

The bile acid-activated retinoic acid response in dendritic cells is involved in food allergen sensitization

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Abstract

Background: Alteration of commensal microbiota is highly correlated with the prevalence of allergic reactions to food in the gastrointestinal tract. The mechanisms by which microbiota modulate food allergen sensitization in the mucosal site are not fully understood.

Methods: We generate DCs specific knockout of retinoic acid receptor α (Rara) gene mice (DC KO Rara) to evaluate food sensitization. The bile acid-activated retinoic acid response was evaluated by flow cytometry, real-time RT-PCR and Illumina transcriptome sequencing. The global effect of Abx treatment on BA profiles in the mucosal lymph tissue mLN in mice was examined by UPLC-MS analysis.

Results: In this study, we demonstrate that depletion of commensal gut bacteria leads to enhanced retinoic acid (RA) signaling in mucosal dendritic cells (DCs). RA signaling in DCs is required for the production of food allergen-specific IgE and IgG1. Antibiotics induced an enlarged bile acid (BA) pool, and dysregulated BA profiles contributed to enhanced RA signaling in mucosal DCs. BA-activated RA signaling promoted DC up-regulation of interferon I signature, RA signature, OX40L, and PDL2, which may lead to T helper 2 differentiation of CD4⁺ T cells. BA-activated RA signaling involved the farnesoid X receptor and RA receptor α (RARa) interaction. Depletion of bile acid reduces food allergen specific IgE and IgG1 levels in mice.

Conclusion: Our research unveils a mechanism of food sensitization modulated by BA-RA signaling in DCs, which suggests a potential new approach for the intervention of food allergic reactions.

KEYWORDS

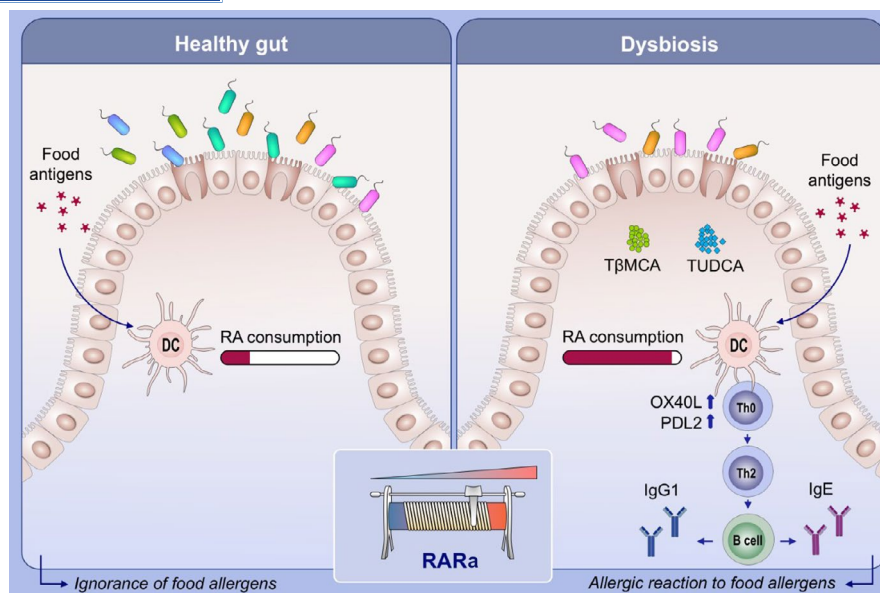
animal models, basic immunology, basic mechanisms, dendritic cells, food allergy, immunoglobulin E, microbiome, mucosal immunity

Abbreviations: DCs, dendritic cells; Abx, antibiotic; BA, bile acids; CA, cholestyramine; DCs, dendritic cells; DDAO, DDAO galactoside; ELISA, enzyme-linked immunosorbent assay; FXR, farnesoid X receptor; IgE, immunoglobulin E; mLN, mesenteric lymph node; OX40L, OX40 ligand; RA, retinoic acid; RARa, retinoic acid receptor alpha; RARE, retinoic acid responsive element; SCFAs, short-chain fatty acids; spDCs, dendritic cells isolated from spleen; Th2, T-helper 2; TLR, toll-like receptor; TUDCA, tauroursodeoxycholic acid; T β MCA, tauro-beta-muricholic acid.

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Renlan Wu and Xiefang Yuan contributed equally to this study.

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GRAPHICAL ABSTRACT

- Dysbiosis leads to enhanced retinoic acid (RA) signaling in mucosal dendritic cells (DCs) and induce food allergens sensitization.
- Antibiotic-induced dysregulated bile acid profiles contribute to enhanced RA signaling in mucosal DCs.
- Retinoic acid receptor alpha act as a “rheostat” and modulate food sensitization in mucosal dendritic cells.

1 | INTRODUCTION

The colonization of microbiota in humans and animals is essential for proper immune reactions.¹ Lack of microbiota or dysbiosis is highly correlated with allergic reactions.^{2,3} Specific commensal bacteria have been reported to protect against food allergies in mice and humans.^{4–7} Toll-like receptor (TLR) ligands⁸ and short-chain fatty acids (SCFAs)⁹ from commensal bacteria can decrease the circulating levels of immunoglobulin E (IgE) and thus ameliorate allergic lung inflammation. In contrast, the mechanism by which gut microbiota inhibit food allergic reactions remains unclear.

Allergen-specific IgG1 and IgE production is the hallmark of the allergic response. The differentiation of CD4+T cells into T-helper 2 (Th2) cells is required for the class switching recombination of IgE and IgG1.¹⁰ Dendritic cells (DCs) can stimulate CD4+T cells toward Th2 differentiation by multiple mechanisms, such as OX40 ligand (OX40L) upregulation,^{11,12} the transcription factors IRF4 and Programmed cell death 1 ligand 2 (PDL2),^{13,14} and the recently identified DC-intrinsic type I interferon (IFN-I) signature.^{15–17} However, the mechanism by which gut microbiota modulate DC phenotypes and promote allergen-specific IgG1 and IgE production remains incompletely understood.

The vitamin A metabolite, retinoic acid (RA), has a broad impact on immune reactions. RA deficiency often leads to immune defects, whereas excess RA can potentially promote inflammatory disorders.¹⁸ Previously, we reported that gut fungi are essential for maintaining DC RA signaling in lymph nodes.¹⁹ However, overgrowth of gut fungi induced M2 macrophage polarization and exacerbated pulmonary allergic reactions.^{20,21} Moreover, RA has been reported to promote M2 macrophage polarization^{22,23} and boost Th2 cytokine

production,^{24,25} which are critical for the class switching recombination of IgE and IgG1. Furthermore, gut microbiota has been shown to modulate RA signaling in intestinal epithelial cells.²⁶ Bacterial quorum sensing molecules acyl-homoserine lactones promote allergic airway inflammation by activating the RA response in DCs.²⁷ However, it has not been determined yet whether gut microbiota can modulate DC RA signaling nor which factors are involved.

Bile acids (BA) are cholesterol derivatives that are synthesized in the liver, conjugated with glycine or taurine, and stored in the gallbladder.²⁸ After secretion in the small intestine, BA undergo extensive enterohepatic recycling and gut microbial modification.²⁹ An “orphan” nuclear receptor, named the farnesoid X receptor (FXR), has been identified as the principal BA receptor.³⁰ FXR plays multiple roles in regulating inflammatory responses, gut barrier function, and the homeostasis of BAs, lipids, and glucose.³¹ It has been reported that BA excretion depends on gut microbiota, and that microbial deconjugation prevents BA uptake from the small intestine.³² Germ-free mice have an increased proportion of tauro-beta-muricholic acid (TβMCA) in the BA pool.³³ Furthermore, antibiotic (Abx) treatment or dysbiosis profoundly changes the BA profile^{34,35} and may contribute to gut inflammation. However, whether microbiota-modulated BA metabolism contributes to mucosal allergen sensitization remains elusive.

In this study, we examined the mechanisms via which gut microbiota regulate food allergen sensitization. Abx treatment provoked the RA responsive element (RARE) response in mucosal DCs, which contributes to food allergen-specific IgG1 and IgE production in mice. Abx treatment changed the BA profile related to the RARE response in DCs. BA-stimulated DCs to upregulate the IFN-I signature and OX40L and PDL2 gene expression, which may promote Th2 cell differentiation.

2 | RESULTS

2.1 | Abx treatment induces a RARE response in mucosal DCs

Previous studies on a mouse model have demonstrated that microbiota can regulate food sensitization, as Abx-treated mice and germ-free mice were more susceptible to food allergic reactions.^{4,36} To confirm these results in our laboratory, we sensitized and challenged Abx-treated and germ-free mice with intragastric co-administration of purified peanuts protein (PN) and mucosal adjuvant cholera toxin (CT), which induce PN-specific IgE and IgG1.

