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The Survival of Memory CD4+ T Cells within the Gut Lamina Propria Requires OX40 and CD30 Signals[1]

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Abstract

Although CD4+ memory T cells reside within secondary lymphoid tissue, the major reservoir of these cells is in the lamina propria of the intestine. In this study, we demonstrate that, in the absence of signals through both OX40 and CD30, CD4+ T cells are comprehensively depleted from the lamina propria. Deficiency in either CD30 or OX40 alone reduced CD4+ T cell numbers, however, in mice deficient in both OX40 and CD30, CD4+ T cell loss was greatly exacerbated. This loss of CD4+ T cells was not due to a homing defect because CD30 × OX40-deficient OTII cells were not impaired in their ability to express CCR9 and $\alpha_4\beta_7$ or traffic to the small intestine. There was also no difference in the priming of wild-type (WT) and CD30 × OX40-deficient OTII cells in the mesenteric lymph node after oral immunization. However, following oral immunization, CD30 × OX40-deficient OTII cells trafficked to the lamina propria but failed to persist compared with WT OTII cells. This was not due to reduced levels of Bcl-2 or Bcl-XL, because expression of these was comparable between WT and double knockout OTII cells. Collectively, these data demonstrate that signals through CD30 and OX40 are required for the survival of CD4+ T cells within the small intestine lamina propria.

The small intestinal mucosa, including the lamina propria (LP),[4] contains the largest reservoir of CD4+ effector/memory cells in the body (1, 2). The CD4+ T cells residing in the LP are of essential importance for protection against infections as well as maintaining tolerance (3–6). Survival of effector and memory cells at the LP site is therefore of crucial importance for the maintenance of mucosal immunity (6–8).

Within secondary lymphoid tissue, signals from common cytokine-receptor γ-chain family cytokines such as IL-2, IL-7, and IL-15 promote T cell proliferation, differentiation, and

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Disclosures
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[4]Abbreviations used in this paper: LP, lamina propria; TNFR, TNF receptor; KO, knockout; dKO, double KO; WT, wild type; PP, Peyer's patch; mLN, mesenteric LN; DC, dendritic cell; LTi, lymphoid tissue inducer cell; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride.

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survival during an immune response (9). It has also become increasingly evident that the survival of activated T cells is controlled through their expression of TNF receptor (TNFR) family members such as OX40, CD30, and 4-1BB (10, 11). OX40, CD30, and 4-1BB are type I transmembrane molecules with transient expression, that provide costimulatory signals during T cell activation as well as generating signals enabling the survival of effector T cells (3). Although signals through 4-1BB are important for $CD8^+$ memory T cells, CD4 responses in 4-1BB- knockout (KO) mice were only slightly impaired (12). Secondary $CD8^+$ T cell responses were not impaired in OX40KO mice (12), however, signals through OX40 were critical in promoting the survival of $CD4^+$ T cells (13-16). Further, signals through CD30 also contribute to $CD4^+$ T cell survival (17, 18). To confer survival, the TNFR family members, including OX40 and CD30, signal through TNFR-associated factors, which link into different signaling pathways resulting in expression of anti-apoptotic proteins such as Bcl-x_L and Bcl-2 (15, 19). Because different TNFRs use the same TNFR-associated factors, redundancy in the signals generated through these receptors is likely (20).

The severe loss of $CD4^+$ memory T cells from the spleen of mice lacking both OX40 and CD30 signals (17, 18) led us to investigate whether memory $CD4^+$ T cells outside secondary lymphoid tissue were similarly dependent on signaling through these pathways. Here we report the selective loss of the $CD4^+$ T cell compartment from the LP of CD30 and OX40 double knockout (dKO) mice. Although deficiency of either CD30 or OX40 significantly reduced the number of $CD4^+$ T cells in the LP, the loss of both genes resulted in the almost complete loss of LP $CD4^+$ T cells. This was not attributable to a defect in either proliferation or the ability of dKO $CD4^+$ T cells to traffic to the small intestine in vivo. Rather, dKO $CD4^+$ T cells failed to survive having reached the LP, despite comparable levels of Bcl-2 and Bcl-x_L expression to wild-type (WT) LP $CD4^+$ T cells.

Materials and Methods

Mice

All experiments were performed in accordance with U.K. laws and with the approval of the University of Birmingham or the Lund/Malmö animal ethics committees. WT (C57BL6 and BoyJ background), CD30KO (C57BL6), OX40KO (C57BL6), CD30KO × OX40KO (dKO, C57BL6), WT × OTII (BoyJ), dKOxOTII (C57B6), and CD3εTg26 mice (BoyJ) (21) were bred and maintained in the animal facilities of the University of Birmingham, U.K., or the Lund University Biomedical Center, Sweden.

