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Deoxycholic Acid Impairs Immune Tolerance during DC and CD4 T Cell Interaction through FXR-Dependent Signaling

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ABSTRACT: Deoxycholic acid (DCA) was implicated in gut dysbiosis and inflammation, but its immunoregulatory mechanisms in allergic sensitization remain unclear. This study investigated how DCA disrupts intestinal immune tolerance, thereby promoting an immune response of lymphocytes to allergens. *In vivo*, the functions of intestinal lymphocytes were characterized through transcriptomic profiling and spectral flow cytometry after DCA gavage. Intestinal lymphocytes were isolated and exposed to DCA *in vitro* to further describe the dose-response immunoregulation of DCA. Additionally, bile acid receptor antagonists were applied to explore the potential target of DCA administration *in vitro*. Gavage of DCA resulted in elevated IgE expression within intestinal B cells (mean difference = 27.21%, 95% CI: 11.22 to 43.19, $P < 0.05$) and a significant reduction (almost 50%) of Foxp3, Helios, and PD-1/PD-L1. Transcriptomic analysis further revealed that DCA downregulated markers associated with immune tolerance. 50 μM DCA suppressed PD-L1 expression on DCs (mean difference = -11.74%, 95% CI: -21.44 to -2.041, $P < 0.005$) and PD-1 on CD4 T cells (mean difference = -5.56%, 95% CI: -4.47 to -6.66, $P < 0.005$), while concurrently promoting Th2 polarization and IgE production in B cells i*n vitro*. FXR antagonism reversed these DCA-induced effects, indicating their dependence on FXR signaling. Collectively, this study unveils a novel mechanism whereby the secondary bile acid DCA impairs intestinal immune tolerance by inhibiting the PD-L1/PD-1 immune checkpoint axis via FXR signaling, thereby promoting allergic sensitization.

Keywords: Deoxycholic acid; Food allergy; Immune tolerance; PD-L1; Farnesoid X receptor; Th2 polarization.

1. Introduction

The rising global prevalence of food allergy currently affects over 10% of pediatric populations. Clinical manifestations ranging from gastrointestinal disorders to anaphylactic shock are elicited by dietary antigens such as milk and egg proteins [1]. The breakdown of intestinal immune tolerance has been identified as a central to food allergy pathogenesis by emerging evidence [2]. Intestinal immune homeostasis is orchestrated by dynamic tripartite interactions among dietary factors, microbiome-derived metabolites, and host signaling pathways. Within this network, the principal safeguard against pathogenic Th2 polarization is provided by dendritic cell (DC)-mediated instruction of T cell responses [3]. Bile acids, key amphipathic components of intestinal digestion, have recently emerged as potent immunomodulators. DC and T helper (Th) cells could be

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regulated by flora-metabolized derivatives from bile acids [4, 5]. However, the specific immunoregulatory roles of bile acids in allergic sensitization remain incompletely defined.

Deoxycholic acid (DCA), a hydrophobic secondary bile acid synthesized via microbial 7α-dehydroxylation of cholic acid, is recognized as a potent Farnesoid X receptor (FXR) agonist and is associated with cytotoxic effects [6]. Elevated intestinal DCA levels – potentially driven by gut dysbiosis, prolonged high-fat dietary patterns, or bile acid dysmetabolism – are clinically associated with colonic inflammation and epithelial barrier disruption in irritable bowel syndrome and colorectal carcinogenesis [7]. Increased fecal DCA concentrations have been epidemiologically linked to food allergy susceptibility, suggesting that intestinal mucosal immunity may be perturbed by DCA, contributing to allergic pathogenesis [8, 9]. Furthermore, dendritic cell maturation, T helper cell polarization, and regulatory T cell (Treg) differentiation are modulated by bile acids via nuclear receptors, including FXR and the vitamin D receptor (VDR) [4, 10]. DCA may thus be regarded as a crucial link through which dietary and pathological factors promote food allergy development [11]. Present studies have established epidemiological links between DCA and allergy, and described the general immunomodulatory potential of bile acids. The precise mechanistic pathway by which DCA subverts intestinal immune tolerance remains a critical unanswered question.

Given these knowledge gaps, we hypothesized that intestinal immune tolerance is disrupted by DCA via receptors, thereby promoting Th2-polarized allergic responses. This study was designed to unravel the molecular mechanisms by which intestinal immune tolerance is compromised by DCA, with a focus on its interaction with the FXR-PD-L1 axis and downstream Th2 polarization. To delineate alterations in intestinal lymphocyte populations and function induced by DCA gavage, transcriptomic analysis was performed on the murine jejunum, and full-spectrum flow cytometry was conducted on mesenteric lymph nodes (MLN). Subsequently, MLN lymphocytes isolated from OVA-induced food-allergic mice were stimulated in vitro with DCA to clarify both the inhibitory on the immune tolerance signaling and the role of bile acid receptors in this process. Actionable insights for the development of precision dietary interventions and FXR-modulating therapies aimed at mitigating allergic sensitization risks are expected to be provided by this work, thereby advancing both mechanistic and translational research in food allergy management.