Abx-treated and germ-free mice exhibited a marked elevation in PN-specific IgE and IgG1 after sensitization and challenge (Figure S1A). Gut microbiome sequencing revealed that Abx treatment resulted in a significantly reduced bacterial diversity of both the ileal and colon contents (Figure S1B). However, Abx treatment did not alter the diversity of mouse commensal fungi (Figure S1C).

Next, we examined whether Abx treatment alters RA signaling in DCs *in vivo*. We first tested the reliability of the RA response in reporter transgenic mice, which have a b-galactosidase (LacZ) reporter under the control of RARE (Figure S2A). As such, b-galactosidase activity can be measured by the conversion of DDAO galactoside (DDAOG) to DDAO.³⁷ RA stimulates DDAO fluorescent signals in cultured BMDCs from RA reporter transgenic mice (Figure S2B). Intraperitoneal injection of RA upregulates DDAO fluorescent signals in DCs in mesenteric lymph node (mLN) (Figure S2C). Other retinoids, acitretin, bexarotene, and tamibarotene maintain the ability to stimulate DDAO fluorescent signals in cultured BMDCs (Figure S2D). These results verify that RA reporter mice can serve as a tool to assess RA signaling both *in vitro* and *in vivo*.

Treating RA reporter transgenic mice with Abx for 4 weeks increases DDAO fluorescent signals in DCs from lamina propria and intestinal epithelial cells (Figure 1A). Abx treatment also upregulated DDAO fluorescent signals in DCs from other mucosal lymphoid tissues, such as mLN (Figure 1B) and Peyer's patches (Figure 1C). However, Abx treatment did not alter DDAO fluorescent signals in DCs from peripheral lymphoid tissue, such as the spleen (Figure 1B) and inguinal lymph node (Figure 1C), or in CD8⁺ T, CD4⁺ T, and B220⁺ B cells from peripheral and mucosal lymphoid tissues (Figure 1B–C). These results indicate that microbiota influence RA signaling in mucosal DCs.

2.2 | RA signaling in DCs is essential for food allergic reactions

Next, we investigated whether RA signaling in DCs contributes to PN-specific IgE and IgG1 production. We established DCs from specific RA receptor α (RARa) mice (CD11c^{cre}RARa^{fl/fl} or DC KO RARa) knockouts by crossbreeding CD11c^{cre} mice with RARa^{fl/fl} mice, as RARa was the dominant RA signaling transcription factor in DCs.³⁸ First, we examined whether DC KO RARa mice bear innate immune

defects, considering the general effect of RA on the immune system.³⁹ The weights of the immune organs (spleen and mLN) of DC KO RARa mice were normal (Figure 2A), and the populations of total DCs (CD11c⁺) were consistent between DC KO RARa and RARa^{fl/fl} mice in the spleen (peripheral lymphoid tissue) and mLN (mucosal lymphoid tissue) (Figure 2B). The frequencies of B and T cells were unchanged in DC KO RARa and RARa^{fl/fl} mice in the spleen and mLN (Figure 2C). The frequencies of CD4⁺ and CD8⁺ T cells in the spleen and mLN were unchanged in DC KO RARa mice (Figure S3A). Also, subsets of CD8⁺ and CD11b⁺ DCs were unchanged in DC KO RARa mice (Figure S3B). However, DC KO RARa mice exhibited reduced regulatory T cells in the spleen, but not in mLN (Figure S3C). DC KO RARa mice exhibited reduced cell surface activation marker CD40 and CD80 with or without LPS stimulation (Figure S4C). The antigen processing ability was unchanged in DC KO RARa mice, as the uptake of fluorescent-labeled OVA was similar between tamibarotene-stimulated groups or DMSO controls (Figure S4D).

Next, we sensitized and challenged DC KO RARa mice by intragastric co-administration with PN plus CT. PN-specific IgE and IgG1 levels and the mouse mast cell activation marker mast cell protease-1, were decreased in DC KO RARa mice (Figure 2D). Collectively, these data indicate that RA signaling in DCs is essential for food allergic reactions.

2.3 | BAs activate the RARE response in mucosal DCs

Next, we determined the factors involved in microbiota-regulated RARE responses in mucosal DCs. First, we stimulated DCs from RA reporter mice with TLR ligand-1, -2, -3, -4, -5, -6, -7, and -9. However, these TLR ligands did not change the RARE response (DDAO, LacZ) in BMDCs or DCs isolated from spleen (spDCs) (Figure 3A–B). Multiple microorganisms cohabit within the gut, which may suggest a synergic effect of TLRs on the RARE response in DCs. We stimulated BMDCs with different combinations of TLR ligands; however, none of these combinations altered the RARE response (Figure 3C). Next, we tested the effect of SCFAs, the main metabolites produced by gut bacteria in the gastrointestinal tract. Neither single SCFAs nor the combination of acetate (C2), propionate (C3), or butyrate (C4) altered the RARE response in DCs (Figure 3D–E).

To test whether gut bacteria-derived molecules modulate the RARE response in DCs, we used 0.22 μ m filter-sterilized cecum content as a control, as it has been reported to contain high SCFA levels⁴⁰ and multiple TLR ligands. Unexpectedly, LPS and SCFAs did not stimulate a RARE response, whereas cecum content stimulated a RARE response in a concentration-dependent manner (Figure 4A–B). Meanwhile, we observed that Abx treatment could influence BA pool size by increasing gallbladder weight (Figure 4C). We suspected that BA may be involved in the RARE response in DCs. To test this possibility, different BA concentrations were added to BMDCs from RA reporter mice, which stimulated a RARE response (Figure 4D). Next, depleting total BAs by incubating with

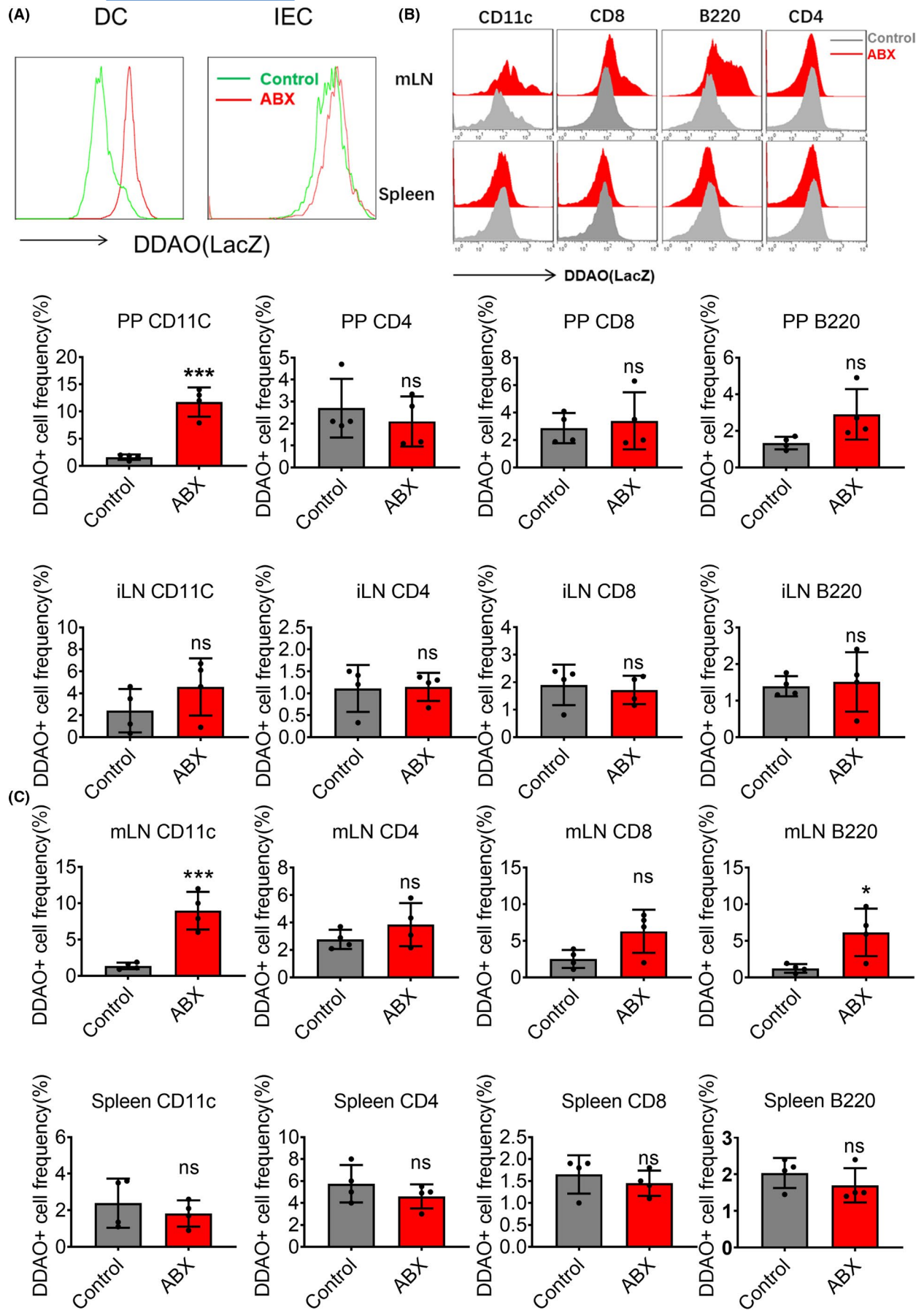


FIGURE 1 Microbiotas modulate retinoic acid (RA) signaling from mucosal dendritic cells (DCs). RA reporter mice were treated with antibiotics (Abx) for 3–4 weeks. DDAO fluorescent signals were analyzed by flow cytometry in DCs from lamina propria, and intestinal epithelial cells (A), mesenteric lymph node, and spleen (B), Peyer's patches and inguinal lymph node (C). Data are presented as mean $\pm$ SD; *p*-values were calculated using the two-tailed Student's *t* test. **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001, n.s.: not significant