Analysis of murine small intestine using immunofluorescence

Gut tissue was flushed clean with RPMI and frozen sections were prepared and stained as described previously (22). Primary Abs used were anti-B220 FITC (RA3–6B2, eBioscience), anti-CD3ε FITC (145-2C11, BD Pharmingen), anti-CD4 Alexa Fluor 647 (GK1.5, eBioscience), and anti-CD11c biotin (N418, eBioscience). Biotinylated Abs were detected with streptavidin Alexa Fluor 555 (Invitrogen). FITC-conjugated Abs were detected using rabbit anti-FITC Abs (Sigma-Aldrich), then goat anti-rabbit-FITC Abs (Southern Biotechnology). Sections were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and mounted using DABCO (Sigma-Al-drich). Confocal images were obtained using a LSM 510 Meta microscope (Zeiss) equipped with 405, 488, 543, and 633 nm lasers and image analysis used the Zeiss LSM software. For T cell quantitation, villi were divided into LP and epithelium areas and individual T cells ($CD3^+$ $CD4^+$ and $CD3^+$ $CD4^-$) counted manually.

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Cell preparations

Small intestine—Cells were prepared from small intestine as described previously (23). Intestinal tissue was rinsed with cold HBSS/HEPES and Peyer's patches (PP) and fat carefully removed. The tissue was cut longitudinally and then into smaller pieces of ~5 mm. The tissue pieces were then transferred to HBSS/HEPES/2 mM EDTA, incubated at 37°C for 15 min then shaken vigorously and the resulting mixture was filtered with the tissue pieces collected. This process was repeated a total of three times and the remaining tissue was washed in cold RPMI/HEPES/10% FCS and transferred to urine beakers which contained 20 ml RPMI 1640/HEPES/10% FCS supplemented with 250 μg/ml collagenase VIII and 50 mM $CaCl_2$. The urine beakers were incubated for 1 h in 37°C under magnetic stirring. The digested tissue was then filtered and the resulting cell suspension centrifuged and washed with cold RPMI. Lymphocytes were enriched using a 40:70% Percoll gradient.

Spleen, PP, and mesenteric LN (mLN)—Tissue was crushed through a 70-μm filter, washed, and RBC lysed if necessary.

Flow cytometry

Flow cytometric analysis was performed on cell suspensions as previously described (22). Primary Abs used were: anti-mouse Bcl-2 FITC (3F11, BD Pharmingen), Bcl-XL (7B2.5, Abcam) B220 FITC (RA3–6B2, eBioscience); B220 Pe-Cy7 (RA3–6B2, BD Pharmingen); CD3 PE (145-2C11, eBioscience); CD4 allophycocyanin (GK1.5, eBioscience); CD4 Pacific blue (RM-5, eBioscience); CD11c FITC (N418, eBioscience); anti-$\alpha_4\beta_7$ (DATK32, BD Pharmingen); anti-CCR9 Ab (7E7 purified from hybridomas, gift from Oliver Pabst) (24) then anti-rat IgG2b biotin (G15–337, BD Pharmingen); CD30L biotin (RM153, eBioscience); CD45 PE Cy7 (30-F11, eBioscience); CD45.1 A700 (A20, BioLegend); CD45.1 FITC (A20, BD Pharmingen); CD45.2 PE (104, eBioscience); CD45.2 allophycocyanin-A750 (104, eBioscience), IL-7Rα biotin (A7R34, eBioscience); OX40L biotin (RM134L, BD Pharmingen);Thy1.2 biotin (53–2.1, BD Pharmingen). Biotinylated Abs were detected with streptavidin-PE Cy5.5, streptavidin-QD605 (Invitrogen) or streptavidin-allophycocyanin (BD Pharmingen). DAPI (final concentration 0.25 μg/ml) or propidium iodide were added to samples before acquisition. Data were acquired using a CYAN ADP flow cytometer or a FACSAria (BD Biosciences) and analyzed using Summit software (Beckman Coulter) or FlowJo software (Tree Star).