2. Materials and methods

2.1 Mice

Female C57BL/6 mice (5-6 weeks old) were obtained from GemPharmatech Co., Ltd (Guangzhou, China), and maintained under specific pathogen-free conditions with controlled temperature ((22 ± 1)°C) and 12 h light/dark cycles. All experimental protocols were approved by the Animal Ethics Committee of Shantou University Medicine College (Approval No. SUMCXM2024-221). Female mice were used to maintain consistency with our previous models for more uniform allergic responses [12].

2.2 DCA administration and tissue collection

Mice were randomly assigned to the control or DCA-treated group, with six mice in each group. Mice

received daily oral gavage of DCA (Sigma-Aldrich, St. Louis, Missouri, USA, 2 mg/mouse dissolved in 1% DMSO) or vehicle control (1% DMSO) for 14 consecutive days. After euthanasia, the small intestine was immediately excised, flushed with ice-cold PBS. The jejunal tissues and mesenteric lymph nodes (MLNs) were immediately cut off with scissors and placed in cold RPMI-1640 complete medium and kept on ice prior to mechanical dissociation. The *in vivo* DCA dose is justified by citing previous studies that used comparable doses to mimic pathophysiological conditions without overt toxicity [13].

2.3 OVA-induced food allergy mouse model

Mice were intraperitoneally sensitized with 50 μg ovalbumin (OVA; Sigma-Aldrich, St. Louis, Missouri, USA) emulsified in 1 mg aluminum hydroxide gel (alum; Sigma-Aldrich, St. Louis, Missouri, USA) on days 0 and 10. On days 22, 25, 28, 31, and 34, sensitized mice received five oral challenges with 20 mg OVA in PBS, while control groups received PBS-alum mixtures. MLNs were immediately cut off with scissors and placed in cold RPMI-1640 (Solarbio, Beijing, China) complete medium and kept on ice prior to mechanical dissociation after euthanasia.

2.4 Ex vivo lymphocyte culture and stimulation

Single-cell suspensions from MLNs were prepared using 70 μm cell strainers (Corning, New York, USA) and cultured in RPMI-1640 medium (Solarbio, Beijing, China) supplemented with 10% FBS (Cellmax, Beijing, China) at (5×10^5) cells/well. In stimulation assays, dose-response experiments were conducted using OVA at a concentration of 50 μg/ mL combined with DCA at graded concentrations (10, 20, 50, or 100 μ mol/L). For receptor antagonism studies, cultures were treated with OVA (50 μg/ mL) and DCA (50 μmol/L) in the presence or absence of 1 μmol/L DY268 (MedChemExpress, Monmouth Junction, New Jersey, USA) or 1 μmol/L MeTC7 (MedChemExpress, Monmouth Junction, New Jersey, USA), which were used as receptor antagonists. All cell cultures were incubated under standard conditions at 37℃ in a humidified atmosphere containing 5% carbon dioxide for a duration of 24 hours to evaluate cellular responses under these treatment regimens. The *in vitro* concentration range is justified by referencing its common use in literature to exert biological effects on immune cells without significant cytotoxicity [14].

2.5 FACS and antibody

Cells after stimulation were washed and stained with LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Thermo Fisher, Waltham, Massachusetts, USA), followed by surface/intracellular markers using BD Cytofix/Cytoperm™ kit (BD, Franklin Lakes, New Jersey, USA). Data acquisition was performed on a Cytek Aurora spectral flow cytometer and analyzed with FlowJo v10.9. Key antibody panel included: CD11c-BV605 (N418), MHC II-FITC (M5/114.15.2), and PD-L1-PE (10F.9G2) for Dendritic cells. CD4-FITC (RM4-5), PD-1-PE/Cy7 (29F.1A12), and GATA3-BV421 (L50-823) for Th cells. CD19-PerCP/Cy5.5 (6D5), IgE-APC (RME-1) for B cells. All antibodies from BioLegend (San Diego, California, USA).

2.6 RNA sequencing and data analysis

Total RNA from jejunal tissues was extracted using TRIzol™ (Invitrogen, Carlsbad, California, USA)

according to the manufacturer's instructions, involving phase separation with chloroform, RNA precipitation with isopropanol, and a final wash with 75% ethanol. Following reverse transcription of 1 μg total RNA using the PrimeScript™ RT reagent Kit (Takara, Kusatsu, Shiga, Japan), the synthesized cDNA was quantified with an Agilent 2100 Bioanalyzer (RIN >8). Poly-A selected libraries were constructed using the NEBNext Ultra II RNA Library Prep Kit and sequenced on the DNBSEQ-G400 platform (BGI), generating 150 bp paired-end reads. Bioinformatics analysis included: Read alignment to the mm10 genome using HISAT2. Differential expression analysis with DESeq2 ($|\log2FC| > 0.83$, $Q < 0.05$). Pathway enrichment via KEGG and Gene Ontology databases.