the BA-binding resin cholestyramine (CA) resulted in cecum content that lost its RARE-stimulating effect (Figure 4E). Cecum content from Abx-treated mice exhibited a reduced effect on the RARE response (data not shown). Furthermore, different BAs could stimulate a RARE response in BMDCs (Figure 4F) and spDCs (Figure S4E), but not the BA precursor, cholesterol, and its chemical analog lanosterol (Figure 4F).

Next, we examined whether BA depletion could downregulate the Abx-induced RARE response in mice *in vivo*. After Abx treatment, mice were treated by oral gavage of the BA sequestrant CA. CA reduced the RARE response in CD11c⁺ DCs and B220⁺ and CD8⁺ cells from mLN *in vivo* (Figure 4G). CA also could reduce the RARE response in immune cells from mLN in mice not treated with Abxs (Figure S5C). However, CA treatment does not change immune cells frequency in ABX-treated (Figure S5D) or ABX-untreated mice (Figure S5A–B). These results indicate that BAs can modulate the RARE response both *in vitro* and *in vivo*.

2.4 | BAs stimulate the RARE response via FXR-RAR α interactions

To understand the cellular mechanism of the BA-activated RARE response in DCs, we stimulated RA reporter BMDCs with chenodeoxycholic acid (CDA), a hydrophobic primary BA that activates the nuclear receptor FXR. CDA stimulated a RARE response in DCs; however, its effect was inhibited by guggulsterone (Figure 5A), a canonical FXR antagonist.⁴¹ Next, we stimulated RA reporter BMDCs with 3-(2,6-dichlorophenyl)-4-(3'-carboxy-2-chlorostilben-4-yl) oxymethyl-5-isopropylisoxazole (GW4064), a synthetic, selective, and non-steroidal FXR agonist. GW4064 stimulated a RARE response, and its effect was inhibited by guggulsterone (Figure 5A). Furthermore, DY268, another potent FXR antagonist,⁴² inhibited the GW4064-evoked RARE response (Figure 5B). These results indicate that FXR is involved in the BA-activated RARE response in DCs. Next, we investigated which transcriptional factors of the RA signaling pathway play a role in the BA-activated RARE response. The RARE response in DCs was inhibited by the RAR α antagonist AR7,⁴³ but not the retinoid X receptor antagonists HX531⁴⁴ or UVI300 3⁴⁵ (Figure 5B). Moreover, the CDA-activated RARE response was inhibited by the FXR antagonist DY268, RAR α antagonist AR7, pan-RAR inverse agonists BMS493,³⁹ AGN 193109,⁴⁶ and CD2665,⁴⁷ but not the retinoid X receptor antagonists HX531 or UVI3003 (Figure 5C).

Next, we examined whether RAR α and FXR directly interact with each other in DCs by co-immunoprecipitation. When FXR was immunoprecipitated from a whole-cell lysate of BMDCs, the interaction between RAR α and FXR was demonstrated with a RAR α

antibody and Western blot analysis (Figure 5D). Collectively, these results indicate that BAs activate the RARE response in DCs via FXR-RAR α interactions.

2.5 | BA induces the PDL2⁺ OX40L⁺ prime type 2 immune response in DCs

Next, we investigated whether BA activates DCs to prime CD4⁺ T cells to Th2 differentiation, which is essential for the type 2 immune reaction. Isolated OT-II cells were co-cultured with DCs in the presence of the OVA antigen. After exposure to BA, DCs stimulated CD4⁺ T cells toward Th2 differentiation, as IL-4⁺ CD4⁺ T cells were upregulated, and IFN- γ ⁺ CD4⁺ T cells were downregulated (Figure 6A). To gain further insight into the mechanism underlying the BA-activated RARE response in DCs, and to identify the cellular targets of BA that are involved in CD4⁺ T-cell Th2 differentiation, we isolated mRNA from CDA-activated, tamibarotene-activated, and control DCs and then subjected it to Illumina transcriptome sequencing. Transcripts with *p*-values < .05 between the groups were graphed by functions. CDA and tamibarotene-activated DCs with a similar gene expressing signature (Figure 6B). Among the genes differentially expressed, the RA signatures HIC1, RALDH2 (Aldh1a2), GOS2, Xaf1, Stra6, and Tgfb2 were upregulated in CDA- and tamibarotene-activated DCs (Figure 6C), consistent with the result that BA can stimulate a RARE response in DCs. Unexpectedly, the IFN-I signatures Oasl1, Ifit207, Ifit47, Mx1, and IRF7 were upregulated in CDA- and tamibarotene-activated DCs (Figure 6D). The IFN-I signature has been well defined as a critical player in DCs for the initiation of the Th2 response.^{15–17} Next, we observed a gene expression signature in CDA- and tamibarotene-activated DCs, which was very similar to Th2-promoting PDL2⁺ OX40L⁺ DCs,¹³ (Figure 6E). This was further confirmed by Q-PCR, which demonstrated that CDA and tamibarotene induce PDL2 and OX40L and are dependent on the transcription factor RAR α , as CDA and tamibarotene were unable to upregulate these genes in DC RAR α knockouts (Figure S4A). Next, we assessed whether BA could induce PDL2⁺ OX40L⁺ DCs *in vitro*. As shown, GW4064 upregulated PDL2⁺ OX40L⁺ DCs and was dependent on RAR α (Figure 7A).

Next, we examined the global effect of Abx treatment on BA profiles in the mucosal lymph tissue mLN in mice. Abx treatment upregulated several BAs in mLN, including T β MCA and tauroursodeoxycholic acid (Figure S4B). However, gavage of CA reduced the levels of T β MCA and tauroursodeoxycholic acid and the weight of gallbladder (Figure S4B). T β MCA also induced PDL2⁺ OX40L⁺ DCs (Figure 7B).

Next, we determine whether depletion of BA could reduce allergen-specific IgG1 and IgE levels in mice. ABX-treated mice

