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OX40 ligand regulates splenic CD8$^-$ dendritic cell-induced Th2 responses *in vivo*

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ABSTRACT

In mice, splenic conventional dendritic cells (cDCs) can be separated, based on their expression of CD8α into CD8$^-$ and CD8$^+$ cDCs. Although previous experiments demonstrated that injection of antigen (Ag)-pulsed CD8$^-$ cDCs into mice induced CD4 T cell differentiation toward Th2 cells, the mechanism involved is unclear. In the current study, we investigated whether OX40 ligand (OX40L) on CD8$^-$ cDCs contributes to the induction of Th2 responses by Ag-pulsed CD8$^-$ cDCs *in vivo*, because OX40–OX40L interactions may play a preferential role in Th2 cell development. When unseparated Ag-pulsed OX40L-deficient cDCs were injected into syngeneic BALB/c mice, Th2 cytokine (IL-4, IL-5, and IL-10) production in lymph node cells was significantly reduced. Splenic cDCs were separated to CD8$^-$ and CD8$^+$ cDCs. OX40L expression was not observed on freshly isolated CD8$^-$ cDCs, but was induced by anti-CD40 mAb stimulation for 24 h. Administration of neutralizing anti-OX40L mAb significantly inhibited IL-4, IL-5, and IL-10 production induced by Ag-pulsed CD8$^-$ cDC injection. Moreover, administration of anti-OX40L mAb with Ag-pulsed CD8$^-$ cDCs during a secondary response also significantly inhibited Th2 cytokine production. Thus, OX40L on CD8$^-$ cDCs physiologically contributes to the development of Th2 cells and secondary Th2 responses induced by Ag-pulsed CD8$^-$ cDCs *in vivo*.

1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells critical for the induction of adaptive immune responses. Conventional DCs (cDCs) are specialized for antigen processing and presentation to T cells and can be subdivided by their surface expression of CD8α and CD4 as CD8$^-$CD4$^+$, CD8$^-$CD4$^-$, and CD8$^+$CD4$^-$ cDCs in the spleen [1–4]. Both CD8$^-$CD4$^+$ and CD8$^-$CD4$^-$ cDCs appear functionally similar and are referred to as CD8$^-$ cDCs [2,3]. In contrast, the physiologic functions of both CD8$^-$ cDCs and CD8$^+$ cDCs markedly differ. *In vivo* experiments demonstrated that injection of antigen-pulsed CD8$^-$ cDCs induced CD4 T cell differentiation toward Th2 responses (high levels of IL-4, IL-5, and IL-10) whereas antigen-pulsed CD8$^+$ cDCs induced Th1 responses (high levels of IFN-γ [5]. The ability of CD8$^+$ cDCs to induce Th1 differentiation is explained by their ability to produce IL-12 efficiently [6,7]. However, the mechanisms of Th2 responses induced by CD8$^-$ cDCs are not understood.

CD4 T cell differentiation might be regulated by cytokines and various costimulatory molecules expressed on CD4 T cells, and their cognate ligands expressed on DCs such as OX40 (CD134) costimulatory molecule, a member of the TNF receptor superfamily, and its ligand, OX40L (CD252) [8,9]. OX40 is preferentially expressed on activated CD4 T cells and OX40L is mainly expressed on antigen-presenting cells, including activated DCs, B cells, and macrophages. Recent studies emphasized the role of OX40L on DCs for Th2 polarization. In humans, schistosomal egg antigen induced monocyte-derived DCs to express OX40L, which contributed to the induction of Th2 responses [10]. IL-3-treated plasmacytoid DCs expressed OX40L and induced Th2 responses by promoting CD4 T cells to secrete IL-4, IL-5, and IL-13. Blockade of OX40L significantly inhibited this ability of IL-3-treated plasmacytoid DCs [11]. Moreover, OX40L expressed on thymic stromal lymphopoietin (TSLP)-activated DCs induced naïve CD4 T cells to differentiate into TNF-α$^+$ IL-10$^-$ inflammatory Th2 cells [12]. In mice, OX40L expression on bone marrow-derived DCs (BMDCs) is upregulated downstream of CD40 signaling and is critical for optimal Th2 priming *in vivo* [13]. In contrast to these studies, the use of agonistic anti-OX40 mAb revealed OX40-mediated costimulation enhanced the

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development of Th1 responses induced by splenic CD8$^-$ cDCs *in vivo* [14]. Thus, the function of OX40L on splenic CD8$^-$ cDCs is still controversial. In this study, we examined the physiological contribution of OX40–OX40L interactions on CD8$^-$ cDCs-induced Th2 responses by using blocking anti-OX40L mAb.