2.7 Statistical analysis

Data are presented as mean ± SEM. Comparisons between two groups used an unpaired two-tailed Student's *t*-test. Multiple group comparisons employed two-way ANOVA with Sidak's (dose-response) or Tukey's (antagonist studies) post-hoc tests. Statistical significance was defined as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. All analyses were performed using GraphPad Prism 9.4.1 (San Diego, California, USA).

3. Results

3.1 Transcriptomic Alterations in Intestinal Responses to DCA Treatment

Transcriptomic profiling identified 112 differentially expressed genes (DEGs; $|\log_2FC| > 0.83$, $Q < 0.05$) in DCA-treated versus control jejunum. Comparative analysis revealed a 2.3-fold enrichment of upregulated genes (n = 84) over downregulated counterparts (n =38) through normalized read count quantification (Fig. 1A). Hierarchical clustering analysis demonstrated distinct segregation of experimental groups in the correlation heatmap (Fig. 1C), confirming treatment-specific transcriptional reprogramming. Notable upregulated DEGs included xenobiotic-metabolizing enzymes, like *Cyp2c38*, *Gsta4*, *Ces2a*, epithelial antimicrobial effectors, like *Reg3a*, *Reg3d*, and retinoid metabolic regulators (*Rdh16f2*). Conversely, Volcano plot analysis identified significant downregulation of early immune activation markers (*Cd69, Tnfaip3*) and stress-responsive transcription factors (*Fos, Fosb*) (Fig. 1B). KEGG pathway enrichment highlighted dual regulatory patterns: significant association with retinol metabolism and IL-17 signaling, contrasted by suppression of steroid hormone biosynthesis and TNF-mediated signaling pathways (Fig. 1C and 2D). Validation of mRNA expression levels for immune tolerance genes, including *PD-1*, *PD-L1*, *TNFp3*, *TNFp2*, *NR4A1*, and *Foxp3*, was performed in Supplementary Figure 1 and Supplementary Table 1.

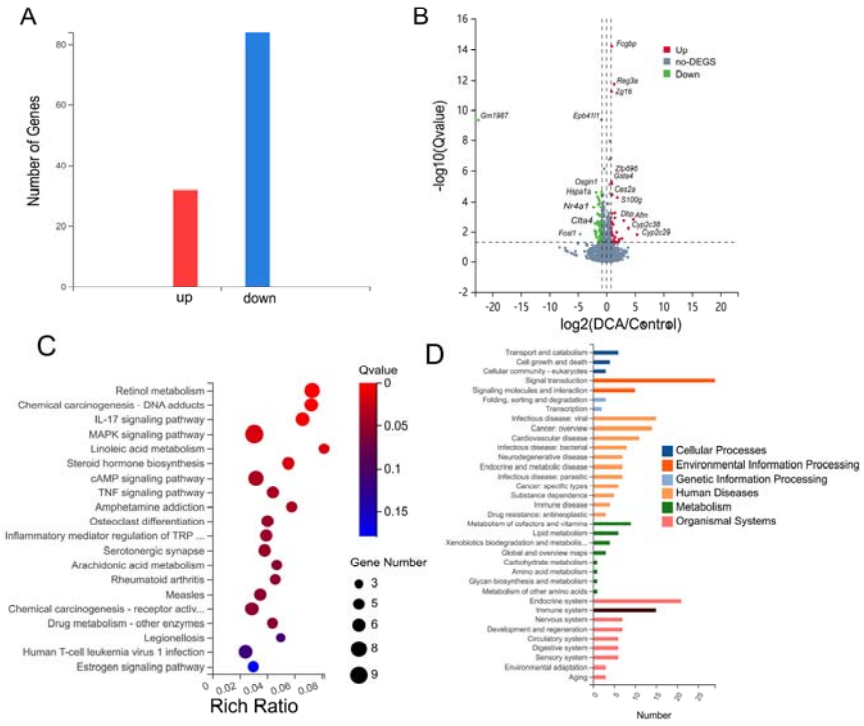

Fig. 1. Oral deoxycholic acid administration induces transcriptomic alterations associated with intestinal barrier disruption and pro-inflammatory responses in murine jejunum.

Quantification of differentially expressed genes (DEGs, |Log2FC| > 0.83, Q < 0.05), (B) Volcano plot of DEG distribution, (C) KEGG pathway and (D) Gene Ontology (GO) enrichment analyses of upregulated (red) and downregulated (blue) DEGs in jejunal tissues from C57BL/6 mice (n=5) receiving daily oral gavage of DCA (2 mg/mouse) or vehicle control for 14 days. Tissues were harvested 24 h post-final administration. RNA sequencing was performed on Illumina NovaSeq 6000. Data expressed as mean ± SEM; dashed lines in B/D/E indicate significance thresholds (Q < 0.05).

