Basic nutritional investigation

Gestational vitamin A deficiency reduces the intestinal immune response by decreasing the number of immune cells in rat offspring

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**Abstract**

**Objectives:** Vitamin A (VA) is a critical micronutrient for life, especially during growth and development. There is a close relationship between VA deficiency (VAD) and the morbidity of diarrhea in the clinical setting. However, the regulatory mechanisms of VA are not clearly understood.

**Methods:** Specific-pathogen-free Wistar rats received a diet with or without VA before gestation. The offspring were submitted to an abdominal injection of *Escherichia coli* lipopolysaccharide. After the challenge, which lasted for 12 h, the serum retinol was detected by high-performance liquid chromatography, and the level of immunoglobulin A in the stool was analyzed by enzyme-linked immunosorbent assay. The lymphocyte immunophenotypes were evaluated with the use of flow cytometry with samples collected from the spleen, the mesenteric lymph nodes, Peyer patches, and intestinal intraepithelial lymphocytes.

**Results:** Early life VAD, independent of the lipopolysaccharide challenge, significantly decreased serum retinol level and CD8+ intestinal intraepithelial lymphocytes. The level of immunoglobulin A secretion and percentages of splenic CD4+CD8+ T cells were affected by the interaction effects of the lipopolysaccharide challenge and VAD treatment. Gestational VAD significantly increased the percentages of B cells in the mesenteric lymph nodes and decreased the percentages of CD11c+ dendritic cells and CD4+CD25+ T cells from the Peyer patches. The lipopolysaccharide challenge only significantly increased percentages of splenic CD4+CD25+ T cells. The intestinal tissue of the pups with VAD displayed mild inflammation.

**Conclusions:** Gestational or early life VAD decreases the numbers of immune cells in offspring, which may partly suppress the activities of the mucosal immune responses in the intestine. This suggests that more attention should be given to the VA nutritional state of children and women of reproductive age.

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**Introduction**

Vitamin A (VA) and its metabolites play a critical role in development during the gestational and the early postnatal period [1]. VA deficiency (VAD) is known to be a major public health and nutrition problem worldwide, especially in developing countries. VAD can limit growth, cause blindness, weaken innate and acquired host defenses, exacerbate infection, and increase the risk of death. Our previous studies have demonstrated that supplementation with VA or with VA in combination with multiple micronutrients can improve the physical growth levels [2], anemia status [3], and infection morbidity of preschool children [4]. Many studies have suggested that a lower nutritional level of VA is correlated directly with
the pathogenesis of diarrhea [5–7]. However, few studies have been conducted on the effect of VAD on the mucosal immune response of the intestine.

Increasing evidence has revealed that VAD decreases the intestinal barrier function, thereby increasing the intestine’s susceptibility to infection by pathogens, which may result in diarrhea [6,8]. Supplementation with the appropriate amount of VA acts as an anti-infection agent, which reduces infant mortality rate [9] and the morbidity associated with diarrhea [10]. Several studies have demonstrated the roles of VA and retinoic acid (RA) receptors in T-cell differentiation and of immunoglobulin A (IgA) switching and production [11–15]. However, the regulatory functions of VA in the intestinal mucosa are unknown (especially Peyer patches and intestinal intraepithelial lymphocytes [IELs]). Few researchers have studied the effects of VAD from the beginning of gestation on the postnatal intestinal mucosal immunity.

A rat model of VAD during the gestational period was used in the present study to investigate whether gestational VAD affected immune activity and impaired the capacity of the immune response in offspring after the challenge. We first observed the differences in the intestinal IgA levels and the pathologic changes of the intestinal mucosa between the VAD and VA normal (VAN) pups. The percentage changes in the various lymphocyte subsets were analyzed in the spleens of the VAD offspring after lipopolysaccharide (LPS) challenge. Second, we focused on the study of gut-associated lymphocyte tissues (GALT)—including the mesenteric lymph nodes (MLNs), Peyer patches (PPs), and IELs—to investigate the immunomodulatory mucosal immune response of the intestine. Third, we used the pups of the VAD gestational rats that were fed with the maternal milk of VAN rats to elucidate the importance of the VA nutritional level during the gestational period for postnatal immune defense. This study provides a new insight into the role that VAD during pregnancy plays in the immunosuppression of the mucosal immune response in the intestine during postnatal development.