2. Materials and methods

2.1. Animals

Female BALB/c mice were purchased from Charles River Laboratories (Kanagawa, Japan). OX40L-deficient mice were generated as previously described [15] and backcrossed for seven generations with BALB/c mice purchased from Oriental Yeast Co. (Tokyo, Japan). All mice were 6–8 week old at the start of experiments and kept under specific pathogen-free conditions during the experiments. All animal experiments were approved by Juntendo University Animal Experimental Ethics Committee.

2.2. Antibodies and reagents

An anti-mouse OX40L (RM134L) mAb was previously generated in our laboratory [16]. Control rat IgG was purchased from Sigma–Aldrich (St Louis, MO, USA). Purified anti-CD40 (HM40-3), allophycocyanin (APC)-conjugated anti-CD8α (53-6.7), and rat IgG isotype control were purchased from eBioscience (San Diego, CA, USA). Purified anti-CD16/32 (2.4G2) and FITC-conjugated anti-CD11c (HL3), recombinant mouse GM-CSF, IL-4, and IFN-γ were purchased from BD Biosciences (San Jose, CA, USA).

2.3. Preparation and stimulation of splenic DCs

To isolate splenic DCs, spleens from BALB/c or OX40L-deficient mice were digested with 400 U/ml of collagenase (Wako Biochemicals, Tokyo, Japan), further dissociated in Ca^{2+}-free medium in the presence of 5 mM EDTA, and separated into low- and high-density fractions by Optiprep-gradient (Axis-Shield, Oslo, Norway) as described previously [17]. Low-density cells were pulsed overnight with 50 μg/ml of keyhole limpet hemocyanin (KLH) in culture medium supplemented with 20 ng/ml of GM-CSF as described previously [5]. After overnight culture, splenic CD11c$^+$ DCs were isolated by incubation with anti-CD11c-coupled magnetic beads and positive selection by autoMACS column (Miltenyi Biotec, Bergisch Gladbach, Germany). CD11c$^+$ DCs were further separated according to CD8α expression by FACS sorting. CD11c$^+$ cells were incubated with FITC-conjugated anti-CD11c and APC-conjugated anti-CD8α mAbs, and two populations (CD8$^+$CD11c$^+$ DCs and CD8$^-$CD11c$^+$ DCs) were sorted by FACSVantage (BD Biosciences). To examine OX40L expression, separated DC populations were incubated with anti-CD40 mAb (10 μg/ml) with IL-4 (20 ng/ml) or IFN-γ (20 ng/ml) in the presence or absence of GM-CSF (20 ng/ml) at 37 °C for 24 h.

2.4. Flow cytometric analysis

Cells were pre-incubated with unlabeled anti-CD16/32 mAb to avoid non-specific binding of Abs to FcγR, incubated with FITC- or APC-labeled mAbs, or biotinylated mAb followed by PE-labeled streptavidin. Stained cells (live cells gated by forward and side scatter profiles and propidium iodide exclusion) were analyzed by FACSCalibur (BD Biosciences), and data were processed by CellQuest (BD Biosciences).

2.5. Immunization protocol

KLH-pulsed splenic cDCs were washed in PBS and immunized (3×10^5 cells) into the hind footpad of BALB/c mice. Some groups of mice ($n = 5$–6) were administered 400 μg of anti-OX40L mAb or rat IgG intraperitoneally (i.p.) at days 0, 1, and 3, or daily from days 0 to 3 and days 14–17. Popliteal lymph node (LN) cells were harvested 5 days after primary or secondary immunizations.