3.2 Oral DCA administration promoted B cell maturation and impairs immune tolerance in MLNs

Spectral flow cytometric analysis of mesenteric lymph nodes (MLNs) was performed to assess the impact of oral DCA administration on immune cell differentiation. DCA exposure significantly promoted B cell activation and antibody class switching, as evidenced by increased expression of IgE, IgD, and IgM in CD38+CD19+B cells (Fig. 2A to 2C). At the same time, lower expression PD-L1 in B cells was observed in the DCA group (Fig.2D). In CD11c+ DCs, the expression of MHC-II was significantly ($P < 0.005$) up-regulated and the expression of PD-L1 was significantly ($P < 0.005$) downregulated in CD11c+DC, suggesting enhanced antigen presentation capacity coupled with diminished immunosuppressive signaling in DCA group compared to Control (Fig.3A and 3B). In addition, the expression of immunotolerance surfacers including PD-1, Foxp3, and Helios in CD4+Th cells was significantly inhibited after DCA oral exposure (all $P < 0.01$, Fig.3C to 3E).

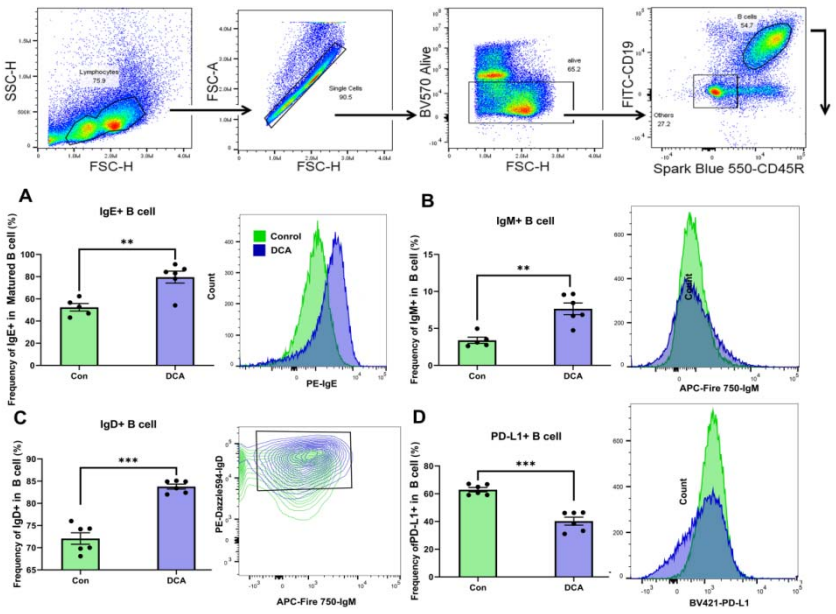

Fig. 2. Oral administration of deoxycholic acid alters B cell activation profile in murine mesenteric lymph nodes.
Oral gavage of DCA (2 mg/mouse daily for 14 consecutive days) enhances immunoglobulin expression, IgE (Fig.2A), IgM (Fig.2B), and IgD (Fig.2C), in MLN B cells while downregulating PD-L1 expression (Fig. 1D) in C57BL/6 mice (n=6). MLNs were harvested 24 hours after the final administration. Data expressed as mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$ vs control by two-tailed Student's *t*-test.