Materials and methods

Animals, diets, and the lipopolysaccharide challenge

The use and reference numbers of the animals were approved by the Animal Experimentation Ethical Committee of Chongqing Medical University (Chongqing, China). Forty 6-wk-old female specific-pathogen-free Wistar rats obtained from the Experimental Animals Center of the Third Military Medical University (Chongqing, China) were randomly selected and divided equally into two groups of maternal VAN and maternal VAD. The maternal VAD rats were fed a VAD diet that contained 400 IU/kg of VA for 4 wk to construct the VAD animal model before gestation, whereas the VAN rats received VAN food that contained 6500 IU/kg of VA as the control [16,17]. When the serum retinol levels of blood samples taken from the tails of the VAD rats decreased to 1.05 μmol/L, the 20 maternal VAD rats were mated with normal males. The pregnant maternal rats were fed either the VAD diet or the VAN diet during both the gestational and lactation periods to maintain the retinol levels in the serum. All rats were housed at a constant temperature of 22 °C with 60% relative humidity, with artificial daylight from 07:00 to 19:00 every day and with VAD or VAN food and water available ad libitum. The specific-pathogen-free animal house was certified for experimental animals. Each dam nursed eight pups from her litter during the lactation period. After weaning, the eight pups were randomly separated into two cages. After a weaning period of 21 postnatal days, the pups were fed continuously for 3 wk with the VAD diet and designated as the VAD group (n = 60) or the VAN diet and designated as the VAN group (n = 60). After birth, the VAD pups were cross-fostered to VAN dams to make the VAD-N pups (n = 60) during the lactation period and then fed the VAN diet after weaning (Fig. 1). Approximately half of the total pups in each group were randomly selected to be injected abdominally with Escherichia coli LPS (i.e., 3 mg of LPS per kilogram of animal weight). The other half of the pups were injected with approximately 0.25 to

Fig. 1. Schematic diagram showing the three offspring groups: VAN, VAD, and VAD-N. The VAN pups were delivered and nursed by maternal VAN rats and then fed a VAN diet for 3 wk after weaning. The VAD pups were birthed and nursed by maternal VAD rats and fed a VAD diet for 3 wk after weaning. The VAD-N pups were delivered from maternal VAD rats and then nursed by maternal VAN rats and fed with a VAN diet until they were 6 wk old. VAD, vitamin A deficiency; VAD-N, VAD during gestation and fed the milk of VAN rats after birth; VAN, vitamin A normal.

0.4 mL (i.e., about the same volume as the LPS injection) of phosphate-buffered saline (PBS) to serve as the sham group for the LPS challenge. After the LPS challenge had lasted 12 h, the 6-wk-old pups were anesthetized with chloral hydrate, and the blood was collected immediately from the femoral artery. The isolated tissues of the spleen, the MLN, and the intestine were extracted and placed into PBS until they were used in the following steps.

Detection of serum retinol

The concentration of serum retinol was determined with the use of high-performance liquid chromatography according to our previously described methods [18] with slight modifications. Briefly, 200 μL of serum was deproteinized with dehydrated alcohol, and then the retinol was extracted with hexane and evaporated with nitrogen gas. The residue was dissolved in 100 μL of the mobile phase mixture (methanol:water ratio of 97:3), and the entire sample was transferred to a bottle installed in the high-performance liquid chromatography apparatus (DGU-20 As, Shimadzu Corporation, Japan). The retinoids were separated by chromatography on an analytical column (Hypersil phenyl 120 A 5 μm, 250 × 4.6 mm, Phenomenex, USA) via gradient elution of the mobile phase in a liquid chromatograph equipped with a 315 nm ultraviolet photodiode array detector.

Hematoxylin and eosin staining and enzyme-linked immunosorbent assay measurements

After the samples of fresh intestine were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and sectioned in accordance with standard methods, the serial sections, which were 4-μm thick, were stained with hematoxylin and eosin to be examined under a light microscope for pathologic observation. As described for the reported method of Frossard et al. [19], 100 mg of feces from the ileocecum were resuspended in 1 mL of 0.01 M PBS that contained 1% fetal bovine serum (FBS) and 0.1 mM phenylmethylsulfonyl fluoride (Solarbio, Amresco). The concentration of total IgA in the supernatants was measured with the use of an enzyme-linked immunosorbent assay kit (Bethyl Lab, Inc, Montgomery, Texas, US) according to the manufacturer’s instructions.