In vitro culture of OTII cells with all-trans retinoic acid

Splenic dendritic cells (DCs) and OTII $CD4^+$ T cells were isolated as described (23). DCs (10^5 cells) were incubated with OVA (1 mg/ml), 10 nM all-trans retinoic acid, and WT or dKO OT-II cells (2×10^5 cells) in flat-bottom 96-well plates (Nunc) in 200 μl of complete RPMI/HEPES/10% FCS. After 4 days, cells were resuspended in RPMI/HEPES/10% FCS to a total volume of 500 μl supplemented with 10 ng/ml IL-7 and 10 ng/ml IL-15 (R&D Systems) and transferred into 48-well plates. Six days after culture setup, cells were analyzed for their expression of $\alpha_4\beta_7$ and CCR9.

Cell isolation, labeling, and adoptive transfers for in vivo activation of OTII cells

$CD4^+$ T cells were isolated from WT × OTII and dKO × OTII spleens (23), labeled with 2.5 μM CFSE in PBS for 8 min at RT and washed twice. For adoptive transfers, $CD4^+$ T cells (3×10^6) were injected i.v. into recipient mice which 1 day later received oral OVA (50 mg/mouse, Sigma-Aldrich) and R848 (10 μg/mouse, Alexis Biochemicals). Twenty hours after OVA administration, mice where indicated received FTY720 i.p. (1 mg/kg, Cayman Chemical). Mice were sacrificed at 3, 4, 5, or 7 days after OVA administration.

Statistics

For quantitation of T cell numbers in the LP and epithelium, the Wilcoxon Two Sample Test was applied. For WT and dKO OTII cell survival the *t* test for paired samples was used.

Results

CD4$^+$ T cells in the LP depend on both OX40 and CD30 signals

To investigate whether CD4$^+$ T cells in the LP depend on OX40 and CD30 signals, sections of small intestine prepared from WT, CD30KO, OX40KO, and dKO mice were stained for the expression of CD3 and CD4 (Fig. 1*A*). In WT mice, CD4$^+$ T cells were clustered specifically within the LP core of each villus, whereas CD8β^+ and particularly $\gamma\delta^+$ T cells were mostly associated with gut epithelium. Compared with WT mice (mean = 2315.6 cells per mm^2, STDEV = 802.8), both CD30KO (mean = 1211.9 cells per mm^2, STDEV = 507.7) and OX40KO (mean = 766.2 cells per mm^2, STDEV = 366.8) mice had significantly reduced numbers of CD4$^+$ T cells in the LP (Fig. 1*B*). In mice deficient in both OX40 and CD30, the effects on the LP CD4$^+$ T cell compartment were dramatic. Many sections of villi in dKO mice contained no CD4$^+$ T cells and this was reflected in a severe reduction in villus CD4$^+$ T cell numbers (mean = 156.9 cells per mm^2, STDEV = 203.9). This represents an ~14.8-fold loss compared with WT mice and a ~7.7- and ~4.9-fold further reduction compared with CD30KO and OX40KO mice, respectively.

The effects of OX40 and CD30 deletion was most evident for CD4$^+$ T cells, CD4$^-$ T cell numbers in the LP were only slightly reduced in dKO mice compared with WT mice (WT mean = 1250.6 vs dKO mean = 1064.1, Fig. 1*C*). The intraepithelial lymphocyte population was modestly affected by the absence of both CD30 and OX40 genes (Fig. 1*D*), however, both CD8β^+ and $\gamma\delta^+$ T cells were readily detected in the villi of dKO mice (Fig. 1*E*). Therefore, combined signals through both CD30 and OX40 appear critical specifically for the presence of the CD4$^+$ T cell compartment of the small intestine LP.

dKO CD4$^+$ T cells show normal activation and gut homing ability

Our previous studies have shown that dKO CD4$^+$ T cells initially proliferate and differentiate normally both in vitro (16) and in vivo (17). To exclude a specific defect related to their gut homing capacity during priming in the mLN, CFSE-labeled CD45.1$^+$ WT and CD45.2$^+$ dKO transgenic OTII cells (25) were transferred in a 1:1 ratio into CD45.1$^+$CD45.2$^+$ recipient mice, allowing host T cells to be discriminated from donor T cell populations. After 24 h, these mice were given 50 μg OVA orally, followed by FTY720 at 20 h after OVA to prevent lymphocyte egress from the mLN (26). Three days later, the ratio of dKO:WT OTII cells in spleen and mLN remained ~1:1 (Fig. 2*A*). Furthermore, the proliferation of these cells, assessed by dilution of CFSE, was comparable between WT and dKO OTII cells, as was the expression of CCR9 and $\alpha_4\beta_7$ (Fig. 2*B*).

