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Prenatal vitamin A deficiency impairs adaptive immune responses to pentavalent rotavirus vaccine (RotaTeq®) in a neonatal gnotobiotic pig model

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ABSTRACT

Vitamin A deficiency (VAD) is associated with increased childhood mortality and morbidity in impoverished Asian and African countries, but the impact of VAD on rotavirus (RV) vaccine or infection is poorly understood. We assessed effects of gestational and dietary induced pre- and post-natal VAD and vitamin A supplementation on immune responses to a pentavalent rotavirus vaccine, RotaTeq® in a neonatal gnotobiotic pig model. Vaccine efficacy was assessed against virulent G1P[8] human rotavirus (HRV) challenge. VAD and vitamin A sufficient (VAS) piglets were derived from dietary VAD and VAS sows, respectively. VAD piglets had significantly lower levels of hepatic vitamin A compared to that of VAS piglets. RotaTeq®-vaccinated VAD piglets had 350-fold higher fecal virus shedding titers compared to vaccinated VAS piglets post-challenge. Only 25% of vaccinated non-vitamin A supplemented VAD piglets were protected against diarrhea compared with 100% protection rate in vaccinated non-supplemented VAS piglets post-challenge. Intestinal HRV specific immune responses were compromised in VAD piglets. Vaccinated VAD piglets had significantly lower ileal HRV IgG antibody secreting cell (ASC) responses (pre-challenge) and duodenal HRV IgA ASC responses (post-challenge) compared to vaccinated VAS piglets. Also, intestinal HRV IgA antibody titers were 11-fold lower in vaccinated VAD compared to vaccinated VAS piglets post-challenge. Persistently elevated levels of IL-8, a pro-inflammatory mediator, and lower IL-10 responses (anti-inflammatory) in vaccinated VAD compared to VAS piglets suggest more severe inflammatory responses in VAD piglets post-challenge. Moreover higher IFN-γ responses pre-challenge were observed in VAD compared to VAS piglets. The impaired vaccine-specific intestinal antibody responses and decreased immunoregulatory cytokine responses coincided with reduced protective efficacy of the RV vaccine against virulent HRV challenge in VAD piglets. In conclusion, VAD impaired antibody responses to RotaTeq® and vaccine efficacy. Oral supplementation of 100,000 IU vitamin A concurrent with RV vaccine failed to increase the vaccine efficacy in VAD piglets.

1. Introduction

Rotavirus (RV) is a leading cause of morbidity and mortality in children worldwide. Two oral human RV vaccines, RotaTeq® (Merck and Co., Inc.) and Rotarix® (GlaxoSmithKline Biologicals) are currently licensed to prevent severe RV disease. RotaTeq® is a pentavalent human-bovine reassortant RV vaccine that contains the dominant circulating human RV parent strain G and P types; G1, G2, G3, G4 and P1A[8], and G6 and P[7] from bovine rotavirus parent strain [1].

Rotavirus is a major cause of severe diarrhea and mortality in children in impoverished countries, where RV vaccine efficacy is lowest. The efficacy of RotaTeq® against severe RV gastroenteritis in Africa is 39% [2] and 48.5% in Bangladesh and Vietnam [3]. In comparison, the efficacy of RotaTeq® is >90% in developed countries such as the US and Finland [4,5]. Several factors were attributed to the lower efficacy of RV vaccines, such as higher prevalence of malnutrition, vitamin A deficiency (VAD), higher RV disease burden, presence of diverse RV strains, and interference by maternal antibodies.

Vitamin A deficiency is prevalent in economically poor countries [6–9]. According to the World Health Organization (WHO), an estimated 250 million preschool-age children in low income countries are affected by VAD. A recent study revealed that 66% of young children were affected by VAD in Guinea-Bissau [6]. The WHO has recommended supplemental vitamin A (50,000–100,000 IU)

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for children at immunization contacts to overcome vitamin A deficiency in these countries [10]. Vitamin A and its metabolite retinoic acid (RA) play a significant role in development of gastrointestinal immune responses to antigens or vaccines. VAD is associated with reduced immunogenicity of different vaccines such as diphtheria [11] measles [12] and tetanus toxoid [13]. Retinoic acid has been shown to mediate mucosal IgA production [14] and intestinal homing of IgA^+ plasma cell [15] and T-cells [16]. In addition to mediating these responses, vitamin A is also essential for normal T-cell responses in the gut mucosa [16]. VAD alters cytokine responses such as polarization toward increased T-helper cell type-1(Th1) cytokine responses in infants [17] and mice [18]. In addition, treatment of T cells with RA enhanced Th2 development and suppressed Th1 development. Thus, vitamin A is involved in regulation of intestinal mucosal immunity. Based on these previous observations, we hypothesized that vitamin A may act as an adjuvant and enhance efficacy of the pentavalent RotaTeq® vaccine and induce increased RV immunity in infants.

The WHO has recommended inclusion of RV vaccines in national immunization programs worldwide. The impact of VAD on RV infection and on efficacy of RotaTeq vaccine is unknown. Moreover the modulation by supplemental vitamin A of RV immunity in neonates has not been investigated. Neonatal gnotobiotic (Gn) piglets are susceptible to HRV induced diarrhea and their mucosal immune systems and gut physiology are similar to that of infants. Retinol metabolism in pigs is comparable with that in humans [19]. In addition, vitamin A content in liver and milk of pigs is similar to that in humans [19–21]. Because of the similarity in vitamin A metabolism, pigs have been previously used to evaluate vitamin A supplementation programs in lactating women [19,22] and children [23]. Also piglets are outbred and similar in size to newborn infants. In this study, we used Gn piglets to investigate effects of gestational and dietary induced pre- and post-natal vitamin A deficiency and supplemental vitamin A (as recommended by WHO at immunization contacts) on RotaTeq® vaccine, virulent HRV challenge and the adaptive immune responses induced.