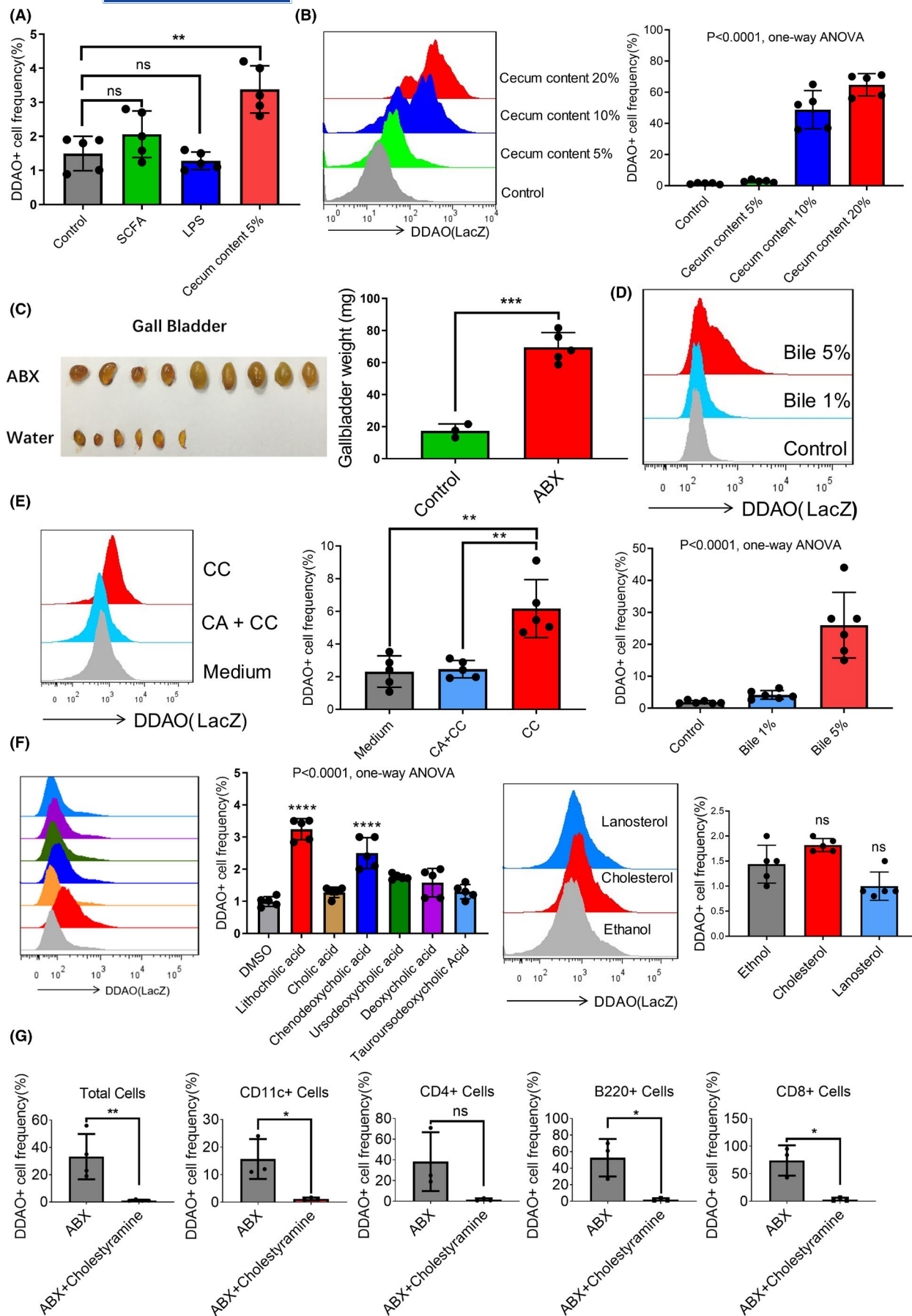


FIGURE 2 Retinoic acid signaling in dendritic cells (DCs) is essential for food allergic reactions. Mice with a DC-specific knockout of the retinoic acid receptor α (RAR α) gene were used (CD11c^{cre}RAR $\alpha^{fl/fl}$ or DC KO RAR α). The weights of immune tissues (spleen and mLN) from DC KO RAR α mice and RAR $\alpha^{fl/fl}$ mice (A). Single cells from lymphoid tissues were analyzed by flow cytometry. Populations of total DCs (CD11c+) were analyzed in spleen and mesenteric lymph node (B). The frequencies of B cells (B220) and T cells (CD3) were determined in spleen and mesenteric lymph node (C). DC KO RAR α mice and RAR $\alpha^{fl/fl}$ mice, after intragastric administration of PN+CT, mice serum was collected, and PN-specific IgE and IgG1 and mouse mast cell protease-1 levels were analyzed by ELISA (D). Data are presented as mean $\pm$ SD; *p*-values were calculated using the two-tailed Student's *t* test. **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001, n.s.: not significant

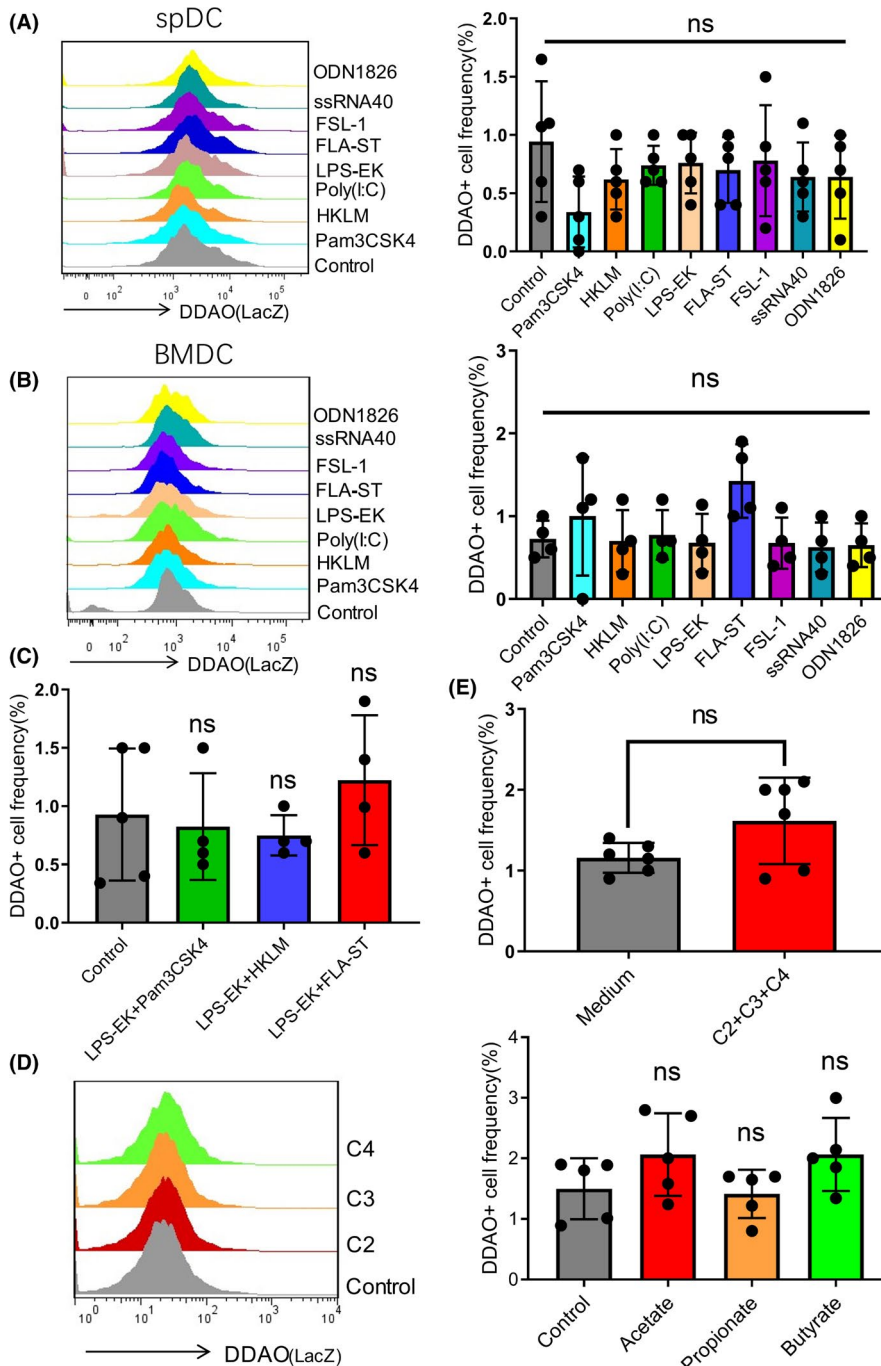


FIGURE 3 TLR ligands and short-chain fatty acids (SCFAs) are not involved in the retinoic acid (RA) responsive element response in dendritic cells (DCs). spDCs (A) isolated from the spleen of RA reporter mice with magnetic-activated cell sorting, and BMDCs (5–7 days) (B) cultured from RA reporter mice were separately stimulated with different TLR ligands or the combination of two TLR ligands (C). BMDCs (5–7 days) from RA reporter mice were separately stimulated with different SCFAs (D), or the combination of three SCFAs (E). DDAO fluorescent signals were analyzed by flow cytometry. Data are presented as mean $\pm$ SD; *p*-values were calculated using the two-tailed Student's *t* test. **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001, n.s.: not significant

were fed with the diet containing CA, sensitized, and challenged by intragastric co-administration with OVA plus CT. As shown, depletion of BA by CA reduces OVA-specific IgG1 and IgE levels in mice (Figure 7C). Meanwhile, CA treatment downregulates

PDL2⁺ OX40L⁺ DCs in mLNs (Figure 7D). Collectively, these results indicate that the BA-activated RARE response induces the IFN- γ signature, and PDL2⁺ OX40L⁺ DCs, which promotes Th2 differentiation.