To test the capacity of dKO OTII cells to traffic to the LP, WT and dKO OTII cells were primed in vitro with splenic DCs and OVA in the presence of retinoic acid, conditions that induce a gut homing phenotype (27). Six days later, almost all (~95%) dKO and WT OTII cells expressed $\alpha_4\beta_7$ and CCR9 (Fig. 2*C*). Both primed CD45.1$^+$ WT and CD45.2$^+$ dKO OTII cells were then transferred (in 1:1 ratio) into CD45.1$^+$CD45.2$^+$ recipients. After 24 h, the distribution of WT and dKO OTII cells was analyzed (Fig. 2*D*), revealing no significant differences between the mouse strains in spleen, mLN, and LP. Combined, these data indicate that dKO CD4$^+$ T cells display normal activation in mLN in vivo as well as no disadvantage in ability to home to the small intestinal LP after in vitro activation.

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Survival of LP OTII cells depends on OX40 and CD30

To analyze whether dKO T cells had a defect during the in vivo generation of gut homing, we adoptively transferred dKO and WT OTII cells into recipient mice, which were subsequently immunized by oral administration of OVA. Three days after immunization, the mLN was analyzed. Comparing immunized and unimmunized mice, we could clearly see an expansion of both dKO OTII ($CD45.2^+$) and WT ($CD45.1^+CD45.2^+$) cells after OVA administration, while the ratio in immunized and naive mice was similar (Fig. 3, *A* and *B*). Furthermore, the CFSE profile of dKO and WT OTII cells as well as expression of CCR9 and $\alpha_4\beta_7$ of dKO and WT cells was similar (Fig. 3, *C*–*E*), indicating normal in vivo generation of gut homing dKO $CD4^+$ OTII cells.

Analysis of the mLN at 3, 5, and 7 days after OVA administration (Fig. 4*A*) revealed that the ratio of dKO:WT OTII cells in the mLN remained fairly constant, albeit skewed in favor of WT OTII cells. However, the ratio was significantly decreased in the LP compared with the mLN at day 7 ($p = 0.03$ and $p = 0.047$ in two separate experiments, Fig. 4*B*). Together, these results provide evidence that OX40 and CD30 are required for the survival of $CD4^+$ T cells within the LP.

Normal levels of Bcl-2 and Bcl-x_L in dKO LP $CD4^+$ T cells

Activation of $OX40^{-/-}$ T cells in vitro has indicated that in the absence of this receptor, expression of both Bcl-2 and Bcl-XL is reduced (15). To investigate whether the failure of survival of $CD4^+$ T cells in the LP of dKO mice was due to reduced levels of Bcl-2 and Bcl-x_L, their expression was analyzed by intracellular flow cytometry. Surprisingly, no differences in the expression of Bcl-2 or Bcl-x_L were detected in $CD4^+CD62L^-CD44^{high}$ T cells isolated from the LP (Fig. 5*A*), spleen, mLN, or PP of WT and dKO mice (data not shown). Expression of Fas by $CD4^+$ T cells in the mLN was also comparable between WT and dKO mice (data not shown).

To further analyze expression of Bcl-2 or Bcl-x_L by responding dKO $CD4^+$ T cells that had been induced to traffic to the LP, WT OTII cells ($CD45.1^+$) and dKO OTII cells ($CD45.2^+$) were transferred (1:1 input ratio) into CD3εTg26 mice (which lack T and NK cells). The mice were then orally immunized with OVA and the spleen, mLN, PP, and LP analyzed 4 days after immunization. A clear population of OTII cells was detected in the LP, however, expression of Bcl-2 and Bcl-x_L was comparable between WT and dKO-derived OTII cells (Fig. 5*B*), despite skewing of the WT: dKO OTII cell ratio in the gut (Fig. 5*C*). Transfer of WT and dKO OTII cells into WT ($CD45.1^+CD45.2^+$) mice with subsequent OVA administration yielded similar results (data not shown). In summary, dKO OTII cells fail to survive in the LP compared with WT OTII cells, however, this is not due to impaired expression of either Bcl-2 or Bcl-x_L.

Discussion

In this study, we describe the severe depletion of $CD4^+$ T cells from the LP of the small intestine in the absence of OX40 and CD30. The loss of these cells was not due to an inability to traffic to the LP, neither were dKO $CD4^+$ T cells impaired in their ability to proliferate in the mLN following oral immunization. Rather, these data demonstrate the failure of dKO OTII cells to survive within the LP.

