePten+/Th cells, heightened immune responses did. We previously showed that T-dependent humoral immune responses were enhanced in OX40CrePten+/Th mice because of increased number and cytokine production of Tfh cells (24). In this article, we show that OX40CrePten+/Th cells enhanced cellular immune responses to L. monocytogenes and prevent tumor growth. Because Pten deficiency revealed a previously unrecognized potential for Th in promoting primary CD8 T cell responses (52), we propose that OX40CrePten+/Th cells are “superhelpers.” The concept of superhelpers has potential clinical implications because adoptive immunotherapy and prophylactic vaccination is currently aimed at modulating responding CTLs, although interest is growing in exploiting CD4 T cells as well (4). Pten-deficient superhelper Th could potentially improve clinical outcomes. Ag-specific CD4 cells have previously been shown to promote partial or complete regression in lymphopenic environments or when high numbers
were infused at regular intervals alongside Ag-specific CTLs (60–
62). In this study, we demonstrate that similar results can be
achieved using a single injection of Pten-deficient, Ag-specific
CD4 T cells in normal hosts. Reduction of Pten activity in ma-
ture T cells using genetic or chemical means may, therefore, be
both safe and desirable in some therapeutic settings such as tumor
immunotherapy.

Acknowledgments

We thank Nigel Killeen, Pier Paolo Pandolfi, and Hans Jorg Felhling
for mouse strains, and Hou Shen for the attenuated Listeria-expressing
OVA. We thank Jonathan Clark for the synthesis of IC, the staff at the
Biological Services Unit and Flow Cytometry facility for expert help,
and Anne Segonds-Pichon for statistical advice.

Disclosures

K.O. is a consultant for GlaxoSmithKline. The other authors have no finan-
ccial conflicts of interest.

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