Vitamin A and D deficiencies and insufficiencies are prevalent worldwide in developed and developing countries. Vitamin metabolites are functionally intertwined in that they are high-affinity ligands for related receptors of the nuclear receptor superfamily. The effects of vitamin A deficiencies (VAD) on antibody responses to respiratory virus vaccines have already been demonstrated. Of particular concern was the reduction in IgA, a first line of defense against pathogens in the respiratory tract. Here, we describe the individual and combined effects of vitamin A and D deficiencies in mice immunized with an attenuated influenza virus vaccine. Relative to VAD, vitamin D deficiency (VDD) had a limited effect, but double deficiencies for vitamins A and D (VAD+VDD) further reduced antibody responses in the respiratory tract. The administration of supplemental vitamins A and D to VAD+VDD mice at the time of vaccination restored responses in a dose-dependent manner. Results suggest that vitamin supplementation programs may be beneficial in a clinical setting to promote healthy immune responses to respiratory virus vaccines in vitamin-deficient individuals.

Vitamin A deficiencies (VAD) and vitamin D deficiencies (VDD) have long been considered maladies in the developing world, but recent research demonstrates that children and adults of developed countries also suffer from vitamin deficits (1–4). Individuals with low vitamin levels are particularly vulnerable to infections of the gastrointestinal and respiratory tracts (5–8), the latter of which are responsible for one-fifth of all deaths among children under the age of five (1, 9).

Vitamin A is ingested in the form of retinyl esters or beta-carotene. It is stored in the liver as an ester and is transported through the circulatory system as retinol bound to the retinol binding protein (RBP) (10). Upon cellular uptake, retinol is converted to retinal by ubiquitous alcohol dehydrogenases, but further conversion to retinoic acid (RA), an active metabolite of vitamin A, requires specialized aldehyde dehydrogenases (ALDH1A). ALDH1A enzymes are expressed by a subset of cells, including gut dendritic cells and epithelial cells lining the upper and lower respiratory tracts (URT and LRT) (5, 8). Once converted from retinol to RA, vitamin A in the form of all-trans-RA can be spontaneously isomerized to 9-cis-RA. The two metabolites function as high-affinity ligands, respectively, for the retinoic acid receptor (RAR) and retinoid X receptor (RXR) proteins (11, 12). RAR and RXR are members of the nuclear receptor superfamily. These exist as monomers, homodimers, and heterodimers. DNA motifs for RAR-RXR binding (known as retinoic acid response elements [RARE]) are typically direct repeats of half sites puG(G/T)TCA separated by 2 or 5 nucleotides. RA binding to RAR-RXR (and related heterodimeric receptors) can have profound effects on immune cell development, activation, homing, and function (5, 12–14).

Vitamin D can be synthesized by the body in addition to being obtained through dietary sources. Cholecalciferol (vitamin D$_3$) and ergocalciferol (vitamin D$_2$) are acquired through the diet, but cholecalciferol is also produced photochemically from 7-dehydrocholesterol in the skin. Cholecalciferol is converted to calcidiol [also named 25-hydroxyvitamin D or 25(OH)D$_3$] by vitamin D-25-hydroxylase and is then converted by 25-hydroxyvitamin D-1α-hydroxylase to calcitriol [also named 1,25-dihydroxyvitamin D or 1,25(OH)$_2$D$_3$], an active metabolite (15, 16).

Vitamin A and D metabolites are highly interactive. Calcitriol binds the vitamin D receptor (VDR), which like RAR can heterodimerize with RXR (17). The same DNA binding motifs described for RAR-RXR [puG(G/T)TCA] can be recognized by the VDR-RXR complex, but half sites are often separated by 3 nucleotides (17) rather than 2 or 5. Receptor proteins can bind promiscuously to ligands and to noncanonical DNA motifs. In some instances, vitamin receptors (and related members of the nuclear receptor superfamily) behave synergistically, but in other instances, they antagonize one another (e.g., vitamin D exerts a negative effect on the vitamin A-dependent commitment of bone marrow cells to the granulocytic lineage [13, 17–20]). Complex influences of vitamins A and D thus depend on the immediate environment of target genes.