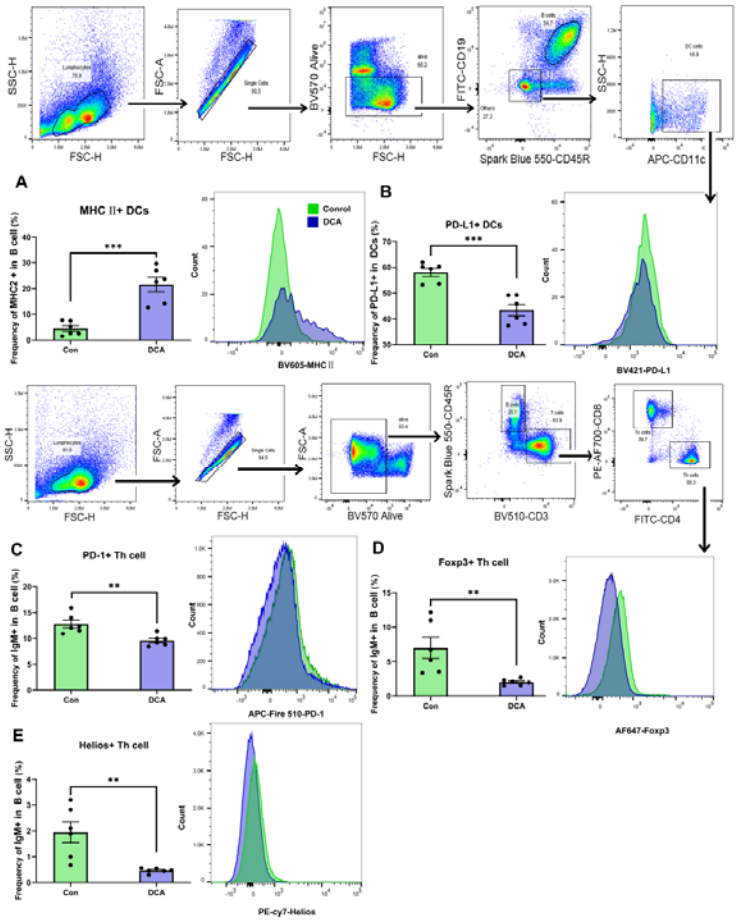

Fig. 3. Oral administration of deoxycholic acid alters activations of dendritic cells and T helper cells in murine mesenteric lymph nodes.
Oral gavage of DCA (2 mg/mouse daily for 14 consecutive days) upregulates MHC class II expression in CD11c+ dendritic cells (Fig.2A) while downregulating PD-L1 (Fig.2B), and reduces PD-1 (Fig.2C), Foxp3 (Fig.2D), and Helios (Fig.2E) expression in CD4+ T cells in C57BL/6 mice (n=6). MLNs were harvested 24 hours after the final administration. Data expressed as mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs vehicle control by two-tailed Student's *t*-test.

3.3 DCA directly impairs immune tolerance in MLN lymphocytes in vitro

To establish a direct causal relationship, lymphocytes isolated from the MLNs of food-allergic mice were stimulated with OVA in the presence or absence of DCA. Consistent with the in vivo findings, DCs stimulated with 50 μmol/L or 100 μmol/L DCA exhibited upregulated MHC-II expression and downregulated PD-L1 expression compared to the OVA group (Fig. 4A, 4B). Furthermore, stimulation with 50 μmol/L DCA led to a significant decrease in PD-1 expression on Th cells and a concomitant increase in the expression of GATA3, a master transcription factor for Th2 cells (Fig. 4C, 4D). This Th2-polarizing effect was further corroborated by a DCA concentration-dependent increase in IgE expression in B cells (Fig. 4E).