2.6. T cell stimulation in vitro

LN cells were isolated and cultured in RPMI1640 medium (containing 10% FCS, 10 mM HEPES, 2 mM L-glutamine, 0.1 mg/ml penicillin and streptomycin, and 50 μM 2-mercaptoethanol) at a density of 6×10^5 cells/well in the presence of indicated doses of KLH. To assess proliferative responses, cultures were pulsed with tritiated thymidine ([^{3}H]TdR; 0.5 μCi/well; PerkinElmer, Winter Street Waltham, MA, USA) for the last 6 h of a 48 h or 72 h culture and harvested on a Micro 96 Harvester (Molecular Devices, Sunnyvale, CA, USA). Incorporated radioactivity was measured using a microplate beta counter (Micro β Plus; PerkinElmer). To determine cytokine production, cell-free supernatants were collected at 48 h or 72 h and assayed for IL-2, IL-4, IL5, IL-10, and IFN-γ by ELISA using Ready-SET-Go! kits (eBioscience) according to the manufacturer's instructions.

2.7. Statistical analysis

Statistical analyses were performed by unpaired Student *t*-test or Tukey's multiple comparison test. Results are expressed as mean ± SEM. Values of $P < 0.05$ were considered significant.

3. Results

3.1. OX40L is required for optimal Th2 responses induced by splenic cDCs in vivo

Because a previous report demonstrated KLH-pulsed CD8$^-$ and CD8$^+$ cDCs differentially regulated Th cell development, we followed the same protocol using KLH as an antigen. To clarify the contribution of splenic cDC OX40L on CD4 T cell differentiation, we examined CD4 T cell responses induced by splenic OX40L$^{-/-}$ cDCs. cDCs were purified from spleens of OX40L-deficient or wild-type BALB/c mice without treatment, pulsed with KLH during overnight culture with GM-CSF, to isolate CD11c^{high} B220$^-$ cells (cDC population). OX40L$^{-/-}$ cDCs or WT cDCs (3×10^5) were injected into hind footpads of syngeneic BALB/c mice. LNs were prepared on day 5 and proliferative responses and cytokine production against various doses of KLH were assessed. KLH-specific proliferative responses and IL-2 production were reduced in LN cells from OX40L$^{-/-}$ cDCs-injected mice compared with WT cDCs-injected mice (Fig. 1). Th2 cytokine production (IL-4, IL-5, and IL-10) was also significantly reduced in OX40L$^{-/-}$ cDCs-injected mice compared with WT cDCs-injected mice. In contrast, Th1 type cytokine IFN-γ production was non-significantly increased in OX40L$^{-/-}$ cDCs-injected mice compared with WT cDCs-injected mice.

Similar results were obtained when KLH-pulsed OX40L$^{-/-}$ bone marrow-derived DCs (BMDCs) were injected into hind footpads of BALB/c mice (Supplemental Fig. S1). KLH-specific proliferative responses and IL-2 production were reduced in LN cells from OX40L$^{-/-}$ BMDCs-injected mice compared with WT BMDCs-injected mice. Th2 cytokine production (IL-4, IL-5, and IL-10) was significantly reduced in OX40L$^{-/-}$ BMDCs-injected mice, whereas IFN-γ production was similar between OX40L$^{-/-}$ BMDCs-injected