Using a mouse model for vitamin A deficiency (VAD), we previously demonstrated that the IgA immune response to respiratory virus vaccines is significantly impaired in the absence of vitamin A (21–23). There was a loss of mucosal, virus-spe-
specific IgA-producing antibody-forming cells (AFCs) and IgA antibody responses. IgG responses were less susceptible to change compared to IgA and were in some cases higher in VAD mice than in controls, resulting in an overall decrease in the IgA/IgG ratio. Supplementation of VAD mice with vitamin A at the time of vaccination rescued the IgA response (23, 24). These results have important clinical implications, as mucosal IgA may provide a first line of defense against infectious respiratory virus diseases in humans (25–27). Although it is not proven, it is possible that individuals with low vitamin levels may respond poorly to influenza virus vaccines in the clinical setting (28–30) and that they may benefit from vitamin supplementation.

Because the functions of vitamins A and D are related (31), we questioned whether double deficiencies for vitamins A and D would exacerbate impaired IgA responses to an influenza virus vaccine in mice. To do so, we developed animal models for vitamin D deficiency (VDD) and double deficiency (VAD + VDD). Experiments presented here show that VAD + VDD mice were more impaired than VAD or VDD mice in immune responses to the influenza virus vaccine. Moreover, we found that vaccine-induced immune responses can be corrected if vitamins A and D are administered to VAD + VDD mice at the time of vaccination.

MATERIALS AND METHODS

Animal model. Animal care practices followed the Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC). To establish vitamin-deficient mice, pregnant female C57BL/6 (H-2b) mice (day 4 to 5 of pregnancy) from Jackson Laboratories (Bar Harbor, ME) were placed on characterized diets (Harlan Laboratories, Madison, WI) in filter-top cages in a biosafety level 2 containment area. The VAD diet (Harlan catalog no. TD.10762) contained casein, α-methionine, sucrose, corn starch, corn seed oil, cellulose, mineral mix AIN-76 (170815), calcium carbonate, vitamin mix (lacking vitamin A) plus choline, and food coloring. The VDD diet was matched but lacked vitamin D rather than vitamin A (Harlan TD.10763). The control diet included vitamin A palmitate at 15 IU/g and vitamin D at 1.5 IU/g (Harlan TD.10764). The vitamin A + D double-deficient diet was Harlan catalog no. TD.10616. For animals depleted of vitamin D (VDD or VAD + VDD), cages were placed in dedicated cubicles with light-emitting diode (LED) bulbs as the source of light to avoid UV-B irradiation.

Pregnant females were maintained on diets throughout their pregnancies and weaned pups were maintained on diets throughout maturation and experimentation. Male and female pups were used. To confirm vitamin depletion, serum samples from vitamin-deficient animals were spot checked for RBP, a surrogate for retinol, by enzyme-linked immunosorbent assay (ELISA) (R&D Systems Mouse RBP4 catalog no. MRBP40, lot no. 327687). Tests were also conducted to measure vitamin D (Michigan University Diagnostic Center for Population and Animal Health [DCPAH], Lansing, MI). All tested mice that were expected to be deficient in vitamin A had RBP levels of <5,000 ng/ml, while levels in control animals were >10,000 ng/ml. All tested mice that were expected to be deficient in vitamin D had serum levels of <15 nmol/liter, while levels in control mice were >50 nmol/liter (32).