Isolation of lymphocytes from the spleen and the mesenteric lymph nodes

The spleens and the MLNs of the rats were cut into pieces with the use of surgical scissors on a 200-mesh metallic grid and then washed with PBS. The individual lymphocytes were collected via centrifugation. After washing, 3 × 10^{7} cells were resuspended in PBS for analysis.

Peyer patch preparation

Lymphocytes from PPs were isolated as described previously [20]. Briefly, the lymphoid follicles of PPs were carefully excised from the intestine. Single lymphocytes were released with 2 mL of PBS after disruption with the use of surgical scissors on a 300-mesh metallic grid. The lymphocyte suspension was slowly added onto the surface of a lymphocyte separation medium for rats (Tianjing-Haoyang Biological Manufacturing Co., China) and centrifuged at 805 g for 15 min. The white floculent at the interface between the PBS and the lymphocyte separation medium was collected into a new tube with a Pasteur pipette, washed twice, and then kept in 100 μL of PBS for flow cytometry.
Isolation of intestinal intraepithelial lymphocytes

With the use of certain modifications that have been described previously [21], the entire intestine was washed with 0.01 M PBS three times to remove the intestinal contents and mucus. The intestine was then incubated in ice-cold PBS that contained 2% FBS for 2 h; the whole intestine without the PPs was washed twice with warmed (37 °C) Dulbecco’s modified Eagle’s medium with 2% FBS and 1% dithiothreitol and then gently squeezed with ophthalmic tweezers from the serosal side to remove the loosened cells on the intestinal mucosal surface. The cell–medium mixture was placed at room temperature for 10 min, and the supernatant was collected into a new tube for centrifugation. The pellet was resuspended in FBS, and the lymphocytes were isolated with lymphocyte separation medium for detection with the use of flow cytometry.

Flow cytometric analysis

The immunophenotypes of the various lymphocytes were used to identify the specific cell types with the following antibodies (BD Pharmingen, Heidelberg). The T lymphocytes were stained with PE-Cy5-anti-CD4, APC-anti-CD3, PE-anti-CD4, and FITC-anti-CD8. The B lymphocytes were stained with PE-Cy5-anti-CD45 and FITC-anti-CD45 R, whereas the dendritic cells (DCs) were stained with PE-Cy5-anti-CD45 and PE-anti-CD11c. The CD4+CD25+ T cells were analyzed with APC-anti-CD3, PE-anti-CD4, and FITC-anti-CD25. The IELs were stained with APC-anti-CD3, FITC-anti-CD8, and PE-anti-TCRγδ. The corresponding isotype-matched monoclonal antibodies were used as negative controls. The percentages of the various lymphocytes were determined with a BD FACSCanto II flow cytometer (Becton Dickinson).

Statistics

All data were expressed as mean ± SD. Significant differences were calculated via two-way analysis of variance with the Bonferroni post hoc test with the use of the GraphPad Prism version 5.0 software package. Statistically significant interactions were analyzed with the Bonferroni post hoc test between LPS and no-LPS treatments. When there was no significant interaction, the main effects were analyzed with the use of the Tukey post hoc test for the three combined VA treatment groups, and the Student t test was used for the two combined LPS treatment groups. Only the relevant comparisons of the combined groups are presented in the Results section [22,23]. P values of <0.05 were accepted as statistically significant.

Results

Lower levels of serum retinols in the early life vitamin A–deficient offspring

We induced the VAD female model before pregnancy in accordance with the international standard for VA levels in humans [24]. The level of serum retinol in the VAD pups (0.641 [0.184] μmol/L) was significantly lower than that of the VAN pups (1.471 [0.337] μmol/L) (P < 0.001) (Fig. 2A). Among the VAD-N pups, serum retinol levels significantly increased (1.45 [0.46] μmol/L) to the levels of VAN pups in the absence of LPS treatment (Fig. 2A). The Tukey post hoc analysis of the three combined VA treatment groups showed that the retinol levels in the combined VAD pups were significantly decreased as compared with those in both the combined VAN and VAD-N groups (Fig. 2B). Although the concentration of retinol in the VAD-N group (1.037 [0.451] μmol/L) was slightly lower than that found in the VAN group after the LPS challenge (Fig. 2A), there was no statistical difference in both of the combined groups without or with LPS treatment (data not shown). These data demonstrated that VAD during the early life period decreased the retinol level of the pups.