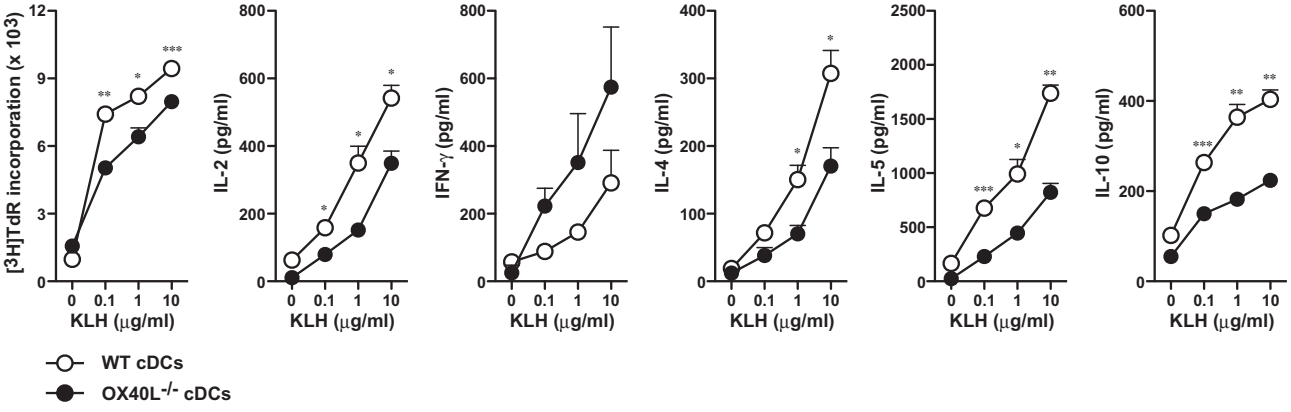

Fig. 1. OX40L is required for optimal Th2 responses by splenic cDCs *in vivo*. BALB/c mouse hind footpads were injected with KLH-pulsed cDCs isolated from the spleen of wild-type BALB/c or OX40L$^{-/-}$ BALB/c mice. LN cells were harvested at day 5 and cultured with indicated doses of KLH. To estimate proliferation, 0.5 μCi ^{3}H-thymidine ([^{3}H]TdR) was added during the last 6 h of a 48 h culture. Production of IFN-γ, IL-2, IL-4, IL-5, and IL-10 in culture supernatants at 48 h was determined by ELISA. Results are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Similar results were obtained in three independent experiments.