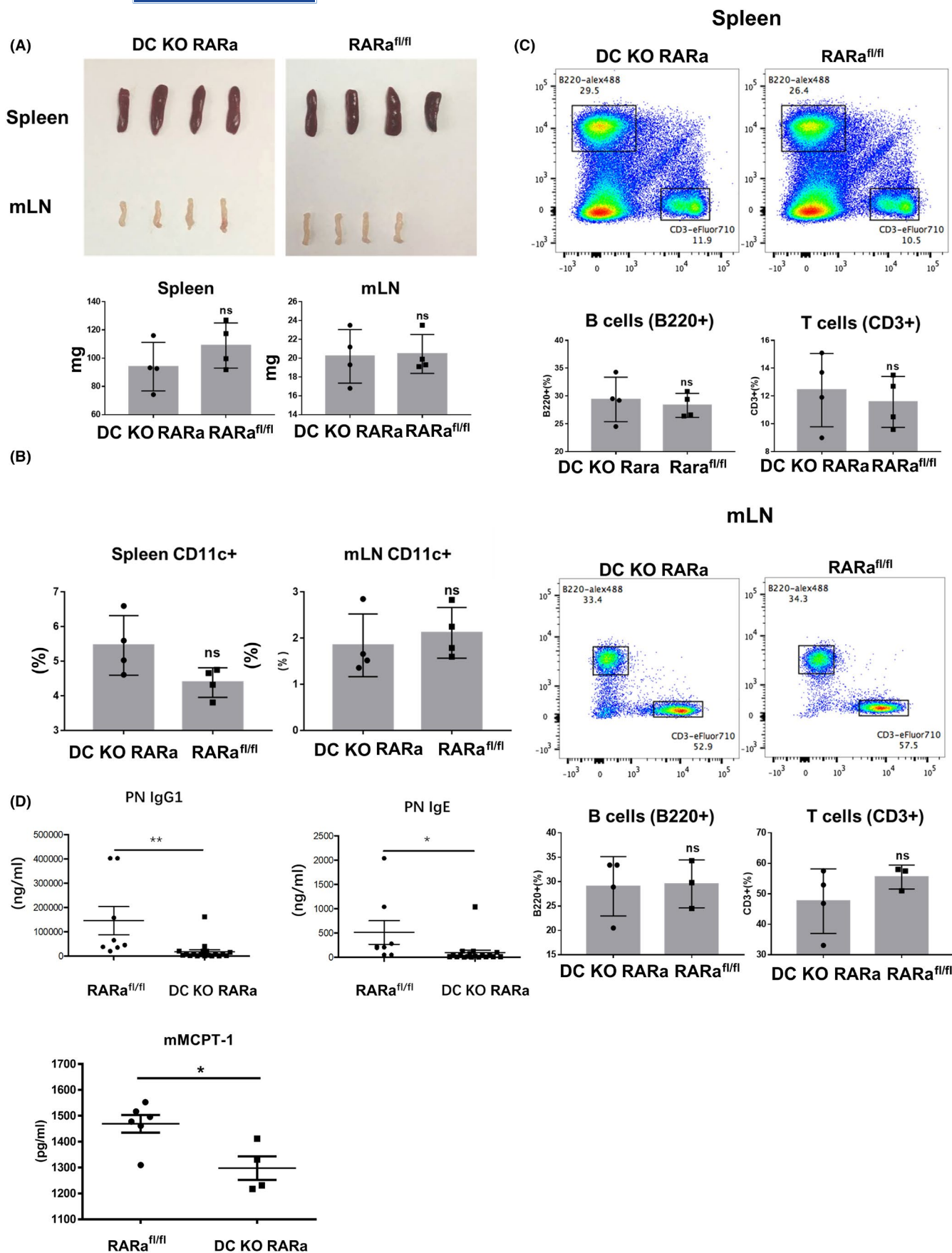


FIGURE 4 Bile acid (BA) stimulates a RARE response in dendritic cells (DCs). BMDCs (5–7 days) cultured from RA reporter mice were stimulated with SCFAs, LPS, and 5% cecum content (A) or with different concentrations of cecum content (B). DDAO fluorescent signals were analyzed by flow cytometry. The weights of gallbladders from antibiotic-treated and control C57BL/6 mice (water) (C). BMDCs (5–7 days) from RA reporter mice were stimulated with BA (1%, 5%) (D), cecum content alone (CC), or cecum content treated with BA sequestrant cholestyramine (CA) (E). BMDCs from RA reporter mice were stimulated with different BA concentrations, cholesterol, and ergosterol (F); antibiotics for 3–4 weeks; gavage of CA (G). DDAO fluorescent signals of cells (Total cells, CD11c+, B220+, CD4+, and CD8+) from lymphoid tissues were analyzed by flow cytometry. Data are presented as mean $\pm$ SD; *p*-values were calculated using the two-tailed Student's *t* test (A, C, E, and G) or one-way ANOVA with Tukey post-test (B, D, and F). **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001, n.s.: not significant

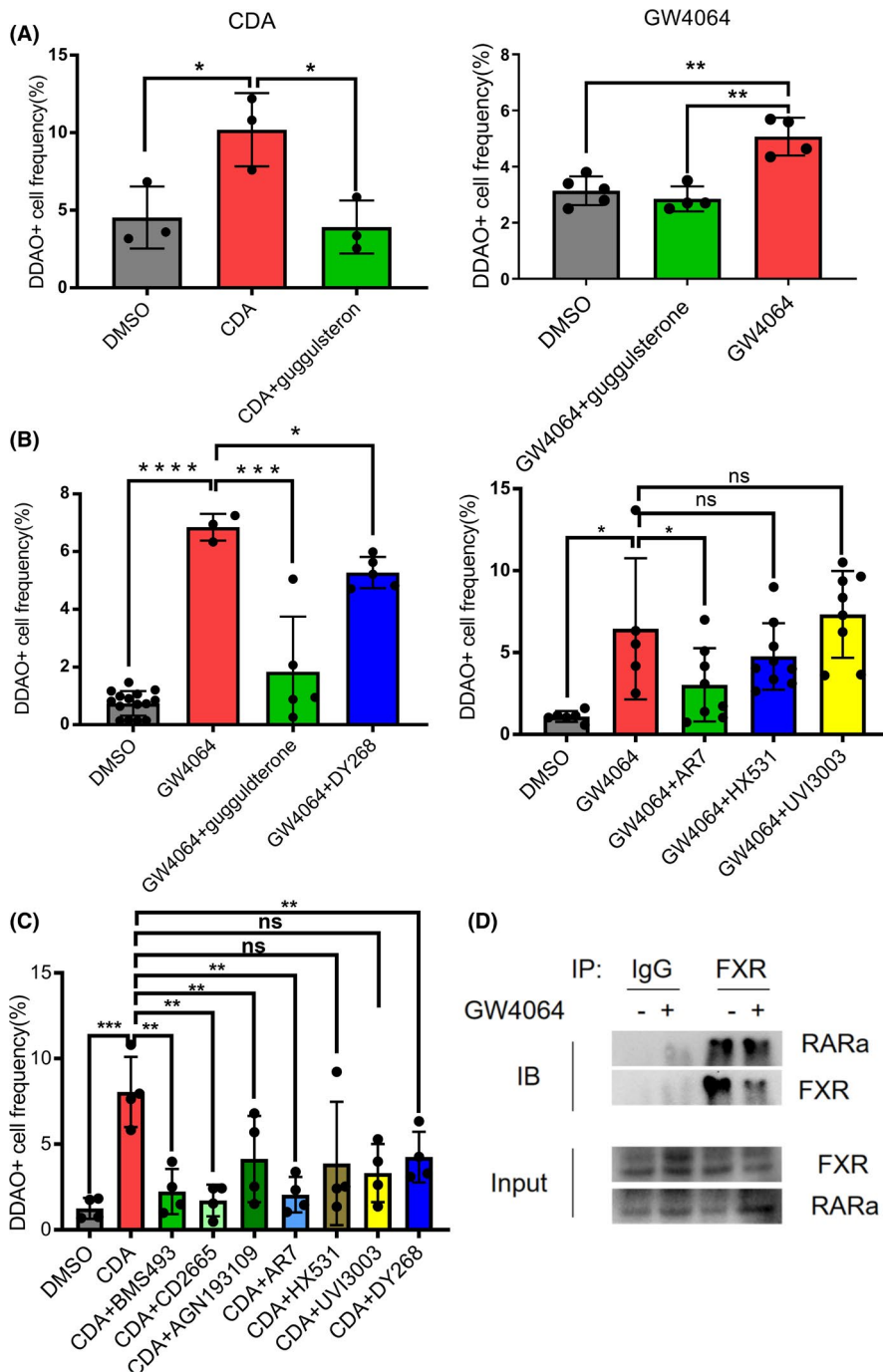


FIGURE 5 Farnesoid X receptor (FXR) and retinoic acid receptor alpha (RARα) are involved in the bile acid-stimulated retinoic acid responsive element response. BMDCs (5–7 days) cultured from retinoic acid reporter mice were stimulated with chenodeoxycholic acid (CDA) and GW4064 alone or combined with guggulsterone (A), GW4064 combined with FXR antagonist (guggulsterone, DY268), RARα antagonist (AR7), retinoid X receptor antagonist (HX531, UVI3003) (B), CDA combined with FXR antagonist (guggulsterone, DY268), RARα antagonist (AR7), retinoid X receptor antagonist (HX531, UVI3003), the pan-RAR antagonist (BMS493, CD2665, AGN 193109) (C). BMDCs from C57BL/6 mice were stimulated with GW4064 and then lysed; RARα and FXR antibodies were used for co-IP (D). Data are presented as mean $\pm$ SD; *p*-values were calculated using the two-tailed Student's *t* test. **p* < .05, ***p* < .01, ****p* < .001, *****p* < .0001, n.s.: not significant

