Vitamin A Deficiency Disrupts Vaccine-Induced Antibody-Forming Cells and the Balance of IgA/IgG Isotypes in the Upper and Lower Respiratory Tract

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Abstract

Vaccination by intranasal (IN) inoculation with a replication-competent virus forms the basis of licensed and novel candidate respiratory viral vaccines (e.g., the cold-adapted influenza virus vaccine). A positive global impact of vaccination depends on vaccine efficacy in developing countries where dietary deficiencies are commonplace. The current study was designed using Sendai virus (SeV) as a model respiratory viral vaccine to test antibody-forming cell (AFC) residence and isotype expression in the context of a vitamin A deficiency (VAD). Samples were taken 1 mo after vaccination when AFCs generally reach their peak in healthy animals. In control animals on a healthy diet, SeV induced an antibody response with a relative bias toward IgA in the upper respiratory tract (URT, as sampled by nasal wash), and IgG in the lower respiratory tract (LRT, as sampled by bronchoalveolar lavage [BAL]). In the context of VAD, the SeV-specific IgA antibodies in the nasal wash were significantly reduced in favor of enhanced IgG antibodies in the BAL. When AFCs were examined in diffuse nasal-associated lymphoid tissues (d-NALT), lungs, cervical lymph nodes (CLN), and mediastinal lymph nodes (MLN), a similar pattern emerged. AFCs were most frequent in the d-NALT and most expressed IgA in control mice. In the context of VAD, these IgA-producing AFCs were significantly reduced in number, skewing the natural balance of IgA and IgG. Taken together, the results show that the VAD diet, which is well known for its association with immune defects in the gut, significantly alters AFC induction and isotype expression in the respiratory tract.

Introduction

VITAMIN A DEFICIENCY (VAD) IS RESPONSIBLE FOR SIGNIFICANT MORBIDITY and mortality in developing countries, particularly in the pediatric health arena. Multiple physiological processes are dependent on vitamin A, including the induction of immune activity. Because the retinal dehydrogenase enzymes (RALDH) that are necessary for catalysis of all-*trans* retinal molecules to the key effector molecule all-*trans*-retinoic acid (RA) are well expressed in gut-associated cells, the effects of VAD on the immune responses of the gastrointestinal tract have been well studied. Research has shown that in the absence of vitamin A, natural processes of oral and gut lymphocyte activation, differentiation, homing, and function, are each significantly altered (2,4–8).

Much less attention has been given to the study of VAD on mucosal tissues other than those of the alimentary canal, particularly of the respiratory tract. Experiments described in this report were therefore designed to examine the residence and function of murine antibody-forming cells (AFC) in-

duced in the upper and lower respiratory tract (URT and LRT) following intranasal (IN) vaccination with replication-competent murine parainfluenza virus (Sendai virus, SeV). Responses were examined 30 d after infection, a time of robust AFC and antibody activity in healthy mice (10).

Materials and Methods

Animals and housing

Pregnant female C57BL/6 (H2^b) mice were purchased from Charles River (Wilmington, MA). The animals were housed in filter-top cages in a Biosafety Level 2⁺ containment area as specified by the Association for Assessment and Accreditation for Laboratory Animal Care guidelines and approved by the Institutional Animal Care and Use Committee.

VAD mice and vaccinations

To establish VAD mice, day 4–5 estrus C57BL/6 females were placed on characterized diets (Harlan Laboratories,

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Madison, WI) upon arrival in the animal facility at St. Jude Children's Research Hospital. The VAD diet (cat. no. TD.10762) was formulated with casein, DL-methionine, sucrose, corn starch, cotton seed oil, cellulose, mineral mix AIN-76 (170815), calcium carbonate, vitamin mix (lacking vitamin A) plus choline, and food coloring. The control diet included vitamin A palmitate at 15IU/g (cat. no. TD.10764). The animals were sustained on the diet throughout their pregnancies and weaned pups were on the diet throughout experimentation. Infections of grown mice involved anesthesia with Avertin®, followed by intranasal (IN) inoculations with 250–500 plaque-forming units (pfu) of SeV.