Although activated and memory $CD4^+$ T cells reside within secondary lymphoid tissue, the main reservoir for these cells is the LP of the small intestine. Because signals through CD30 and OX40 are required for the persistence of memory $CD4^+$ T cells in the spleen (17, 18), we investigated whether these signals were also required for survival in the LP. Immunofluorescence analysis of the small intestine of dKO mice revealed a severe loss of

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$CD4^+$ T cells from the small intestine LP, significantly more dramatic than observed in either OX40KO or CD30KO mice. To exclude the possibility that dKO $CD4^+$ T cells were unable to traffic to the small intestine oral immunization with OVA, in addition to in vitro culture with retinoic acid, was performed. In all assays, WT and dKO OTII cells expressed comparable levels of both CCR9 and $\alpha_4\beta_7$ and migrated as efficiently as the WT $CD4^+$ T cells to the LP at early time points. Therefore, dKO $CD4^+$ T cells were neither impaired in their potential nor ability to relocate to the small intestine.

An alternative explanation for the loss of dKO T cells from the LP was the inability of these cells to proliferate within the mLN. However, CFSE-labeled WT and dKO OTII cells showed comparable dilution of the dye 3 days after oral immunization strongly arguing against this. Despite the initial priming of WT and dKO OTII cells within the mLN being comparable, the skewing of the WT:dKO OTII ratio in favor of WT cells in the mLN does indicate that OX40 and CD30 signals are required either during or soon after priming at this site. Because the ratio of WT:dKO OTII cells remained at the input ratio in the spleen after oral immunization, this provides further evidence that OX40 and CD30 signals are required post activation. Comparison of the ratio of WT:dKO OTII in the mLN and LP at 7 days post immunization, demonstrated a significant loss of the dKO T cells consistent with a further requirement for OX40 and CD30 signals within the LP.

The $OX40L^+CD30L^+$ cells that provide signals for the survival of memory T cells in the LP are currently unclear. Within the mLN, lymphoid tissue inducer cells (LTi) are well placed to support activated $CD4^+$ T cells, constitutively expressing high levels of OX40L and CD30L (16, 28). Within the small intestine, LTi reside within cryptopatches (29) and ILFs where a role in T cell independent switching to IgA was identified (30). Although a $CD4^+CD3^-$ population of cells resides within the LP, these cells are not dependent upon RORγ (29) and lacked expression of IL-7Rα (D. Withers, unpublished observations). This $CD4^+CD3^-$ population is clearly distinct from LTi and although it may be involved in mediating T cell survival, other APCs resident within the LP may also provide OX40L and CD30L signals.

To investigate the mechanisms behind the impaired survival of dKO $CD4^+$ T cells, expression of Bcl-2 and Bcl-x_L were analyzed, because these have been reported to be reduced in the absence of OX40 signals (15). Surprisingly, we found expression of the antiapoptotic factors Bcl-2 and Bcl-x_L to be identical when WT and dKO $CD4^+$ T cells in the LP were compared. Furthermore, when WT and dKO OTII cells were induced to traffic to the LP, levels of Bcl-2 and Bcl-x_L were again comparable, despite the impaired survival of dKO OTII cells. In contrast to our in vivo study, previous studies demonstrated impaired expression of Bcl-2 and Bcl-x_L in in vitro-stimulated $OX40^{-/-}$ T cells, and these experimental differences may explain this discrepancy. Nevertheless, why dKO $CD4^+$ T cells survive less well than WT $CD4^+$ T cells in the LP remains unclear.

In summary, our data demonstrate that signals through OX40 and CD30 are required for the persistence of $CD4^+$ T cells within the small intestine LP. This shows the relevance of the OX40 and CD30 signaling pathways in the survival of tissue memory $CD4^+$ T cells and has implications for the modulation of these pathways in intestinal inflammatory diseases.