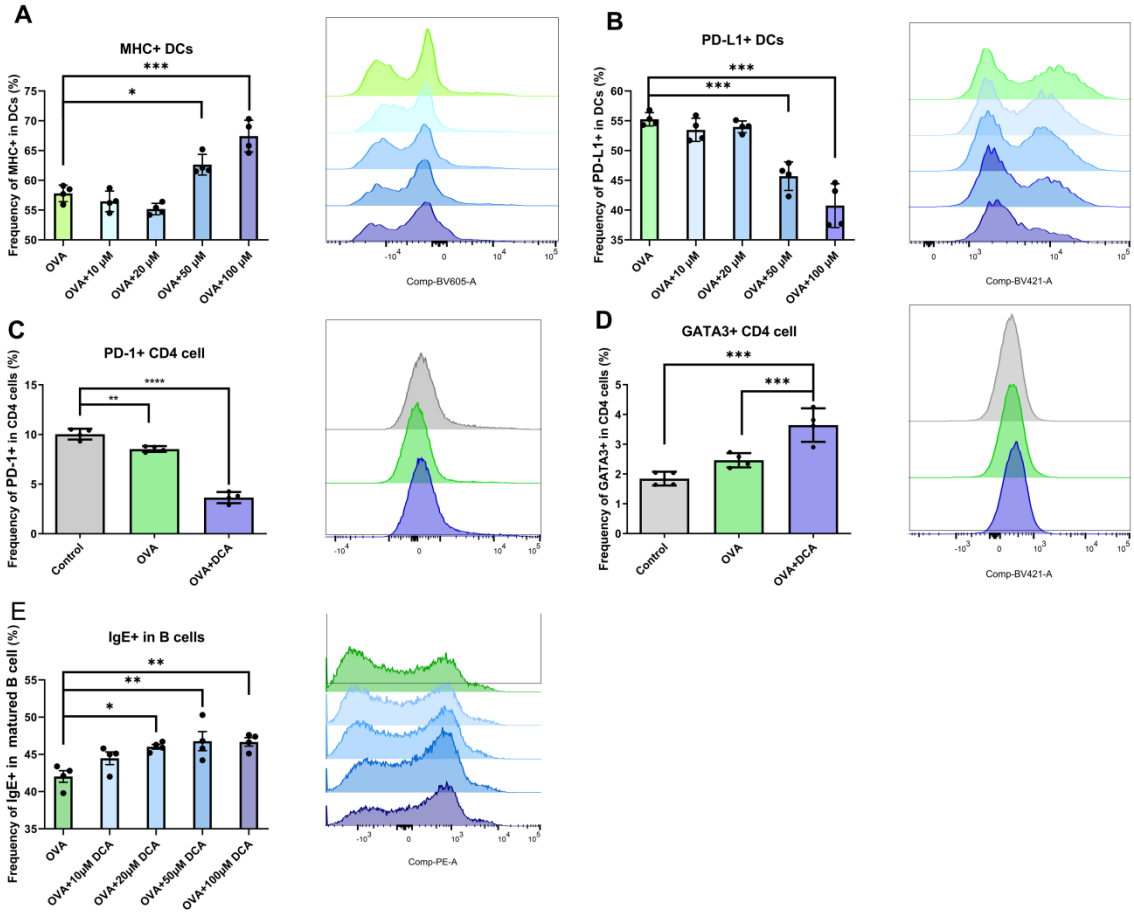

Fig. 4. DCA potentiates ovalbumin-induced hypersensitivity responses in vitro cultured mesenteric lymph node lymphocytes

(A) MHC class II and (B) PD-L1 expression frequencies in CD11c+ dendritic cells, (E) IgE+ B cell proportions, and (C) PD-1/(D) GATA3+ CD4+ T cell ratios following 24 h stimulation with OVA (50 μg/ mL) ± DCA (10-100 μmol/L) in MLN cells from OVA-sensitized C57BL/6 mice (n=6). Co-stimulation groups: OVA (50 μg/ mL), DCA (50 μmol/L), and OVA+DCA. Data acquired by spectral flow cytometry (Cytek Aurora) expressed as mean ± SEM; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs OVA-only group by two-way ANOVA with Sidak's multiple comparisons test.

3.4 FXR Antagonism Reverses the Disruptive Effects of DCA on the PD-L1/PD-1 Axis and Th2 Polarization

To delineate the molecular targets of DCA in modulating immune tolerance, we utilized selective receptor antagonists: DY268 for FXR and MeTC7 for VDR (Fig. 5). Compared to OVA-treated controls, DCA+OVA significantly reduced PD-L1 expression on DCs. Critically, the FXR antagonist DY268 completely reversed this DCA-induced suppression of both PD-L1 and PD-1 (Fig. 5A-5B). Furthermore, DCA promoted Th2 polarization, as indicated by upregulated GATA3 in CD4+ T cells and elevated IgE in B cells (Fig. 5C-5D).

Treatment with the FXR antagonist abolished both of these DCA-induced effects (Fig. 5C, D). In contrast, antagonism of VDR using MeTC7 failed to significantly alter DCA's impact on PD-L1, PD-1, GATA3, or IgE expression (Fig. 5A-D), demonstrating the specific and essential role of FXR signaling in mediating the immunomodulatory effects of DCA.

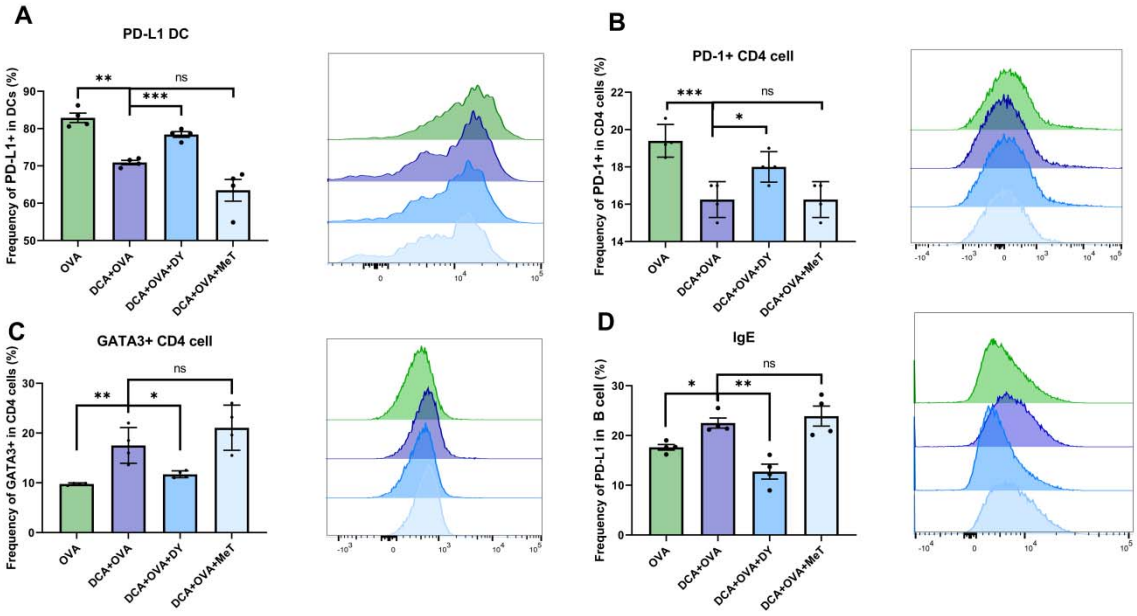

Fig. 5. Pharmacological inhibition reveals FXR/VDR-dependent mechanisms underlying DCA-enhanced hypersensitivity responses

(A) PD-L1+ dendritic cell frequency, (B) PD-1+ and (C) GATA3+ CD4+ T cell proportions, and (D) IgE+ B cell ratios in OVA (50 μg/ mL) and DCA (50 μmol/L) co-stimulated MLN cultures with/without 1 μmol/L DY268 (FXR antagonist) or MeTC7 (VDR antagonist). Cells derived from OVA-sensitized C57BL/6 mice (n=6), analyzed by spectral flow cytometry (Cytek Aurora). Data expressed as mean ± SEM; **P* < 0.05, ***P* < 0.01 between inhibitor groups by two-way ANOVA with Tukey's post-hoc test.