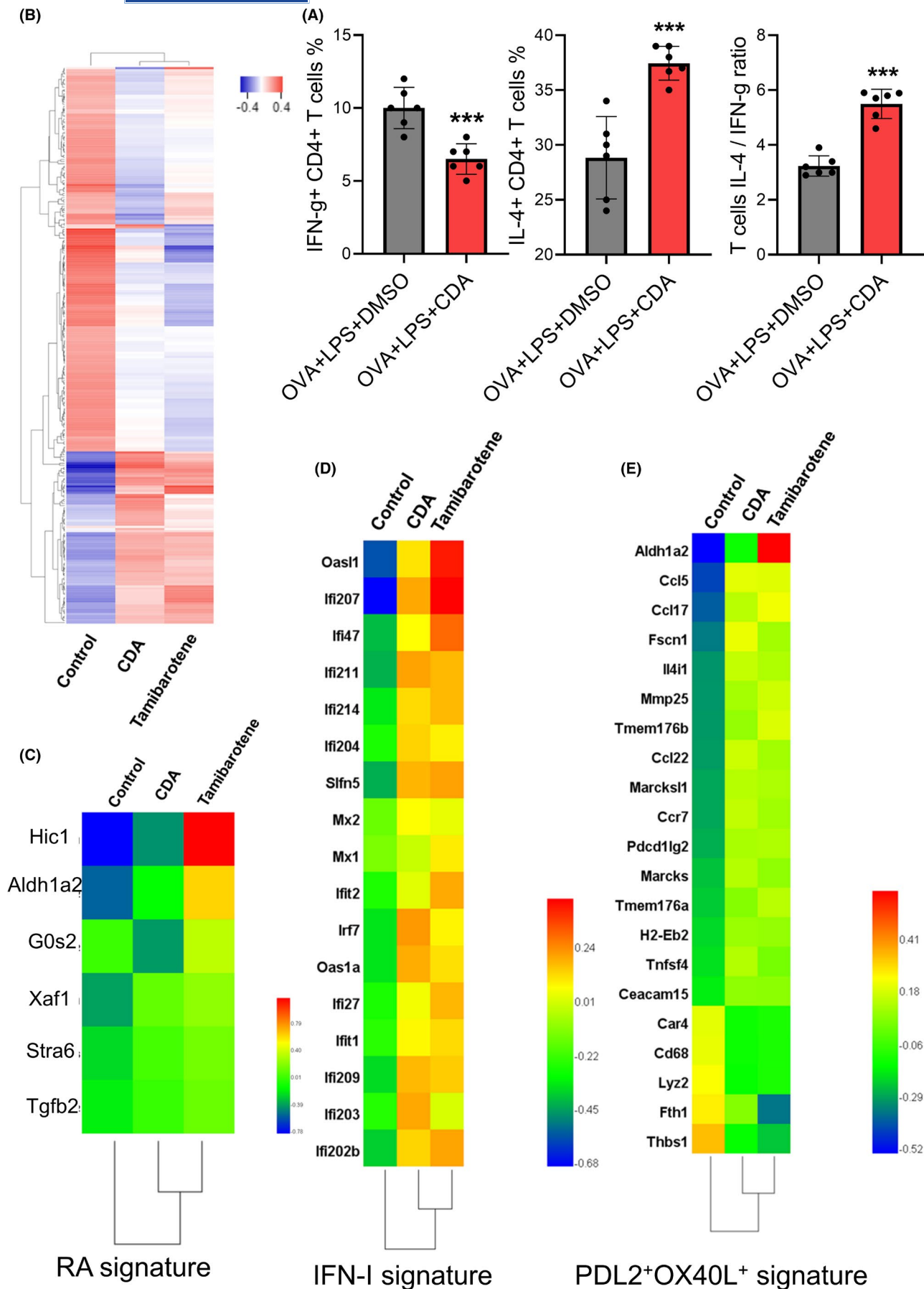


FIGURE 6 Gene expression pattern in dendritic cells (DCs) stimulated with chenodeoxycholic acid (CDA) and tamibarotene. BMDCs (5–7 days) cultured from C57BL/6 mice were loaded with OVA antigen (100 µg/ml) and stimulated with CDA in the presence of LPS; naive OT-II cells were added and co-cultured for 5 d. IL-4- and IFN-γ-positive cells in differentiated CD4+T cells were determined by flow cytometry (A). RNA-seq analysis of global gene expression of CDA and tamibarotene-activated DCs ($n = 4$ per group, pooled together) (B), RA signature (C), IFN-I signature (D), and PDL2⁺OX40L⁺ signature (E). The heatmaps represent fragments per kilobases per million reads values

3 | DISCUSSION

Under physiological conditions, oral tolerance to dietary antigens is established in the gastrointestinal tract by mucosal DCs.² Lack of microbiota or alteration of diversity (dysbiosis) both contributes to food allergen sensitization in mice and humans.^{4,5,48,49} However, the mechanisms by which microbiota modulate food allergic reactions remain poorly understood. Here, we demonstrate that Abx treatment upregulates RA signaling in mucosal DCs, which are responsible for food allergen-specific IgG1 and IgE production. Furthermore, we demonstrated both *in vitro* and *in vivo* that BA stimulates a RARE response in DCs, which involves the transcription factors FXR and RARα. Moreover, RNA-seq analysis revealed that BA and RA similarly stimulated DCs to upregulate the IFN-I signature, OX40L, and PDL2, which may contribute to CD4+T-cell differentiation into Th2 cells.

The gut microbiota constitutes a complex ecosystem that includes bacteria, fungi, viruses, and some archaea. Bacteria and fungi are the most prominent components. The incidence of food allergic sensitization is highly related to differences in the composition of the gut microbiota.⁴⁹ Previous report indicates that greater gut microbiota diversity and genera from *Lachnospiraceae* and *Ruminococcaceae* were associated with egg sensitization in children.⁴⁸ However, in this study, it seems that decreased microbiota diversity in ABX mice contributes to food allergen sensitization. ABX treatment increase *Lactobacillus*, *Akkermansia*, and *Parasutterella*, but decrease *Bacteroidales*, *Erysipelotrichaceae*, and *Lachnospiraceae* in colon and ileum.

RA is an active metabolite of vitamin A, produced through an irreversible enzymatic reaction catalyzed by retinal dehydrogenases.¹⁸ RA exerts its multiple effects on the immune system by binding to nuclear receptors of the RA receptor family.⁵⁰ Vitamin A supplements reduce the morbidity and mortality of children in developing countries and have long been recognized as an “anti-infective” therapy.⁵¹ However, RA has also been proposed as an adjuvant for mucosal DCs that together with IL-15 abolishes tolerance to dietary antigens.⁵² Vitamin A deficiency impairs IgE and IgG1 antibody responses to antigens that require Th2-mediated help,⁵³ alters the pattern of Th1/Th2 cytokines, and produces more IFN-γ and less IL-4 and IL-5.^{54,55} High-dose retinoid treatment may enhance Th2-mediated responses.⁵³ RA induces M2 macrophage polarization,^{22,23} which may promote Th2 cytokine production. In this study, Abx treatment enhanced RA signaling in mucosal DCs, which are essential for food allergen sensitization. DC KO RARα impair PN-specific IgE and IgG1 production in mice. Indeed, also other studies have reported that microbiota can modulate RA signaling in DCs and intestinal epithelial cells.^{19,26}

The relationship between RA and Treg cells is not clearly determined. It is suggested that RA is involved in the induction and stabilizing of regulatory T cells *in vitro*.^{56–58} In this study, we reported

that Treg cell was reduced in the spleen but not in the mLN in DC KO RARα mice, indicate that RA signaling in DCs is involved in maintaining Treg cells in the spleen, but not in mucosal lymph tissue. Food sensitization is initiated in the mucosal lymph tissue.⁵⁹ At the mucosal site, Treg cells may be induced by other factors, like indigenous *Clostridium* Species.⁶⁰

The worldwide prevalence of allergic diseases over a short period of time cannot be fully attributed to genetic variation alone. The “hygiene hypothesis” proposes that environmental factors shape allergic reactions.⁶¹ The influence of the western diet and altered microbiome diversity has received more attention in recent years.⁶² A long-term western diet in mice leads to dysregulated BA signaling and contributes to allergic dermatitis that involves the Th2 and Th17 pathways.⁶³ Furthermore, the lack of microbiota profoundly changes BA profiles. BA may serve as an important mediator of allergic reactions. Our data suggest that BA stimulates RA signaling in mucosal DCs and induces the Th2-evoked IFN-I signature, OX40L, and PDL2. BA-stimulated RA signaling in DCs requires FXR and RARα. Additionally, RA has been reported to upregulate FXR pathway genes and to modulate hepatic BA homeostasis, which suggests the presence of cross-talk between FXR and RARα.⁶⁴ However, our study did not fully exclude other factors that are regulated by microbiota and which may also be involved in regulating RA signaling in DCs.