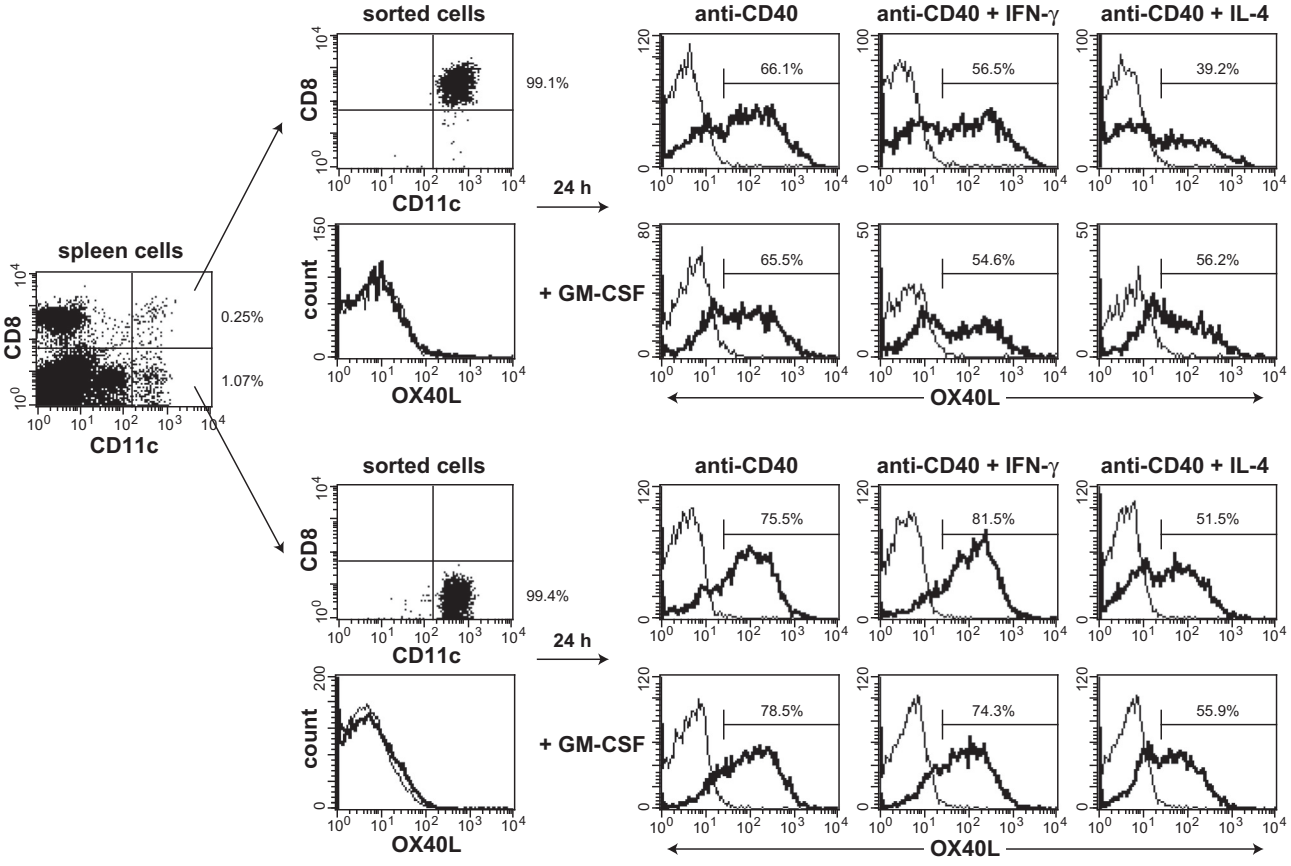

Fig. 2. Expression of OX40L on activated CD8^{-} and CD8^{+} cDCs. Spleen cells were isolated from BALB/c mice and stained with FITC-labeled anti-CD11c, APC-labeled anti-CD8α, and biotinylated anti-OX40L or control IgG followed by PE-labeled streptavidin. CD8^{-}CD11c^{high} and CD8^{+}CD11c^{high} cDCs were isolated from spleens by FACS sorting. Isolated CD8^{-}CD11c^{high} and CD8^{+}CD11c^{high} cDCs were stimulated with anti-CD40 mAb in the presence or absence of GM-CSF, IFN-γ, and IL-4. Cells were harvested at 24 h and stained with anti-OX40L mAb or control rat IgG. Thick lines indicate staining with anti-OX40L mAb and thin lines indicate background staining with control IgG. Data are representative of three experiments.

and WT BMDCs-injected mice. In addition, administration of neutralizing anti-OX40L mAb to WT BMDCs-injected mice significantly reduced Th2 cytokine production similar to OX40L$^{-/-}$ BMDCs-injected mice. Th2 cytokine reduction was also observed in KLH-pulsed WT BMDCs injected with anti-OX40L mAb into IFN-γ-deficient mice (Supplemental Fig. S2). These results indicated a critical role of OX40L in splenic cDCs- and BMDCs-induced Th2 responses *in vivo*. The inhibition of Th2 responses by anti-OX40L treatment was not necessarily a result of a shift to Th1 responses.

3.2. Expression of OX40L on splenic cDCs

The expression of OX40L on two major subsets of splenic cDCs was assessed by flow cytometry. Splenic cDCs were separated

based on CD8α and CD11c expression, into $CD8^{-}CD11c^{high}$ cDCs ($CD8^{-}$ cDCs) and $CD8^{+}CD11c^{high}$ cDCs ($CD8^{+}$ cDCs), and stimulated with agonistic anti-CD40 with or without cytokines (GM-CSF, IFN-γ, or IL-4) for 24 h (Fig. 2). While OX40L expression was not observed on freshly isolated $CD8^{-}$ or $CD8^{+}$ cDCs, it was induced by anti-CD40 mAb stimulation. Addition of IL-4 reduced OX40L expression on anti-CD40-stimulated $CD8^{-}$ and $CD8^{+}$ cDCs, whereas OX40L expression was not affected by the addition of GM-CSF or IFN-γ.