Reduction of intestinal immunoglobulin A and local inflammatory changes after the lipopolysaccharide challenge in the vitamin A–deficient pups

Secretory IgA is the first line of humoral immune defense at the intestinal mucosal surface that provides protection against...
Vitamin A deficiency assessed with the use of the IgA levels in the VAD and VAD-N groups with or without LPS challenge assessed using flow cytometry (n = 8). (B) The LPS main effect, independent of VA treatment, in the percentage of CD4⁺ CD25⁺ T cells in the spleen. *interaction indicates an effect of LPS treatment versus null treatment. P < 0.001; P < 0.01; P < 0.001; n.s. – not significant in post hoc tests. LPS, lipopolysaccharide; VAD, vitamin A deficiency; VAD-N, VAD during gestation and fed the milk of VAN rats after birth; VAN, vitamin A normal.

**Fig. 3.** The percentage of (A) CD4⁺ CD25⁺ T cells and (C) CD4⁺ CD8⁺ double-positive T lymphocytes in the spleen of VAN, VAD, and VAD-N groups with or without LPS challenge assessed using flow cytometry (n = 8). (B) The LPS main effect, independent of VA treatment, in the percentage of CD4⁺ CD25⁺ T cells in the spleen. Interaction indicates an effect of LPS treatment versus null treatment. *P < 0.005, **P < 0.01, ***P < 0.001; n.s. – not significant in post hoc tests. LPS, lipopolysaccharide; VAD, vitamin A deficiency; VAD-N, VAD during gestation and fed the milk of VAN rats after birth; VAN, vitamin A normal.

Increased percentage of B lymphocytes in the mesenteric lymph nodes of vitamin A-deficient pups

The MLN is part of the gut-associated lymphoid tissue, and it acts as a link between the systemic immune response and the mucosal immune response of the intestine. The subsets of lymphocytes in the MLN were observed in this study, and we found that the percentage of B lymphocytes in the MLN of the VAD pups was slightly increased as compared with the VAN pups with or without LPS challenge. The numbers of B cells in the VAD-N group were similar to those of the VAD group in the presence or absence of LPS treatment (Fig. 4A). The two-way analysis of variance showed that VA treatment was the main factor that increased the number of B cells in the MLN (P < 0.0001). The percentage of B cells in the combined VAN group was significantly decreased as compared with the combined VAD and VAD-N groups (Fig. 4B), thereby suggesting that VAD during gestation increased the percentage of B cells in the MLN. This pattern was independent of LPS treatment.

**Suppression of the mucosal immune response in the intestine as a result of vitamin A deficiency**

As an extension of the findings obtained from splenic and MLN tissue, we further determined whether VAD during the gestational period affected the development of the local intestinal immune cells, specifically in the PPs and IELs. PPs are specialized collective lymphoid nodes of the gut that have been implicated as intestinal inductive sites during the mucosal immune response. The data shown in Figure 5A revealed that the percentage of CD11c⁺ DCs within the PPs was decreased in both VAD and VAD-N pups as compared with the VAN pups with or
without LPS challenge. These differences represented the effects of VA treatment \( (P < 0.0001) \) but not of LPS treatment \( (P = 0.1075) \). After combining the two LPS treatments of the VAN, VAD, and VAD-N groups, the percentages of the CD11 c⁺ DCs in the combined VAD and VAD-N groups were significantly decreased as compared with those of the combined VAN pups (Fig. 5B). The trend in the changes of the CD4⁺CD25⁺ T-cell subset in the PPs was identical to that of the CD11 c⁺ DCs among the three groups with or without LPS treatment (Fig. 5C, D); the \( P \) values of the VA and LPS effects were \( <0.0001 \) and \( 0.0685 \), respectively. These data demonstrated that gestational VAD independent of LPS treatment decreased the number of CD11 c⁺ DCs and CD4⁺CD25⁺ T cells in PPs. However, the CD4⁺CD25⁺ T lymphocytes in the PPs were not induced by the LPS exposure in the present study. The reason for this may have been that the LPS challenge was administered for a short period via an abdominal injection, which only resulted in systemic immune responses and not in mucosal-based immunity of the intestine.