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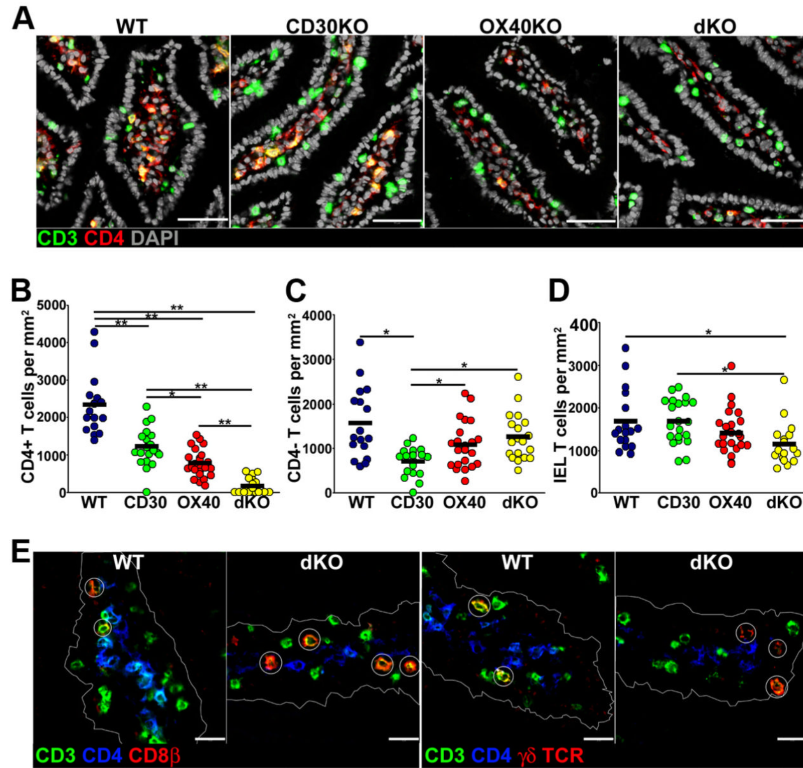

FIGURE 1.

$CD4^+$ T cell numbers in small intestine villi of WT, CD30KO, OX40KO, and dKO mice. Sections of small intestine from WT, CD30KO, OX40KO, and dKO mice were stained for expression of CD3 and CD4 and numbers of $CD4^+$ and $CD4^-$ T cells enumerated. *A*, Expression of CD3 (green) and CD4 (red), counterstained with DAPI (gray). Scale bar, 50 μm. *B*, Numbers of $CD4^+$ T cells per mm^2 small intestine LP. *C*, Numbers of $CD4^-$ T cells per mm^2 small intestine LP. *D*, Numbers of IELs per mm^2 small intestine epithelium. Each data point represents an individual micrograph from which cells were counted. Sections were cut from tissues from at least four mice of each type. Statistical significances of differences shown were obtained using the Wilcoxon two sample test, *, $p < 0.01$; **, $p < 0.00001$. *E*, Expression of CD3 (green), CD4 (blue), and CD8β or $\gamma\delta$TCR (red) in sections of WT and dKO small intestine. CD8β^+ and $\gamma\delta$TCR$^+$ T cells circled, outline of villus shown in white. Scale bar, 50 μm.

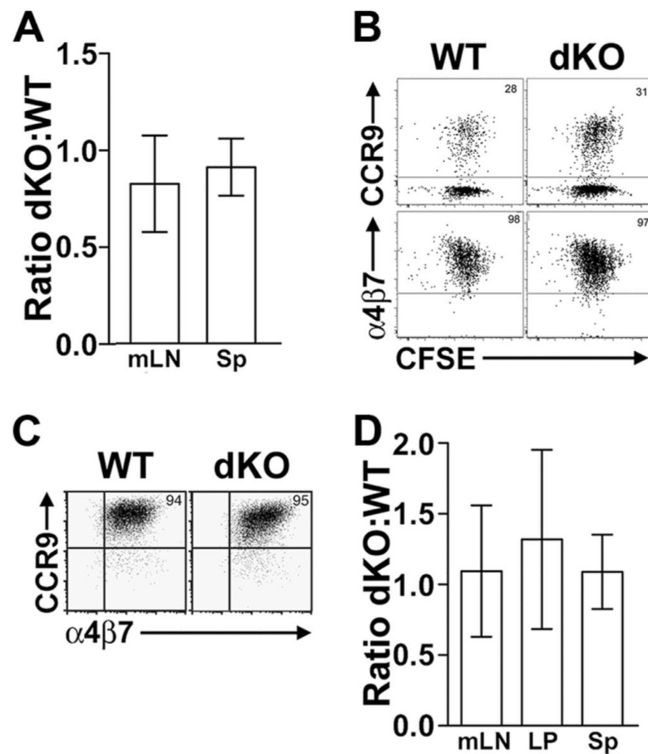

FIGURE 2.