3.3. Effect of anti-OX40L mAb on the development of Th2 responses induced by KLH-pulsed $CD8^{-}$ cDCs in vivo

We next examined whether KLH-pulsed $CD8^{-}$ cDCs could induce Th2 responses compared with KLH-pulsed $CD8^{+}$ cDCs, and whether OX40L contributes to $CD8^{-}$ cDCs-induced Th2 responses. BALB/c mice were injected into the hind footpads with KLH-pulsed $CD8^{-}$ or $CD8^{+}$ cDCs, and treated with anti-OX40L mAb or control IgG at days 0, 1, and 3. LN cells were isolated at day 5 and KLH-specific proliferative responses and cytokine production were assessed. Consistent with previous reports, IL-4 production by LN cells from $CD8^{-}$ cDCs-injected mice was significantly higher than in $CD8^{+}$ cDCs-injected mice (Fig. 3). In contrast, IFN-γ production in $CD8^{+}$ cDCs-injected mice was non-significantly increased compared with the $CD8^{-}$ cDCs-injected mice. Proliferative responses and other Th2 cytokine production (IL-5 and IL-10) were similar between $CD8^{-}$ cDCs-injected and $CD8^{+}$ cDCs-injected mice. Anti-OX40L mAb administration strongly inhibited IL-4, IL-5, and IL-10 production induced by $CD8^{-}$ cDCs injection, while IFN-γ was slightly increased. Thus, OX40L has an important role in the development of Th2 responses induced by KLH-pulsed $CD8^{-}$ cDCs *in vivo*. Furthermore, administration of anti-OX40L mAb reduced IL-4 production induced by $CD8^{+}$ cDCs injection. Therefore, OX40L may also regulate IL-4 production induced by KLH-pulsed $CD8^{+}$ cDCs.

3.4. Effect of anti-OX40L mAb in secondary Th2 responses induced by KLH-pulsed $CD8^{-}$ cDCs in vivo

The OX40–OX40L pathway is crucial for recall responses when memory T cells are reactivated [18]. Therefore, we further examined the role of OX40L in secondary Th2 responses induced by KLH-pulsed $CD8^{-}$ cDCs *in vivo*. BALB/c mice were immunized first into the hind footpads with KLH-pulsed $CD8^{-}$ cDCs at day 0 and then under the same conditions with KLH-pulsed $CD8^{-}$ cDCs at day 14. Some groups of mice were treated with anti-OX40L mAb or control IgG daily from days 0 to 3 in the primary phase and days 14–17 in the secondary phase. LN cells were isolated at day 19 and the KLH-specific Th2 cytokine production was assessed. Anti-OX40L mAb administration during the primary phase only, reduced IL-4 and IL-5 production compared with control IgG (Fig. 4). In addition, anti-OX40L mAb administration in the secondary phase strongly inhibited IL-4, IL-5, and IL-10 production compared with control IgG. The inhibitory effect of anti-OX40 mAb

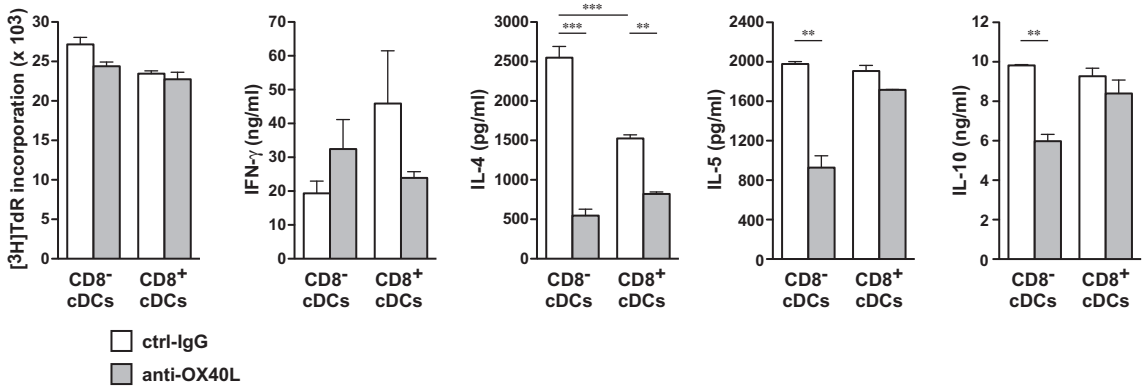

Fig. 3. Effect of anti-OX40L mAb on the development of Th2 responses induced by KLH-pulsed $CD8^{-}$ cDCs *in vivo*. BALB/c mouse hind footpads were injected with KLH-pulsed $CD8^{-}$ or $CD8^{+}$ cDCs. Mice were administered 400 μg of anti-OX40L mAb or control rat IgG (ctrl-IgG) i.p. at days 0, 1, and 3. LN cells were harvested at day 5 and cultured with 20 μg/ml of KLH. To estimate proliferation, 0.5 μCi [^{3}H]TdR was added during the last 6 h of a 72 h culture. Production of IFN-γ, IL-4, IL-5, and IL-10 in the culture supernatants at 72 h was determined by ELISA. Results are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Similar results were obtained in three independent experiments.