Preparation of samples

Animals were sacrificed 1 mo after SeV vaccinations. Immediately prior to sacrifice, the mice were anesthetized with Avertin and exsanguinated. Following the removal of cervical lymph nodes (CLN), nasal wash samples were collected by exposing the trachea and washing the upper trachea and nasal cavity with 200 μ L of PBS. Bronchoalveolar lavage (BAL) samples were collected by inserting catheters into the trachea and washing three times with 1 mL PBS (3 mL total, centrifuged to separate cellular material). Mice were perfused with PBS injected through the retro-orbital sinus and the left ventricle of the heart, after which the mediastinal lymph nodes (MLN), lungs, and diffuse nasal-associated lymphoid tissue (d-NALT) were collected. d-NALT (1,3) were harvested by removing skin, lower jaws, soft palates (including the attached oral NALT), muscles, cheek bones, and incisors from the heads. The remaining snouts were cut into small pieces. The lungs and snouts were digested with 4 mg/mL collagenase in PBS at 37°C for 30 min and purified on Percoll gradients as described previously (10).

Enzyme-linked immunosorbent assay (ELISA)

The SeV-specific ELISA has been described previously (10). Briefly, purified SeV was lysed in disruption buffer (0.05% Triton X-100, 60 mM KCl, and 10 mM Tris [pH 7.8]), and diluted with PBS to 10 μ g/mL for the coating of 96-well ELISA plates for overnight incubation at 4°C. After blocking plates with PBS containing 3% bovine serum albumin, serially-diluted test samples were applied to the plates for a 1-h incubation at 37°C. The plates were washed with PBS-Tween 20, and incubated with alkaline phosphatase-conjugated goat anti-mouse IgG or IgA. The plates were developed with p-nitrophenyl phosphate and read at optical density (OD) 405 nm.

Antibody-forming cell ELISPOT

The ELISPOT plates (multiscreen™-IP; Millipore, Billerica, MA) were coated with purified SeV overnight at 1 μ g/100 μ L/well overnight at 4°C as described previously (10). The wells were washed and blocked with medium containing 10% fetal calf serum (FCS). Then 1×10^5 cells were applied to the wells and incubated for 3 h at 37°C. After washing $3 \times$ with PBS and $3 \times$ with PBS-Tween 20, 100 μ L of alkaline phosphatase-conjugated goat anti-mouse IgA, IgG₁, or IgG_{2b} were added. After overnight incubation at 4°C, the antibodies were removed and the plates were developed with 1 mg/mL BCIP/NBT. The plates were rinsed with water and the spots were counted using a dissecting scope.

Results

To determine if VAD affected antibody responses in the respiratory tract, groups of test and control mice were infected with SeV by the IN route. One month later, when SeV-specific antibody responses were at peak levels in the URT of control animals, the mice were sacrificed for antibody analyses. Both nasal wash and BAL samples were monitored. In each case, serially-diluted samples were scored and OD readings were measured to compare biases in SeV-specific IgA:IgG production. As shown in Fig. 1A and B, IgA was found at greater levels in the nasal wash compared to BAL in control animals, while IgG was observed at greater levels in

FIG. 1. Altered IgA:IgG antibody balance in the URT and LRT of VAD mice. One month after SeV vaccination, nasal wash (NW) and BAL samples from VAD and control mice were tested for SeV-specific IgA (A) and IgG (B) production by ELISA. The mice were sampled and tested individually (3–5 per group). Plotted data represent averages and standard errors of scores from individual mice. Two independent experiments yielded similar results.