IELs reside within the intestinal epithelium to ensure the integrity of the gut barrier function. They form one of the main branches of the immune system, and they play a critical role in the mucosal immune response of the intestine. Most IELs,
especially in the small intestine, constitutively express a hallmark of CD8\(^+\) T cells and TCR\(^{\delta+}\) [25]. As shown in Figure 6A, the ratio of IELs with the CD8\(^+\) phenotype was much lower in the VAD pups as compared with the VAN pups; however, the CD8\(^+\) IELs in the VAD-N group were similar to those of the VAN group with or without LPS treatment. Under the VA main effect (\(P = 0.0002\)) independent of LPS treatment, the number of CD8\(^+\) IELs in the combined VAD pups was significantly decreased as compared with those in the combined VAN and VAD-N pups (Fig. 6B), thereby suggesting that VAD during early life but not during gestation suppressed the percentage of CD8\(^+\) IELs. The VA treatment was also the main effect that impaired the percentage of CD8\(^+\) TCR\(^{\delta+}\) IELs (\(P = 0.0006\)), whereas the LPS effect did not (\(P = 0.1046\)) (Fig. 6C). The numbers of CD8\(^+\) TCR\(^{\delta+}\) IELs were significantly reduced in the combined VAN group as compared with both the combined VAN and VAD groups (Fig. 6D). Taken together, these results indicate that early life VAD could impair the activity of the intestinal mucosal immune response in the offspring by decreasing the CD8\(^+\) IEL percentage.

Discussion

Retinol is the main form of ingested VA, and it has become the diagnostic criteria for the serum in many clinical applications. In the present study, as compared with a VAD model established after weaning [26] or mid gestation [27], we successfully constructed a VAD animal model in the rat from the beginning of the gestation period, which is consistent with Jiang’s model [17], and we obtained VAD offspring with approximately 0.3 \(\mu\)mol/L of serum retinol after birth. There were no significant differences in the VAD and VAN groups with regard to time to delivery, number of pups per litter, birth weight, and pup physiologic state; however, a small body size with messy, dry, and dim fur was observed in the VAD pups 6 wk postnatally (data not shown). To confirm the effects of VAD during the gestational period, we designed the VAD-N group in this study to be deficient for VA during gestation by cross-fostering VAD pups to VAN dams to create the VAD-N pups. The increase in the serum retinol level in the VAD-N pups further indicated that the postnatal feeding of milk from VAN female rats could restore the lower retinol levels caused by gestational VAD (Fig. 2A), thereby providing a useful animal model that simulated VAD in pregnant women. Although the RA levels were affected by both the VA (\(P < 0.0001\)) and LPS effects (\(P = 0.0077\)), VAD during the early life period decreased the serum RA level in the offspring; this pattern was independent of LPS treatment (Fig. 2B).

IgA is synthesized by the plasma cells and epithelium of the intestine, and it prevents pathogens and their toxic products from attaching to the mucosal surface of the intestine [28]. The level of IgA in the feces of VAD rats was markedly lower than that of VAN rats [29]. The article by Quadro et al. [30] showed that the IgA levels in the retinol-binding protein knockout mice were half those of the wild-type mice. In the present study, there were no differences among the VAN, VAD, and VAD-N groups in the absence of LPS treatment. IgA is an important immunoglobulin in the intestine that protects against pathogen invasion, and an increase in the IgA level can be induced by exogenous pathogens. After the injection of 3 mg/kg of LPS for 12 h, we found that the rats displayed obvious congestion and edema of the small intestine, with yellow feces, diarrhea, and even twitch (data not
shown); there was also a prompt increase in the IgA levels of the VAN pups after LPS exposure (Fig. 2C). These results demonstrated that the LPS abdominal challenge caused relevant clinical features. The levels of IgA were considerably suppressed by the LPS challenge both in the BVD and VAD-N pups as compared with the VAN-LPS pups, which suggests that VAD during gestation impairs a normal IgA response in the gut. VAD can result in a decreased level of RA, which impairs the expression of retinol-binding protein, the homing and maturation of B cells, and the synthesis of immunoglobulins [15,30].

CD4+CD25+ T cells play a critical role in maintaining intestinal homeostasis and in suppressing systemic and mucosal immune activities in the gut. The percentage of CD4+CD25+ T cells in the spleen was increased by the LPS challenge (Fig. 3B), which suggests that LPS injection into the abdomen can upregulate the number of CD4+CD25+ T cells and that the LPS main effect on the number change of CD4+CD25+ T cells in the spleen was independent of VAD. The CD4+CD8+ DP T cells, which are antigen-specific effective cells, can activate memory cells, and they are likely involved in the regulation of immune responses [31]. We therefore hypothesized that the depression of CD4+CD8+ DP T cells in both the VAD and VAD-N pups further caused dysfunction of the immune responses. These findings demonstrated that LPS-effect-independent VA treatment could induce an increase in the number of CD4+CD25+ T cells and that both LPS challenge and VAD during gestation may impair the normal systemic immune response through a decrease in the number of CD4+CD8+ DP T cells.