Allergen-specific IgE and IgG1 production is the characteristic of an allergic reaction. Class-switch recombination of IgE and IgG1 is directed by cytokines from Th2 cells. DCs are the prominent antigen capturing and presenting cells that can promote CD4+T cells toward Th1 or Th2 cell differentiation. The DC signal for Th1 cell differentiation has been well defined, whereas the induction of Th2 cell differentiation is relatively unclear.⁶⁵ Recently, a subset of DCs has been identified as Th2-promoting DCs¹³; these cells exhibit high expression of PDL2, the RA catalytic enzyme RALDH2, Mx1 (IFN-I signature), and OX40L. Interestingly, in this study, BA and RA stimulated DCs to upregulate the IFN-I signature, RA signature, PDL2, and OX40L; this phenotype is very similar to the previously identified PDL2+DCs.

Animal BA and gallstone have been used for the treatment of a wide number of disorders in humans for thousands of years in traditional medicine from China and Japan.^{66,67} Our data suggest that BA could stimulate RA signaling in DCs as well as the upregulation of the IFN-I and RA signatures. This BA-RA signaling pathway may partially account for the effect of BA in traditional Chinese medicine. However, the use of BA in traditional Chinese medicine may also contribute to allergic reactions under certain circumstances.

Collectively, our study suggests that the BA-RA signaling pathway boosts food allergen-specific IgE and IgG1 production via FXR-RARα interactions in DCs. BA-RA signaling may change DC phenotype as

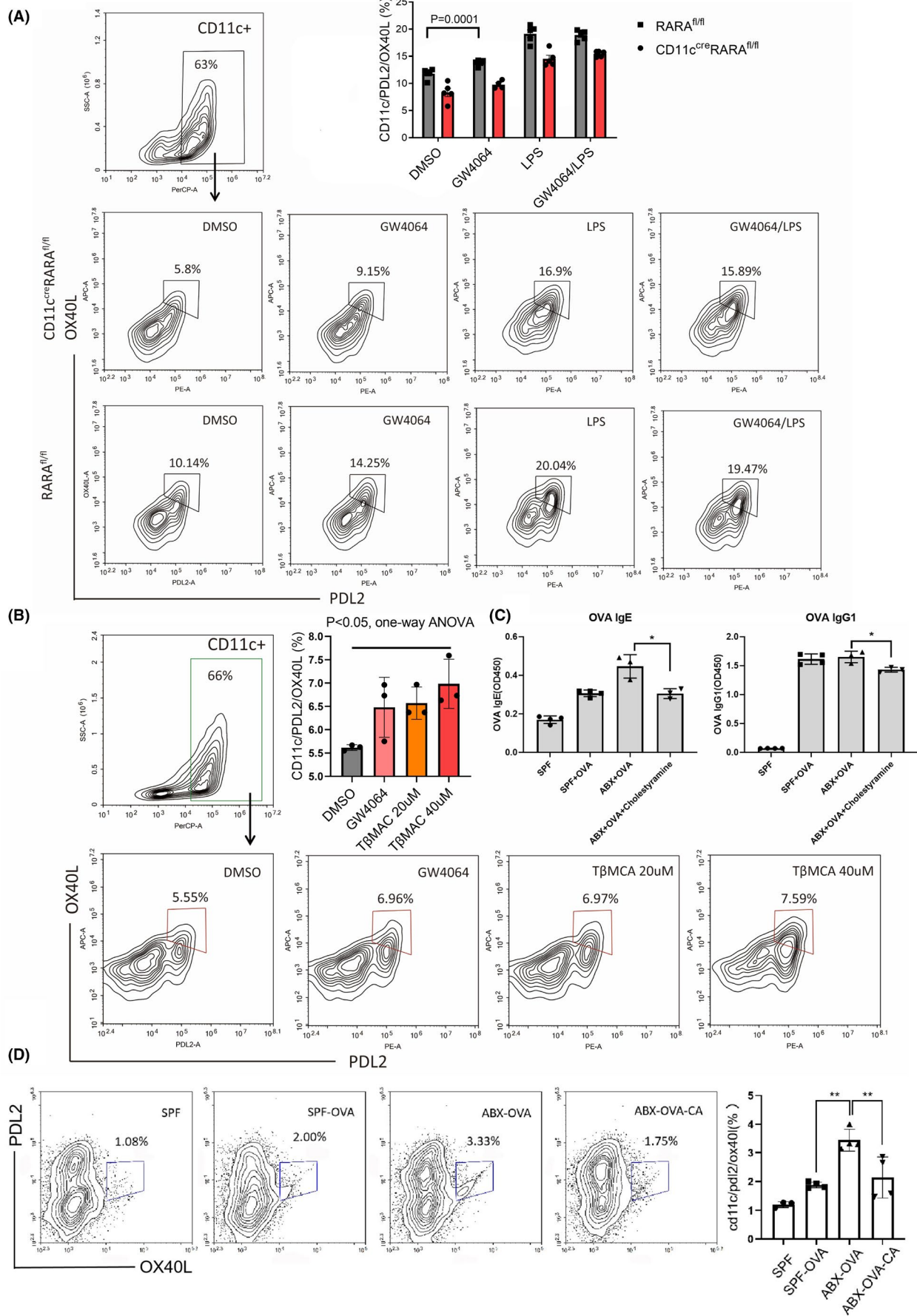


FIGURE 7 Bile acid induces OX40L⁺PDL2⁺ dendritic cells. BMDCs (5–7 days) cultured from CD11c^{cre}RARa^{fl/fl} and RARa^{fl/fl} mice were stimulated with GW4064, LPS, GW4064 plus LPS (A). BMDCs (5–7 days) cultured from C57BL/6 mice were stimulated with tauro-beta-muricholic acid (T β MCA) (B). PDL2⁺OX40L⁺ double-positive cells were determined by flow cytometry. Cells were gated on CD11c⁺ cells. (C) ABX-treated mice were fed with the diet containing cholestyramine, sensitized, and challenged by intragastric co-administration with OVA plus CT. OVA-specific IgG1 and IgE levels in serum were determined by ELISA. (D) PDL2⁺OX40L⁺ double-positive cells in mLN were determined by flow cytometry. Cells were gated on CD11c⁺ cells. Data are presented as mean $\pm$ SD; *p*-values were calculated using two-way ANOVA with Bonferroni correction (A) or one-way ANOVA with Tukey post-test (B, C, and D). **p* < .05, ***p* < .01, ****p* < .0001, *****p* < .001, n.s.: not significant

Th2 cells prime DCs with the following features: upregulated IFN- γ signature, RA signature, PDL2, and OX40L. Our study suggests a potential target within BA-RA signaling for the treatment of food allergies.

4 | MATERIALS AND METHODS

4.1 | Mice

The RA reporter Tg(RARE-Hspa1b/lacZ)12Jrt/J mouse strain as well as OT-II and CD11c^{cre} mice with a C57BL/6 genetic background were obtained from Jackson Laboratories. RARa^{fl/fl} mice were established and maintained in our laboratory as previously reported.⁶⁸ C57BL/6 was purchased from GemPharmatech. Germ-free mice were obtained from the Shanghai Slac Laboratory Animal Center. Mice were kept in a room with 12-h light-dark cycle at 22°C, fed by OVA-free diet sterilized by irradiation. Water was sterilized by autoclaving, and aspen wood shaving was used for bedding.

4.2 | Reagents

The following reagents were purchased: DDAOG (Invitrogen or Setareh Biotech); TLR1–9 ligands (Invivogen); bile acids, lithocholic acid, cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid, tauroursodeoxycholic acid, cholesterol, ergosterol, acitretin, bexarotene, and tamibarotene (Aladdin-e); T β MCA (GlpBio Technology); colestyramine, AR7, AGN193109, and AM580 (MedChemExpress or Sigma); agonist and antagonist, guggulsterone; and GW4064 (Amquar); HX531, UVI3003, and DY268 (Tocris); BMS493; and CD2665 (APExBio).