Adult mice of >6 weeks were used for experimentation. All experiments were repeated to ensure reproducibility. Mice were anesthetized with Avertin and were administered 30 μl of a cold-adapted derivative of influenza virus A/Puerto Rico/8/34 (PR8; kindly provided by J. McCullers) by the intranasal (i.n.) route. Virus was prepared by amplification in hen eggs at 33°C. Virus vaccine was at a concentration of 1.25 × 10^7 50% egg infective dose (EID<sub>50</sub>/ml (33, 34). Unvaccinated mice served as negative controls. Groups of vaccinated test mice received (i) no vitamin supplement, (ii) vitamin A supplements comprising retinyl palmitate (NutriSorb A; InterPlexus Inc., Kent, WA) at various doses, and/or (iii) liquid vitamin D<sub>3</sub> (cholecalciferol; Pure Encapsulations, Sudbury, MA). Dosing was by oral gavage on day 0 (1× regimen) or on days 0, 3, and 7 (3× regimen) relative to vaccination. Approximately 1 month after vaccination, mice were tested for influenza virus-specific antibody-forming cells (AFCs) or antibodies.

Tissue isolation. Mice were sacrificed with Avertin and exsanguination, confirmed by cervical dislocation. Nasal wash (NW) samples were collected by inserting catheters into the trachea and nasal cavity with 200 μl of phosphate-buffered saline (PBS). Bronchoalveolar lavage (BAL) samples were collected by inserting catheters into the trachea and washing three times with 1 ml PBS.

To isolate nasal mucosa-associated lymphoid tissue (NALT) and lungs, mice were first perfused with 5 ml PBS injected through the retro-orbital sinus. NALT was isolated by removing skin, lower jaws, muscles, cheek bones, and incisors. Remaining snouts were cut into small pieces. Lungs were minced with a razor blade. NALT and lungs were then digested with 4 mg/ml collagenase in PBS at 37°C for 30 min. Lymphocytes were isolated on 75%/40% Percoll gradients as described previously (2,000 rpm in a Beckman Coulter Allegra X-14R centrifuge for 30 min at 25°C) (35–38) and were washed prior to testing.

ELISA. Vaccine-induced antibodies in NW, BAL fluid, and serum samples from individual mice were tested with an influenza virus-
specific ELISA. Wild-type PR8 influenza virus was produced in eggs
and was purified by sucrose gradient sedimentation. Purified influenza
virus was lysed in disruption buffer (0.05% Triton X-100, 60 mM KCl,
10 mM Tris, pH 7.8) for 5 min at room temperature and was diluted
with PBS to 10 μg/ml, a stock used for coating 96-well ELISA micro-
titer plates. Plates were blocked with PBS containing 1% bovine serum
albumin (BSA), after which serially diluted test samples were applied.
After overnight incubation at 4°C, plates were washed with PBS-
Tween 20 (0.05%). Assay mixtures were next incubated with alkaline
phosphatase-conjugated goat anti-mouse IgA (catalog no. 1040-04;
Southern Biotech, Birmingham, AL) or goat anti-mouse IgG (IgM and
IgA absorbed; catalog no. 1030-04; Southern Biotech) for 1 h at room
temperature. Assays were developed with p-nitrophenyl phosphate
(catalog no. N2640; Sigma-Aldrich), and optical density measure-
ments were taken at 405 nm. Scores in unvaccinated negative-control
mice or in control ELISAs with plates coated only with BSA and no
virus were <0.05.

**ELISPOT assay for AFCs.** AFCs from NALT and lungs were tested by
enzyme-linked immunosorbent spot (ELISPOT) assay. Tissues from each
group of mice were pooled and tested in multiple wells. Plates (Multi-
Screen-IP, catalog no. MAIPS4510; Millipore, Billerica, MA) were coated
with purified wild-type PR8 influenza virus at 10 μg/ml with 100 μl/well.