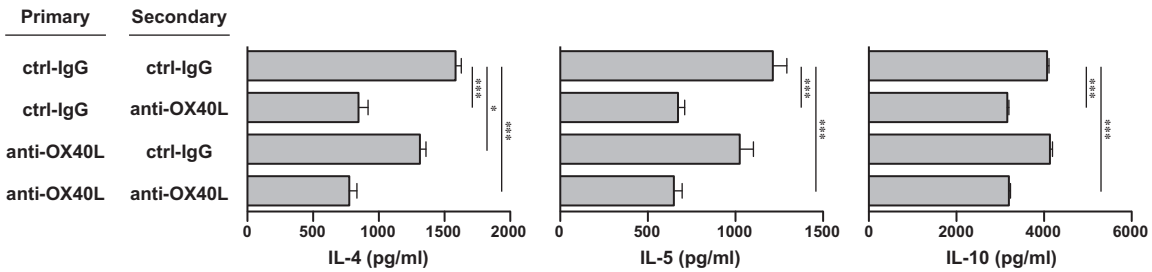

Fig. 4. Effect of anti-OX40L mAb on the development of memory Th2 responses induced by $CD8^{-}$ cDCs *in vivo*. BALB/c mice were immunized first with KLH-pulsed $CD8^{-}$ cDCs at day 0 and boosted with the same KLH-pulsed $CD8^{-}$ cDCs at day 14. Mice were administered 400 μg of anti-OX40L mAb or ctrl-IgG i.p. daily from days 0 to 3 and days 14–17. LN cells were harvested at day 19 and cultured with 10 μg/ml of KLH. To estimate proliferation, 0.5 μCi [^{3}H]TdR was added during the last 6 h of a 72 h culture. Production of IFN-γ, IL-4, IL-5, and IL-10 in culture supernatants at 72 h was determined by ELISA. Results are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Similar results were obtained in three independent experiments.

treatment in the secondary phase was comparable to mice treated with anti-OX40 mAb in both primary and secondary phases. Thus, OX40L might have an important role in both primary and secondary Th2 responses induced by KLH-pulsed CD8$^-$ cDCs *in vivo*.

4. Discussion

The current study investigated the physiological role of splenic CD8$^-$ cDC OX40L to regulate CD4 T cell Th2 differentiation *in vivo*. When antigen KLH-pulsed OX40L-deficient cDCs were injected into BALB/c mice, LN Th2 cytokine production (IL-4, IL-5, and IL-10) was significantly reduced. Splenic cDCs were separated into CD8$^-$ and CD8$^+$ cDCs. A previous study demonstrated that although injection of KLH-pulsed CD8$^-$ cDCs induced CD4 T cell differentiation toward Th2 responses, KLH-pulsed CD8$^+$ cDCs promoted Th1 responses [5]. Consistently, our results indicated that CD8$^-$ cDCs markedly induced IL-4 production and CD8$^+$ cDCs tended to induce IFN-γ production. Administration of neutralizing anti-OX40L mAb significantly inhibited IL-4, IL-5, and IL-10 production induced by KLH-pulsed CD8$^-$ cDCs. Moreover, treatment of anti-OX40L mAb with KLH-pulsed CD8$^-$ cDCs during a secondary response also significantly inhibited Th2 cytokine production. Thus, OX40L contributes to both the development of Th2 cells and secondary Th2 responses induced by KLH-pulsed CD8$^-$ cDCs *in vivo*. However, these findings are inconsistent with a previous report where administration of anti-OX40 mAb enhanced the development of Th1 cells secreting high levels of IFN-γ, but no IL-4 and IL-5, induced by KLH-pulsed CD8$^-$ cDCs *in vivo* [14]. The reason for this discrepancy is not clear, but it may be attributable to differences in experimental conditions. The previous study isolated splenic cDCs from mice treated with FMS-like tyrosine kinase 3 ligand (Flt3L) on 11 days, whereas mice were untreated in our study. Flt3 is a crucial factor in humans and mice to promote the development of cDCs *in vivo* and *in vitro*. However, a bias toward the generation of CD8$^+$ cDCs in the spleen was observed in mice treated with Flt3L [19,20]. The previous study also examined the effect of exogenous OX40 costimulation using agonistic anti-OX40 mAb, suggesting such an effect is not mediated by endogenous OX40–OX40L interactions between CD4 T cells and cDCs. Our results suggest that physiological OX40–OX40L interactions participate in CD4 T cell-CD8$^-$ cDCs interactions, and that OX40L on CD8$^-$ cDCs might contribute to the induction of Th2 responses *in vivo*.