Unlike the decrease in T-cell subsets in the spleen, the number of B lymphocytes in the MLN was significantly increased by gestational VAD: this pattern was independent of LPS treatment (Fig. 4). This is consistent with the findings of Chang et al. [32], in which the number of immunoglobulin G–secreting B cells was increased in the MLN of VAD mice. RA can upregulate the levels of integrin α4β7 and the CCR9 expression of B lymphocytes [33], which enhances the preferential homing of the B lymphocytes to the small intestine and induces the secretion of IgA. We therefore speculate that the decreased homing signaling by RA causes fewer B cells to travel from the MLN to the gut.

The intestinal PPs are collective mucosal lymphoid tissues, and they are the most important inductive sites during the mucosal immune response. The distribution of lymphocyte populations, which was identified with the use of flow cytometry in the present study, was consistent with previous data [34,35]. DCs are the classic antigen-presenting cells, and immature DCs are dispersed within the subepithelial domes of the PPs. Contrary to the report by Dong et al. [27], we found that the number of CD11c+ DCs in the PPs was reduced in the VAD pups (Fig. 5A); this is due to the use of VAT rats from the beginning of gestation and the different markers of CD11c-DCs that were used in the present study, VA is one of the key factors during the differentiation of CD4+CD25+ T cells from naive T cells, and RA activates the transforming growth factor-β signaling pathway to enhance the maturation of Foxp3+ regulatory T cells [36,37]. The data obtained in the present study showed that the percentages of both CD11c+ DCs and CD4+CD25+ T cells were significantly suppressed by gestational VAD independent of the LPS challenge (Fig. 5). These results suggest that VAD during the gestation period limits the activities of inducible CD11c+ DCs and CD4+CD25+ T cells in the PPs.

IELs are effective T lymphocytes that are part of the mucosal immune response of the intestine, and they are scattered in the basal aspect of the intestinal epithelium. Most of the IELs are CD8+ T lymphocytes, and more than 85% of the isolated IELs in this study were CD8+ T cells (Fig. 6A), which is consistent with previous data about lymphocyte populations [21]. The CD8+ IELs display characteristics that are similar to cytotoxic T lymphocytes, and IELs with TCRγδ+ perform activities that are similar to those of natural killer cells. As shown in Figure 6B, the percentage of CD8+ IELs was significantly decreased in the combined VAD offspring, thus indicating that the immunologic activity of the IELs was suppressed by the early life VAD. RA signaling regulates the differentiation of T cells as well as the homing of T and B cells in the intestine [38–40]. Furthermore, IELs have been implicated in antibody class switching and IgA production [25], Fujihashi et al. [41] reported that the gut epithelium was abnormal in TCRγδ-deficient mice, that the level of major histocompatibility complex class II molecule expression in the enterocytes was reduced, and that the mucosal IgA production was impaired. Therefore, the decrease in TCRγδ+ IELs may explain the repression of IgA secretion in the combined VAD-N pups. The findings from the present study suggest that the mucosal immune functions of IELs are weakened by VAD during the early life period. In the present study, the combined VAD-N pups had a lower percentage of TCRγδ+ IELs as compared with the combined VAN pups; this decrease was comparable with or without the LPS challenge.

We were surprised to find that the results of the series of parameters in the VAD-N group were the same as those of the VAD group, including the decrease seen in IgA levels after LPS treatment (Fig. 2C), the depression of splenic CD4+CD8+ DP T cells without LPS challenge (Fig. 3C), the reduction in CD11c+ DCs (Fig. 5B) and CD4+CD25+ T cells (Fig. 5D) in the PP, and the increase in B cells in the MLN (Fig. 4B). These results provide novel evidence that the nutritional level of VA during the gestational period plays a critical role in the immune responses. Further studies involving this approach should focus on the regulatory mechanisms of VA for the mucosal immunity of the intestine and the identification of the best time point and dosage for VA supplementation.

Conclusions

VAD during the gestational period may impair the intestinal mucosal immune responses of offspring by suppressing the numbers of CD11c+ DCs and CD4+CD25+ T cells in the PPs and by increasing the number of B cells in the MLNs. Early life VAD impairs the number of CD8+ IELs and decreases the serum retinol level. IgA secretion and the percentage of splenic CD4+CD8+ T cells were affected by both the VA and LPS treatments. These findings indicate that it is important to focus on the nutritional states of both children and gestating women.

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