Normal homing of dKO CD4$^+$ T cells to small intestine. To investigate whether dKO OTII T cells proliferated normally and expressed gut-homing molecules, CFSE-labeled WT (CD45.1$^+$) and dKO (CD45.2$^+$) OTII T cells were transferred (1:1 ratio) to CD45.1$^+$CD45.2$^+$ mice that were orally immunized with OVA. *A*, Ratio of dKO:WT OTII cells 3 days post immunization in the mLN and spleen. Data represent mean (with SD) of two experiments, each with three mice. *B*, Expression of CCR9 and $\alpha_4\beta_7$ vs CFSE in WT and dKO OTII T cells. Data are representative of three mice. *C*, Expression of CCR9 and $\alpha_4\beta_7$ by WT and dKO OTII T cells cultured in vitro with splenic DCs, OVA, and retinoic acid for 6 days. Data are representative of two experiments. *D*, Ratio of dKO:WT in vitro-cultured OTII cells in mLN, small intestine, LP, and spleen, 24 h after transfer into allotype-marked mice. Data represent mean (with SD) of two experiments, each with three mice.

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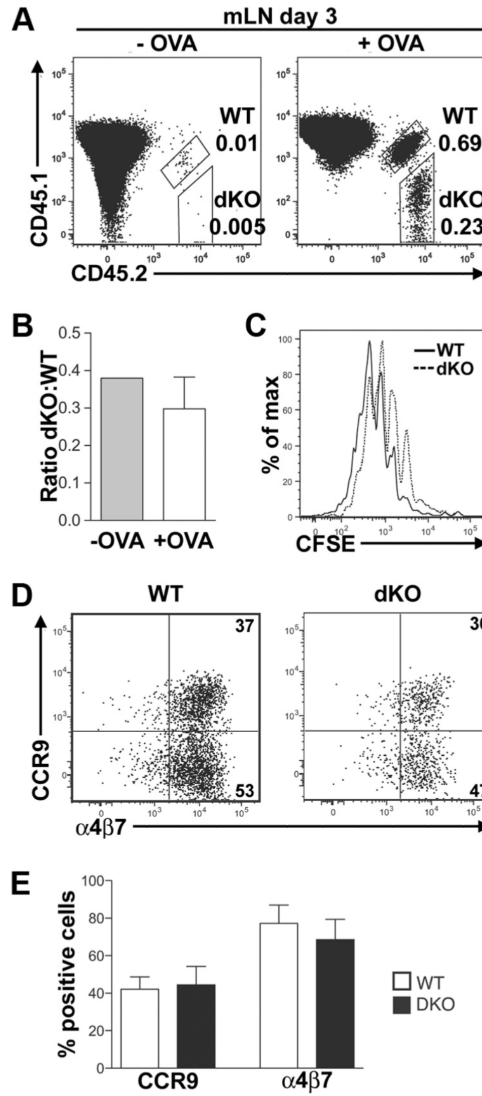

FIGURE 3.

In vivo activated WT and dKO cells acquire similar expression of CCR9 and $\alpha_4\beta_7$ in the mLN. Recipient mice (CD45.1$^+$) were adoptively transferred with dKO (CD45.2$^+$) and WT (CD45.1$^+$CD45.2$^+$) OTII cells, input ratio 0.8. The OTII cells were then activated in vivo by oral administration of OVA and the mLN was analyzed at day 3. *A*, Expansion of both dKO OTII (CD45.2) and WT (CD45.1$^+$CD45.2$^+$) cells after OVA administration. *B*, Ratio of dKO:WT OTII cells with and without OVA administration. *C*, CFSE profile of dKO and WT OTII cells. *D*, Representative expression of CCR9 and $\alpha_4\beta_7$ of dKO and WT cells. *E*, Expression CCR9 and $\alpha_4\beta_7$ of dKO and WT cells, data represent mean (with SD) of three mice per group.