4. Discussion

The elevated fecal DCA levels are associated with children's food allergy [8]. Meanwhile, the underlying mechanisms and associated risks remain unclear. Our study now identifies the bile acid receptor FXR and the PD-L1/PD-1 immune checkpoint axis as the central mechanistic pathway underlying this association. We demonstrate that DCA, by activating FXR, suppresses PD-L1 on dendritic cells and PD-1 on T cells, thereby dismantling a critical tolerogenic circuit and promoting a Th2-polarized response conducive to allergic sensitization.

Intestinal immune tolerance relies on the delicate interplay between dendritic cells (DCs) and helper T cells (Th cells). PD-1 and its ligands play a critical role in regulating the activation and function of DCs and CD4+ T cells [15]. As immune checkpoint molecules, they maintain the balance and homeostasis of immune responses and prevent the excessive accumulation of Th cell responses [16]. Our finding that DCA disrupts this specific checkpoint through FXR activation provides a novel, receptor-mediated explanation for bile acid-driven immunopathology. This is particularly significant as it mirrors mechanisms described in unrelated contexts; for instance, FXR agonism has been shown to downregulate PD-L1 in hepatic stellate cells and certain cancer models [17, 18]. Our data extend this paradigm to intestinal immunity, positioning FXR as a

conserved regulator of this checkpoint.

Bile acids and their metabolites have been suggested by previous studies to be capable of broadly regulating immune cell functions through multiple bile acid receptors [19]. The intestinal mucosa and its associated lymphoid tissues—the primary sites for allergen recognition and the initiation of food allergy [20]—are exposed to high concentrations of these metabolites. Our data demonstrate that DCA administration within this context disrupts intestinal immune homeostasis, promoting a pro-allergic phenotype characterized by B cell activation (Fig. 2), suppression of Treg markers (Fig. 3), and inhibition of the PD-L1/PD-1 checkpoint. These findings position the DCA-driven suppression of immune tolerance as a plausible mechanistic model. We proposed that dysregulated bile acid metabolism, exemplified by elevated DCA, may commonly impair intestinal immune tolerance through an FXR-dependent pathway that converges on the suppression of the PD-L1/PD-1 axis, thereby providing a novel link between metabolic dysfunction and the pathogenesis of not only food allergy but potentially other intestinal inflammatory disorders.

To validate the impact of DCA-FXR axis on intestinal tolerance, lymphocytes from mesenteric lymph nodes (MLNs) of OVA-sensitized allergic mice were co-stimulated with DCA and OVA. Functionally validating the DCA-FXR axis in intestinal immunity, lymphocytes isolated from mesenteric lymph nodes (MLNs) of OVA-sensitized allergic mice were co-cultured with DCA and OVA. Exposure to higher DCA concentrations (50-100 μmol/L) significantly enhanced dendritic cell (DC), T helper (Th) cell, and B cell activation in response to OVA while concurrently suppressing the PD-L1/PD-1 tolerance checkpoint (Fig. 4). This effect was mechanistically attributed to FXR activation, evidenced by complete restoration of PD-L1/PD-1 signaling and suppression of Th2/IgE responses upon FXR antagonism, whereas VDR blockade failed to replicate these outcomes. Direct FXR-mediated attenuation of PD-L1 expression on DCs ($P < 0.01$) was demonstrated, effectively disabling this pivotal regulator of T cell anergy and regulatory T cell (Treg) differentiation. Concomitant reductions in PD-1$^+$ Th cells and Treg markers further confirmed immune polarization toward a Th2-dominant phenotype. Collectively, these data establish that DCA promotes food allergy pathogenesis via FXR-dependent suppression of the PD-L1/PD-1 checkpoint—a conserved mechanism previously observed in oncology models [17, 18]—which synergizes with Treg impairment to disrupt intestinal tolerance, thereby facilitating aberrant Th2 responses and barrier dysfunction as documented in our cellular and transcriptomic analyses (Figs. 1-3).