4.3 | FACS and antibody

DCs were washed and suspended in FACS buffer (PBS, 2% FBS), incubated with fluorochrome-coupled agents for 30 min at 4°C, and then washed in FACS buffer. Data were obtained with the BD LSR Fortessa™ Flow Cytometer (BD Biosciences) and analyzed by FlowJo software v9.6 (FlowJo, Ashland, USA). The following anti-murine antibodies were used for flow cytometry: CD40 (HM40-3), CD80 (16-10A1), CD86 (GL1), CD11c (N418), TCR V alpha 2 (B20.1), IL-4 (11B11), CD4 (RM4-5), B220 (RA3-6B2), and CD3 (17A2) (all purchased from eBioscience); MHCII (M5/114.15.2), OX40L (RM134L), CD273 (B7-DC, PDL2) (TY25), and IFN- γ (XMG1.2) (Biolegend).

4.4 | BMDC culture and isolation of spleen DCs

DCs were generated from bone marrow (BM) cells obtained from 5- to 7-week-old RA reporter mice or C57/B6 mice. Briefly, BM cells were flushed out from femurs and tibias. BM cells were cultured in complete culture medium (RPMI 1640 supplemented with 10% FBS, 25 mM HEPES, 5 mM 2-ME, and Abxs; Hyclone) containing 10 ng/ml GM-CSF and 10 ng/ml IL-4 (Peprotech). Spleen DCs were isolated with magnetic-activated cell sorting by EasySep™ mouse CD11c-positive selection kit (stem cell), according to the manufacturer's instructions.

4.5 | Enzyme-linked immunosorbent assay (ELISA)

Mouse serum was collected after the last gavage, and levels of total PN-specific IgE and IgG1 were determined by ELISA. Briefly, purified PN extracts were coated in a coating buffer (Biolegend). After washing and blocking, diluted serum samples were added, and then primary antibodies against PN-specific immunoglobulin (Chondrex) were added. After washing, HRP-labeled secondary antibodies (Southern Biotech) were added. TMB Reagent (Biolegend) was used for color development, and optical density was read at 450 nm.

4.6 | β -galactosidase activity

SpDCs and BMDCs from RA reporter mice were incubated with the galactosidase substrate DDAOG (10 μ M) in serum-free Hank's buffer for 0.5–2 h at 37°C (Figure S2A). The cells were washed three times with Hank's buffer before flow cytometry. DDAOG yields the hydrolytic product DDAO in DCs, which can be excited with the 633 nm laser (excitation/emission maxima ~645/660).

4.7 | Co-IP and western blots

DCs were collected and lysed on ice with RIPA Buffer (CST) containing protease/phosphatase inhibitor cocktail (CST) and 1 mM PMSF (CST). Cell extracts were loaded onto Biofuraw™ Precast Gel (Tanon), separated by electrophoresis, and transferred onto NC membranes (Millipore). Signals were generated with high-sig ECL Western blotting substrate (Tanon). RARa and FXR polyclonal antibodies were purchased from ABclonal.

4.8 | DC/T-cell co-culture assays

BMDCs were isolated by EasySep™ mouse CD11c-positive selection kit (Stemcell), according to the manufacturer's instructions, loaded with OVA (200 µg/ml), then mixed with CD62L^{hi}/CD44^{low} naive CD4+OT-II T cells (DC/T-cell ratio 1:4) isolated with EasySep mouse CD4+T-cell enrichment kit (Stemcell), and co-cultured for 6 days. Th1/2 differentiation was determined by FACS analysis of intracellular IL-4 and IFN-γ staining in activated CD4+ OT-II T cells.

4.9 | RNA isolation, library construction, and sequencing

Total RNA from DCs was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Poly(A)+ RNA was purified from 5 µg of pooled total RNA using oligo(dT) magnetic beads, sheared into short fragments, and primed for cDNA library synthesis using the TruSeq RNA sample preparation kit per Illumina protocol. After quantitation using a TBS-380 mini-fluorometer (PicoGreen), the samples were clustered (TruSeq paired-end cluster kit, v3-cBot-HS; Illumina) and sequenced on the HiSeq2000 platform (100 bp, TruSeq SBS kit v3-HS 200 cycles; Illumina). Annotations for the entire data set were obtained with Tophat, which was followed by statistical analysis with Cufflink (Cuffdiff module). The read counts were further normalized into fragments per kilobases per million reads values, which were pairwise compared; the fold changes were calculated by RSEM software (version 1.2.7), and DEGs were identified by using the DESeq2 or edgeR software package (version 3.8.2) (if $p < .05$ and FDR < 0.05 , the result was considered statistically significant). The data were analyzed on the free online Majorbio I-Sanger Cloud Platform (www.i-sanger.com). The heat maps were generated by Heml (Heatmap Illustrator) software from the collected gene ($p < .05$).

4.10 | Antibiotic treatment

RA reporter mice and C57BL/6 mice were given an Abx cocktail containing ampicillin (1 g/l), vancomycin (500 mg/l), neomycin sulfate (1 g/l), and metronidazole (1 g/l) in drinking water for 3 wks after weaning.

4.11 | rRNA sequencing analysis of bacterial and fungal diversity

Mice fecal samples were collected, and DNA was extracted using the TIANamp Stool DNA Kit (TIANGEN Biotech) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop spectrophotometer (Thermo Scientific). The hypervariable regions of the bacterial or fungal rRNA gene were amplified by a thermocycler PCR system. The resulting PCR products were extracted from a 2% agarose gel and further purified

using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) and quantified using QuantiFluor™-ST (Promega) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina). Raw fastq files were demultiplexed, quality filtered by Trimmomatic, and merged by FLASH based on the following criteria: (1) the reads were truncated at any site that received an average quality score < 20 over a 50 bp sliding window; (2) the primers were exactly matched, allowing a 2-nucleotide mismatch, and reads containing ambiguous bases were removed; and (3) sequences with overlaps of > 10 bp were merged according to their overlap sequence. Operational taxonomic units were clustered with a 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva 16S rRNA database (silva 132/16s bacteria) using a confidence threshold of 70%. The data were analyzed on the free online platform of the Majorbio I-Sanger Cloud Platform (www.i-sanger.com).

4.12 | UPLC-MS analysis of BAs

Tissue samples were processed as previously reported.³³ The detection of BAs was carried out on UPLC (Shim-pack UFLC SHIMADZU CBM30A) and MS/MS (Applied Biosystems 4500 QTRAP). The analytical conditions were as follows: HPLC column, waters ACQUITY UPLC HSS T3 C18 (1.8 µm, 2.1 mm*100 mm); solvent system, water (0.04% acetic acid): acetonitrile (0.04% acetic acid); gradient program, 100:0 V/V at 0 min, 5:95 V/V at 10.0 min, 5:95 V/V at 11.0 min, 95:5 V/V at 11.1 min, 95:5 V/V at 15.0 min; flow rate, 0.35 ml/min; temperature, 40°C; and injection volume: 5 µl. The effluent was alternately connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS. The UPLC-MS datasets for hierarchical cluster analysis were processed through R software (<https://www.r-project.org/>).

4.13 | Purified peanut extract and intragastric sensitization.

Purified peanut (PN) extract was prepared from roasted, unsalted PN according to a modified protocol.⁶⁹ PN sensitization was performed by intragastric gavage with 6 mg of PN +15 µg CT (List Biologicals) (Figure S4F). Similarly, ovalbumin (OVA) sensitization was performed by intragastric gavage with 5mg OVA (Grade V, Sigma) +15 µg CT for 5 weeks, and challenging with 40mg OVA.

4.14 | Quantitative real-time PCR

BMDC cDNA was synthesized from total RNA by the Superscript III reverse transcription system (Invitrogen). qPCR was performed with the SYBR green PCR mix and StepOnePlus machine (Applied

Biosystems). PCR primers of mouse genes were purchased from OriGene. GAPDH or actin was used as an internal control.

4.15 | Statistical analyses

Statistical analyses were performed with the SPSS statistical software version 13.0 (SPSS Inc., Chicago). All plot graphs display means with standard deviation. Statistical significance was determined with the two-tailed Student's *t* test, one-way ANOVA with Tukey post-test, or two-way ANOVA with Bonferroni correction, as appropriate to the number of comparisons to be made. A *p*-value of $<.05$ was considered statistically significant. **p* $<.05$, ***p* $<.01$, ****p* $<.001$, not significant (N.S.).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Z.Z. conceived the study and wrote the manuscript. R.W., X.Y., Z.Z., H.J., X.L., N.M., H.T., and G.X. performed the experiments. Z.L. helped with manuscript writing and data discussion.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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