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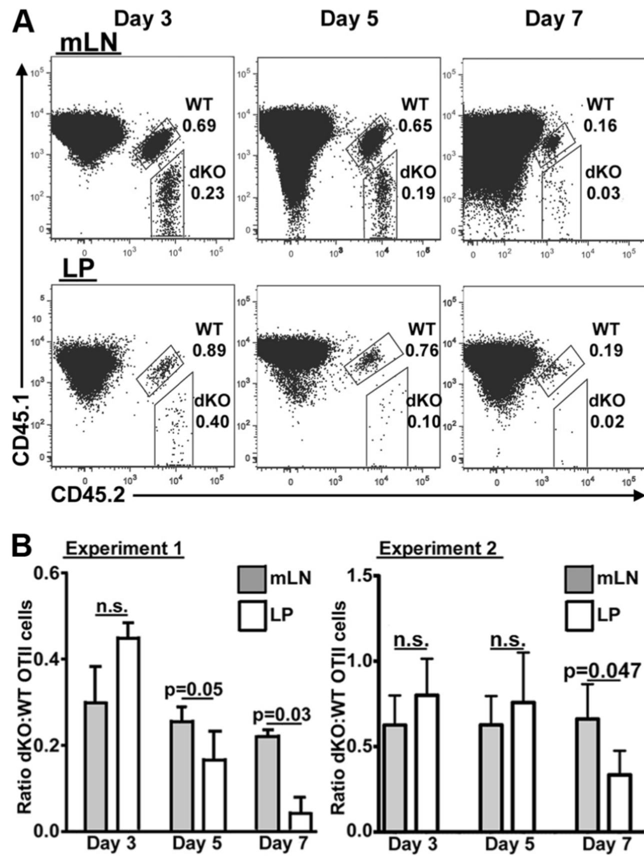

FIGURE 4.

dKO OTII cells fail to persist in the LP after in vivo activation. Recipient mice (CD45.1) were adoptively transferred with dKO ($CD45.2^+$) and WT ($CD45.1^+CD45.2^+$) cells, input ratio 0.8. The OTII cells were then activated in vivo by oral administration of OVA. The ratio of dKO OTII cells to WT OTII cells in the mLN and LP was then assayed at days 3, 5 and 7 post immunization. *A*, Detection of WT and dKO OTII cells in the mLN and LP, numbers show percentage of $CD4^+$ T cells. *B*, Ratio of dKO:WT OTII cells in mLN and LP from two independent experiments; data is mean (with SD) of two to three mice per group.

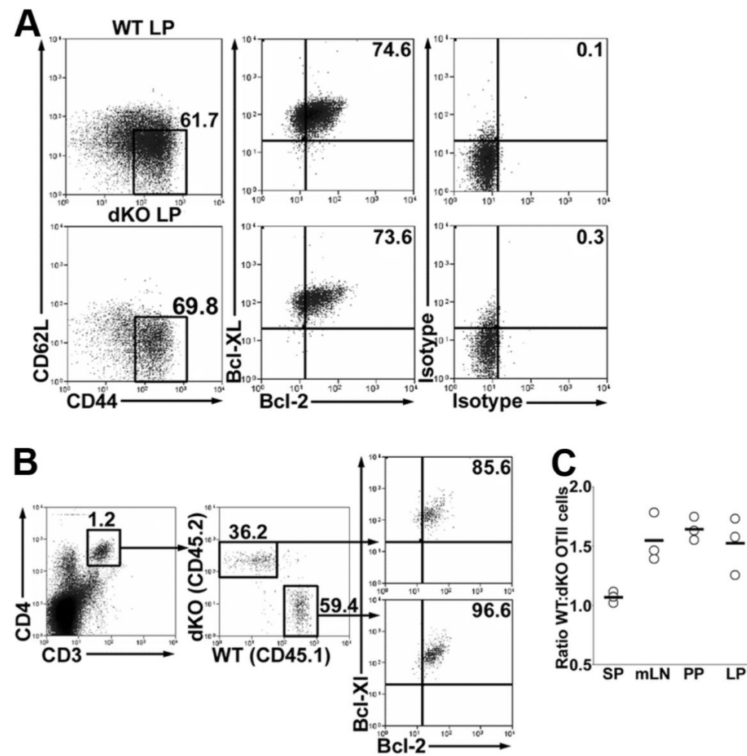

FIGURE 5.

Normal Bcl-2 and Bcl-x_L expression in dKO LP $CD4^+$ T cells. To investigate whether the failure of dKO $CD4^+$ T cells to survive in the LP was due to impaired expression of Bcl-2 and Bcl-x_L, intracellular staining was done. *A*, Expression of Bcl-2 and Bcl-x_L by $CD62L^-CD44^{high}$ $CD4^+$ T cells of LP isolated from WT and dKO mice. *B*, WT (CD45.1) and dKO (CD45.2) OTII cells were transferred into CD3εTg26 mice which were then orally immunized with OVA and analyzed 4 days later. Expression of Bcl-2 and Bcl-x_L by WT and dKO OTII cells of LP. *C*, Ratio of WT:dKO OTII cells in spleen, mLN, PP, and LP at 4 days after oral immunization. Numbers show percentage of cells in gate, data from three mice.

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