The synergy between DCA and OVA in promoting Th2 polarization (Fig. 4D) is mechanistically rooted in DCA's action as an FXR ligand. This is substantiated by FXR antagonist-mediated reversal of Th2 responses (Fig. 5C-D) and enrichment of nuclear receptor pathways in transcriptomic data (Fig. 3D). The reversal of DCA effects by FXR/VDR antagonists (DY268/MeTC7, Fig. 5), combined with enriched nuclear receptor pathways in RNA-seq (Fig. 3D), highlights FXR signaling as a central regulator. Previous reports have shown FXR-mediated suppression of Th17 differentiation, suggesting context-dependent immunomodulatory properties of bile acids. Furthermore, the results of the intestinal transcriptome analysis showed that DCA significantly downregulated immune tolerance-related genes such as *CTLA4*, *Tnfaip3*, and *NR4A1* in the

jejunum. And CTLA4 and NR4A1 are indispensable in the establishment of Tregs and immune tolerance homeostasis [21, 22]. Critically, DCA downregulated *NR4A1* (Fig. 3B)—a transcription factor essential for Treg function [21]—while suppressing PD-L1 via FXR (Fig. 5A). This dual impairment of checkpoint molecules and Treg stabilizers creates a permissive milieu for Th2 responses.

Notably, a mechanistic study in non-small cell lung cancer directly supports our findings, demonstrating that FXR binds to a specific FXR element in the PD-L1 promoter to transcriptionally repress its expression, while also implicating downstream SHP and EGFR signaling in this regulation [23]. This parallel from oncology validates the physiological relevance of FXR-mediated PD-L1 suppression. The critical insight from comparing these studies is the context-dependent outcome of this regulation. In NSCLC, FXR-driven PD-L1 downregulation defines an immunosuppressive yet anti-PD-1-responsive tumor subtype. In our model of intestinal tolerance, the same mechanism—DCA activating FXR to suppress PD-L1 on DCs—disrupts immune homeostasis and promotes allergic sensitization. We therefore hypothesize that in our system, FXR may similarly repress PD-L1 through direct promoter binding or via intermediary signals like SHP. Elucidating whether this occurs through a conserved or gut-specific mechanism will be a major focus of our subsequent work, solidifying the molecular bridge between bile acid metabolism and food allergy pathogenesis.

The upregulation of detoxification enzymes (*Cyp2c38*, *Gsta4*) and antimicrobial peptides (*Reg3a*, *Reg3d*) in DCA-treated mice supports its role in enhancing intestinal barrier integrity. *Cyp2c38*, a cytochrome P450 enzyme critical for xenobiotic metabolism and ROS scavenging [24], suggested that DCA may exacerbate food allergy pathogenesis via oxidative barrier damage [25], thus facilitating allergen penetration. Concurrently, the downregulation of immune checkpoint genes Cd69 and Tnfaip3 points to systemic immune dysregulation. Tnfaip3, a ubiquitin-editing enzyme, constrains NF-κB signaling to prevent excessive TNF-α production and aberrant Th17 differentiation [26]. Loss of Tnfaip3 may thus amplify TNF-driven inflammation, synergizing with DCA's cytotoxic effects to disrupt epithelial tight junctions—a key event in allergy development.

Our findings focus on the direct immunomodulatory effect of DCA on host immunity, they must be considered within the established triad of gut microbiota, bile acid metabolism, and immune homeostasis. The gut microbiota is a master regulator of bile acid composition, transforming primary bile acids into immunologically active secondary forms like DCA. Our discovery that DCA disrupts tolerance via the FXR-PD-L1 axis provides a missing mechanistic link, explaining how microbial dysbiosis, often associated with food allergy, can directly promote a pro-allergic immune milieu. Thus, elevated DCA serves as both a marker of dysbiosis and a functional effector molecule in allergy pathogenesis. Future studies investigating the dietary and microbial drivers of DCA fluctuations are warranted.

Collectively, these results establish a mechanistic bridge between epidemiological observations of fecal DCA elevation and pediatric allergic sensitization [8], implicating dysregulated bile acid metabolism as a potential dietary determinant of immune hyperreactivity. These findings corroborate emerging evidence positioning bile acids as immunomodulators acting through nuclear receptors, including FXR [27]. This study elucidates a novel mechanism whereby DCA compromises intestinal immune tolerance through FXR-

mediated suppression of PD-L1 signaling, consequently driving Th2 polarization.

5. Conclusion

This study unveils a novel mechanism of food allergy pathogenesis by demonstrating that deoxycholic acid DCA impairs intestinal immune tolerance via an FXR-dependent pathway that suppresses the PD-L1/PD-1 checkpoint. This discovery provides a mechanistic link between dysregulated bile acid metabolism and allergic sensitization, thereby identifying FXR as a potential target for innovative therapeutic interventions aimed at restoring gut immune homeostasis.

Conflict of Interest

The authors declare that they have no conflict of interest in this manuscript.

Data Availability Statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center [28], China National Center for Bioinformation Beijing Institute of Genomics, Chinese Academy of Sciences that are publicly accessible at https://ngdc.cncb.ac.cn/gsa (GSA: CRA027605).

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