![Graphs showing antibody forming cells](image-url)
Plates were incubated for 3 h at 37°C. Wells were next washed with PBS and blocked with a medium containing 10% fetal calf serum (FCS) for 1 h at 37°C. Cells were then applied to the wells (1.25 × 10^4 to 1 × 10^5 cells/well) and were incubated for 3 h at 37°C. After washing the plates with PBS 4 times and then PBS-Tween 20 4 times, alkaline phosphatase-conjugated goat anti-mouse IgA or goat anti-mouse IgG (IgM and IgA absorbed, as above) was added in PBS-Tween 20 with 1% BSA. After overnight incubation at 4°C, plates were developed with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) substrate (catalog no. B5655; Sigma-Aldrich). Plates were rinsed with water, and spots were counted using a Nikon dissecting scope. Background AFCs with tissues from unvaccinated mice had a value of ≤3 AFCs/100,000 cells.

**RESULTS**

VAD, VDD, VAD + VDD, and control mice were vaccinated with a cold-adapted influenza virus for comparisons of immune responses. After 1 month, NALT and lungs were evaluated by ELISPOT assay to measure responses in the URT and LRT, respectively. As shown in Fig. 1, the VDD mice were more similar to controls than were other test groups, but AFC levels were significantly lower in the NALT of VDD mice compared to those of controls. VAD animals, as described previously, exhibited poor IgA AFC in NALT and in lungs. IgG responses in VAD animals were affected to a lesser extent than IgA responses (and were in some cases improved compared to controls), resulting in a decrease in the IgA/IgG ratio. Vitamin A was the dominant regulator, but VAD + VDD animals were the most impaired.

We next asked if weak resident antibody responses in the respiratory tracts of VAD + VDD animals could be corrected with vitamin supplementation. We dosed VAD + VDD mice orally with vitamin D, vitamin A, or both on days 0, 3, and 7 relative to vaccination (Fig. 2). Doses were 600 IU retinyl palmitate and/or 40 IU vitamin D. Vitamin A was again the dominant regulator, particularly for IgA AFC re-

![Graph showing correction of AFCs with different vitamin dosing regimens.](http://cvi.asm.org/)
sponses. The best improvements were in VAD+VDD animals that received vitamins A and D as supplements. The degree of correction varied among experiments, and in some experiments, responses in vitamin-supplemented VAD+VDD mice exceeded those of controls.

In a clinical setting, administering vitamin supplements more than once poses logistical difficulties, and very high doses of vitamins, although recommended for use in young children by the World Health Organization (39), can be toxic (40). We therefore asked if a mixture of vitamins A and D could be administered just once on day 0 rather than three times on days 0, 3, and 7 and if the doses of vitamins could be reduced. We tested groups of VAD+VDD animals with the following regimens of vitamin supplementation: (i) 6 IU vitamin A plus 4 IU vitamin D on day 0 (termed 6/4 1X and most comparable to the recommended daily dose of vitamins by weight in clinical pediatrics [41]), (ii) 60 IU vitamin A plus 40 IU vitamin D on day 0 (termed 60/40 1X), (iii) 600 IU vitamin A plus 40 IU vitamin D on day 0 (termed 600/40 1X), and (iv) 600 IU vitamin A plus 40 IU vitamin D on days 0, 3, and 7 (termed 600/40 3X). Vitamin formulations in mice were tested with an understanding that the vitamin A content in a standard laboratory mouse diet is more than 10-fold that recommended for humans by weight (41) and that when vitamin A content is reduced in the C57BL/6 mouse diet, there is a significant fall in retinol equivalents in the kidney (32).

As shown in Fig. 3, the 600/40 1X and 600/40 3X regimens each sufficed to enhance IgA and IgG responses in NALT and lungs following vaccination with the cold-adapted influenza virus vaccine. In some cases, responses in supplemented animals exceeded those of controls. When smaller amounts of vitamins were tested in VAD+VDD groups, we found that the lowest dose (6/4 1X) was sufficient to correct IgG AFC but not IgA AFC in the NALT and lungs. Altogether, results demonstrated that vitamin A and D supplements administered with influenza virus vaccination assisted vaccine-induced immune responses in VAD+VDD animals in a dose-dependent manner and that IgG was easier to correct than IgA.