2. Materials and methods

2.1. Experimental design

This study was approved by The Ohio State University Institutional Animal Care and Use Committee. Cesarean-derived Gn piglets from near-term sows were maintained in sterile isolators as described previously [24]. VAD and vitamin-A sufficient (VAS) piglets were derived from 2 paired VAD and VAS sows (both Landrace × Yorkshire White × Duroc crossbred), respectively, as described previously [25]. VAD pigs were defined on the basis of their origin (from VAD sows) and hepatic vitamin A levels. Briefly, pregnant VAD and VAS sows were kept on vitamin A restricted or supplemented diets, respectively, for approximately 80 days (until piglet derivation) starting from gestation day 34. VAS sows were given an additional 500,000 IU injectable vitamin A (NDC50989-178-12; Vedco) during gestation to maintain vitamin A levels. Gestational and dietary induced VAD and VAS piglets were fed commercial ultra-high temperature-treated sterile bovine milk (Parmalat) containing low amounts of vitamin A for maintaining low levels of vitamin A in VAD piglets. The VAD and VAS piglets were assigned to one of the following four groups: 3XRotaTeq® vaccinated (Vac); 3XRotaTeq® vaccinated and vitamin A supplemented (100,000 U) (Vac + VitA), unvaccinated control (Control), and unvaccinated and vitamin A supplemented (100,000 IU) controls (Control + VitA). RotaTeq® vaccine with and without supplemental vitamin A was given orally at 6- (Post inoculation day 0, [PID0]), 16- (PID10) and 28- (PID21) days of age. Vitamin A supplemented groups received oral supplementation of retinyl palmitate (Sigma–Aldrich, R3375) at a dosage of 100,000 IU with each vaccine dose.

Serum samples were collected to assess cytokine and HRV-specific antibody responses at multiple time-points. Subsets of pigs were euthanized at PID28/post-challenge day 0 (PCD0) to assess pre-challenge responses and the remaining were challenged with 10^5 FFU of virulent HRV Wa strain (VirHRV) and were euthanized at PID35/PCD7 to assess post-challenge immune responses. Mononuclear cells (MNC) were isolated from euthanized pigs to measure HRV specific antibody secreting cell (ASC) responses in intestinal tissues [25,26].

2.2. Clinical signs and virus shedding

VirHRV challenged piglets were examined daily post-challenge to assess diarrhea and fecal HRV shedding, as previously described [27].

2.3. B cell and cytokine responses and vitamin A levels

HRV specific IgA and IgG antibody responses were measured by ELISA as described previously [27,28], with slight modifications. Enumeration of IgA and IgG isotype-specific HRV ASC was performed by enzyme-linked immunosorbent spot (ELISPOT) assay as previously described [25,27], with slight modifications. RotaTeq® vaccine was used as antigen in both HRV specific ELISA and ELISPOT assays. Serum IFNγ, IFNα, TGFβ, IL4, IL12, IL10 and IL8 levels at PID0, PID2, PID6/7, PID28/PCD0, PID30/PCD2 and PID35/PCD7 were measured by ELISA as described previously [25,29,30]. Hepatic and serum vitamin A levels were assessed as described previously [25].

2.4. In vitro stimulation with RA and RV antigen

Frozen splenic MNC (in 90% FBS/10% dimethylsulfoxide [DMSO]) from four unrelated RV vaccinated Gn piglets (7-week old) were thawed and washed immediately to remove residual DMSO. In each culture, 2×10^6 cells were stimulated with semi-purified group A RV antigen (12 μg/ml), alone or in combination with RA at 10 nm, 100 nm and 1000 nm or mock stimulated for 6 days. Following incubation, supernatants were collected to measure total IgA titers by ELISA and cells were harvested and washed to determine frequency of IgA^+ B cells ($CD79\beta^+IgA^+$) by flow cytometry. Briefly, for ELISA, goat anti-pig IgA antibody (cat #A100-102A, Bethyl) was used as coating antibody. The HRP conjugated goat anti-pig IgA antibody (cat #A100-102AP, Bethyl) was used as detection antibody. Standard curves were generated using pig reference serum (RS10-107, Bethyl) to extrapolate the IgA levels in the samples. For flow cytometry, the stimulated MNC were stained with mouse monoclonal anti-porcine IgA (Clone K61 1B4, Serotec) followed by anti-mouse IgG1-Allophycocyanin (BD Biosciences, CA) antibodies. Subsequently, samples were permeabilized and stained intracellularly for porcine cross-reactive anti-mouse CD79β antibody (Clone AT107-2, Serotec, NC). The samples were acquired and analyzed using Accuri C6 flow cytometer and C6 flow sampler software, respectively.

2.5. Statistical analysis

Log_{10} transformed isotype-specific ELISA antibody titers were analyzed using one-way ANOVA followed by Duncan's multiple range test. The area under the curve (AUC) for diarrhea severity and shedding was calculated as previously explained [31]. The HRV-specific ASC, cytokine concentrations, AUC, total IgA concentration and frequencies of $CD79\beta^+IgA^+$ cells were compared among groups using the Kruskal–Wallis rank sum test. All