In humans, TSLP-activated DCs can promote the differentiation of naïve CD4 T cells into a Th2 phenotype and the expansion of CD4 Th2 memory cells in an unique manner dependent on OX40L in the absence of IL-12 [12]. TSLP, an IL-7-like cytokine, is produced mainly by damaged epithelial cells and is a key molecule that links epithelial cells and DCs at the interface of allergic inflammation by participating in the programming of DC-mediated Th2 polarization [21–24]. TSLP activates STAT1, STAT3, STAT4, STAT5, and STAT6, whereas the contributions of individual STAT proteins to the activation of DCs is unclear [25]. Most recently, a mouse study demonstrated that DC-specific deletion of STAT5 was critical for TSLP-mediated Th2 differentiation, but not Th1 differentiation [26]. Loss of STAT5 in DCs affected upregulation of OX40L expression in response to TSLP. However, DC subsets in *Stat5*$^{-/-}$ chimeric mouse spleens had a higher proportion of CD8$^+$ cDCs and a reduced frequency of CD4$^+$ CD8$^-$ cDCs compared with *Stat5*$^{+/+}$ chimeras, suggesting STAT5 signaling regulates a balanced production of these splenic DC subsets *in vivo* [27]. Thus, STAT5 may be required for OX40L-dependent Th2 cell differentiation induced by KLH-pulsed CD8$^-$ cDCs. To confirm this, further studies are required using STAT5-specific deleted CD8$^-$ cDCs. In this study, we demonstrated that KLH-pulsed OX40L$^{-/-}$ BMDCs injected into hind footpads of BALB/c mice significantly reduced Th2 cytokine production (IL-4, IL-5, and IL-10) in LN cells compared with WT BMDCs-injected mice. Consistent with these observations, it was reported that OX40L expression by GM-CSF-induced BMDCs is required for optimal induction of primary and memory Th2 responses *in vivo* [13]. GM-CSF can activate STAT5, and GM-CSF-activated STAT5 inhibits the transcription of *Irf8* [27], which encodes interferon regulatory factor 8 (IRF8). IRF8 is required for IL-12 production [25], an essential cytokine required for the induction of Th1 responses [28]. Therefore, OX40L-dependent Th2 responses induced by KLH-pulsed CD8$^-$ cDCs might depend on the absence of IL-12, as IL-12 has a dominant effect over OX40L in Th cell differentiation [12]. Indeed, we observed that CD8$^+$ cDCs produced high amounts of IL-12p40 after stimulation with agonistic anti-CD40 mAb, whereas IL12p40 production on CD8$^-$ cDCs was markedly lower (unpublished observation). Taken together, these findings suggest that the development of Th2 responses by KLH-pulsed CD8$^-$ cDCs requires two conditions: the expression of OX40L and the absence of IL-12.

However, whether OX40 signaling on CD4 T cells directly induces Th2 differentiation is still unclear. It is well known that OX40 can bind to TNF receptor-associated factor (TRAF) 2, TRAF3, and TRAF5. However, these molecules also can bind to other TNF receptor family molecules. On a transcriptional basis, it was determined that OX40L expressed by TSLP-DCs induced the expression of GATA-3 in CD4 T cells, supporting their critical role in Th2 polarization [12]. Another study indicated that OX40 enhanced TCR-induced calcium influx, leading to the enhanced nuclear accumulation of NFATc1 and NFATc2, that likely regulates the production of cytokines [29]. More studies are required to determine how OX40 signaling promotes Th2 differentiation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.060.

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