To determine if the virus-specific AFC results predicted virus-specific antibody responses in VAD+VDD animals, we measured antibodies in the serum samples, NW, and BAL fluid of test and control mice. As shown in Fig. 4A, IgG responses in serum samples were not significantly impaired in VAD+VDD animals compared to those in controls, which is similar to the situation for VAD animals described previously (42). IgA antibody levels, like IgA AFC levels, were significantly reduced in VAD+VDD animals compared to those in controls (Fig. 4B and D). However, unlike the situation for AFC levels, the IgG antibody levels in NW and BAL fluid did not drop significantly in VAD+VDD mice (Fig. 4C and E). This is likely because serum IgG is readily transmitted to the respiratory tract and particularly the LRT (43, 44).

Figure 5 shows antibody results following vitamin supplementation of VAD+VDD mice. Similar to the situation for AFC (Fig. 3), the lowest dose of vitamins (6/4) heightened IgG responses better than IgA responses in the VAD+VDD animals. Higher vitamin doses were required for full correction of IgA. Upper (NW) and lower (BAL fluid) respiratory tract antibodies were improved and sometimes exceeded the levels of controls. The highest dose of vitamins, administered one or three times, yielded the highest antibody levels in URT and LRT secretions.

To determine how vitamin-deficient mice would fare following a lethal challenge with wild-type influenza virus after vaccination, we vaccinated animals, rested animals for at least 3 weeks, and then infected animals with wild-type PR8. We found that some vaccinated control mice lost weight after this challenge but then cleared the virus and recovered prechallenge weight (to a level of ≥90%) within a 2-week time period. The majority of mice in the VAD+VDD group also cleared the virus (not shown) and recovered ≥90% prechallenge weight by day 14 (Fig. 6A). In one experiment (experiment 1), there were more deaths among VAD+VDD mice compared to those among controls (Fig. 6A and B). Overall, there were fewer VAD+VDD animals that recovered ≥90% of their weight by day 14 postchallenge compared to controls. Subtlest in group differences likely reflected the retention by vitamin-deficient mice of serum IgG, which may have assisted in virus clearance (43, 44).
DISCUSSION

Here we describe three mouse models of vitamin deficiencies to compare mucosal and systemic immune responses generated against an i.n. cold-adapted influenza virus vaccine. Results revealed a significant impairment of vaccine-induced respiratory antibody responses in VAD/H11001-VDD mice. Vitamin A was the dominant regulator, but responses were lowest when animals were deficient for vitamins A and D. The AFC responses in the URT and LRT were not entirely predictive of antibody responses, and the loss of IgG was less extreme than the loss of IgA. This was likely because serum IgG antibody was sustained in vitamin-deficient animals and may have been transmitted to URT and LRT tissues (43, 44). The serum antibody activities also may have assisted in protection, as vaccinated VAD+VDD animals were usually, but not always, protected from virus challenge. Overall, a return to ≥90% prechallenge weight was less frequent in VAD+VDD animals than it was in controls. We further showed that if VAD+VDD animals were supplemented with vitamins A and D at the time of vaccination, influenza virus-specific responses could be restored. The vitamin levels required for correction of antibody responses were lower for IgG than for IgA. Supplementation with vitamin A had a greater corrective effect than supplementation...
with vitamin D, and best results were with the two vitamins combined.

How do vitamins influence antibody production? We have observed in an in vitro system of B cell activation that interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are upregulated in the presence of vitamin A and that IgA production is dependent upon these cytokines (45). Similarly, vitamin A has been shown to enhance transforming growth factor β (TGF-β), which triggers IgA production by activated B cells (46). Mechanisms of vitamin influences on the immune response are complex, as cytokine production, innate immune cell activation, cell integrity, antigen presentation, and lymphocyte cell homing to mucosal surfaces are all affected by vitamin levels (5). We have recently discovered hot spots for vitamin receptor DNA binding motifs in the switch (S) regions of murine and human immunoglobulin heavy chain genes (particularly Sμ, Sε, and So, but rarely Sγ), which suggests that there may be direct and indirect influences of vitamins on class switch recombinations (CSR) and on B cell antibody isotype expression patterns (47). Hot spots for DNA binding motifs of related nuclear receptors (e.g., estrogen receptors) are also found in S regions, perhaps explaining, in part, the complexity of vitamin influences on their target genes (e.g., males and females respond differently to vitamin supplementation [48]).