BAL compared to nasal wash. In the context of VAD, IgA levels were significantly reduced ($p < 0.05$ by Student's *t*-test), while IgG levels were improved ($p < 0.05$ by Student's *t*-test). Clearly, VAD altered the natural balance of IgA/IgG expression in the respiratory tract by depleting the naturally high IgA levels in the URT in favor of high IgG levels in the LRT.

Passive transfer studies have shown that IgG in the blood can be readily transferred to the respiratory tract, particularly the LRT (9). This explains, at least in part, the relatively high IgG levels in the BAL in both VAD and control mice. The measurement of antibodies in ELISAs may also be biased toward IgG due to the tethering of IgA to the secretory component on the surface of airway epithelial cells, possibly inhibiting IgA release into wash solutions. We therefore questioned how VAD may impact local IgA- and IgG-producing AFCs in the URT and LRT. ELISPOT assays were used to measure AFCs expressing IgA, IgG₁, and IgG_{2b} in d-NALT, lung, CLN, and MLN. As shown in Fig. 2A, AFCs were most prevalent in the control d-NALT, and were predominantly IgA producers. In the context of VAD, there was a significant reduction in IgA-producing AFCs in the d-NALT. The result was statistically significant ($p < 0.05$ for the values shown in Fig. 2), and reproducible in each of four independent experiments. Unlike IgA, IgG-producing cells were slightly improved in numbers in the presence of VAD. For example,

the increases in IgG_{2b} AFCs in the lung and MLN, despite small cell numbers, were each statistically significant ($p < 0.05$ by Student's *t*-test for the values shown in Fig. 2), and reproducible in each of the four independent experiments. In total, the lung, CLN, and MLN exhibited relatively few AFCs, but there was nonetheless a bias against IgA in favor of IgG in VAD animals, as was the case for the ELISAs described in Fig. 1.

Discussion

The data described in this report revealed a significant impact of VAD on antibody and AFC responses, both in terms of cell residence and the balance of IgA:IgG production in respiratory tract tissues. IgA-producing cells and IgA were each reduced, while IgG-producing cells and IgG were enhanced.

These data are reminiscent of studies of gut-associated immune responses. VAD animals are known to exhibit decreased numbers of IgA-AFC in the small bowel. In fact, it was previously proposed that there was no other mucosal compartment for which IgA-AFC differentiation was dependent on RA (2). Studies have shown that RA can promote IgA switching with a reciprocal reduction of IgG in cooperation with IL-5 in tissue-cultured murine spleen cells (12,13), and that gut-associated DCs can induce IgA-AFC by a

FIG. 2. Altered IgA:IgG AFC balance in the URT and LRT of VAD mice. d-NALT (A), lung (B), CLN (C), and MLN (D), were sampled 1 mo after SeV vaccinations. Mouse samples from each group (3–5 mice per group) were pooled to ensure sufficient cell numbers for assay completion. The samples were tested in replicate. Plotted data represent averages and standard errors of scores. Four independent experiments yielded similar results.

process that is at least partially dependent on RA (14). However, it has also been noted that although gut-associated DCs are best known for RA production, extra-intestinal murine DCs can be triggered by cytokines to acquire RA-producing capacity (15), and that in some cases DCs may promote IgA expression in an RA-independent fashion (11). Clearly, the contribution of RA and of various DCs to the differentiation of IgA AFC in the intestine and in the respiratory tract remains a complex topic requiring additional study.

In conclusion, our results show that the respiratory tract AFCs and antibody responses are significantly altered by VAD, encouraging additional studies of resident cells, receptor-ligand interactions, and environmental cytokines (2,8). The results emphasize that VAD-induced AFC defects are not exclusive to the gut, and show that antibody responses in the URT and LRT are non-identical. Finally, our results question: (1) whether IN vaccinations are efficacious in the context of VAD, and (2) whether vitamin A supplementation, which is recommended as an adjunct to oral polio vaccine campaigns, might also benefit respiratory virus vaccine programs in the clinical arena (5).

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Author Disclosure Statement

No competing financial interests exist.

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