Vitamins A and D are each known for their positive effects on the human immune response to viral disease (6, 29, 49). In a recent clinical study, we found that vitamin insufficiencies or deficiencies were prevalent in an inner city of the United States and that vitamin A (measured using RBP as the surrogate [10]) correlated with serum IgA, serum IgG4, and influenza virus-specific neutralizing antibodies; vitamin D was associated with serum IgM and IgG3 (50). A clinical study of vitamin levels or supplements in the context of an i.n. respiratory virus vaccine has never been performed. Previous studies have exhibited benefits of vitamin supplementation in the context of other vaccination programs, but results have been inconsistent (30,51, 52). This may be because (i) previous clinical studies have focused largely on vitamin A or vitamin D without consideration of cross-regulatory capacities, and (ii) vitamin supplementation studies were often conducted without measurement of incoming vitamin levels; some participants may have been vitamin replete and negatively impacted by high vitamin doses (48,53). With these caveats in mind, our results and previous literature encourage careful clinical tests of vitamin A and D supplements in vitamin insufficient and deficient recipients of i.n. influenza virus vaccines.

Though our mouse studies are informative, the selection of a best dose for vitamin supplementation in humans will require empirical testing. Benefits and risks must be contemplated upon clinical trial design (48,54), as an overzealous immune response in the respiratory tract, particularly if comprising T cell responses in the absence of neutralizing antibodies, can lead to a cytokine storm and obstructed airways (55). It is unlikely that the doses described in mice can be directly translated to humans, in part because a normal mouse diet (as was received by control animals in our experiments) contains more than 10 times the recommended daily allowance in humans by weight (41). Our mouse

### Table: Mice with ≥90% pre-challenge weight by day 14 post-challenge

| Group       | Mice with ≥90% pre-challenge weight by day 14 post-challenge |
|-------------|-------------------------------------------------------------|
| Expt        | Expt 1 | Expt 2 | Expt 3 | Total |
| Control     | 9/9 (100%) | 10/10 (100%) | 8/9 (89%) | 27/28 (96%) |
| VAD         | ND     | 6/6 (100%) | ND     | 6/6 (100%) |
| VDD         | ND     | 6/8 (75%)  | ND     | 6/8 (75%)  |
| VAD+VDD     | 1/4* (25%) | 7/8 (88%)  | 4/6 (67%) | 12/18* (67%) |
| VADD+VDD+VitA+VitD | 4/5 (80%) | ND     | 2/2 (100%) | 6/7 (86%) |

**FIG 6** Influenza virus challenge of vaccinated mice. (A) Animals were vaccinated, rested for at least 3 weeks, and then challenged with wild-type influenza virus in three experiments (expt). In two experiments, one group of VAD+VDD mice received no vitamin supplements and one group received supplements with vitamin A (600 IU) and vitamin D (40 IU) on days 0, 3, and 7 relative to vaccination. The numbers of mice per group that experienced recovery of at least 90% prechallenge weight by 14 days postchallenge are shown. ND, not done. An asterisk indicates groups that were significantly different from controls using Fisher’s exact test. (B) Weights (percentage based on prechallenge weight) are shown per day for one experiment after vaccination and challenge of wild-type (n = 9) and VAD+VDD (n = 4) mice.
model also differs from humans in that VAD+VDD mice were deprived of all dietary vitamin A and vitamin D from day 5 gestation until maturity, whereas humans, even in cases of severe deficiencies, might receive some level of vitamins. Despite these recognized differences, mouse data instruct clinical trial design by showing that (i) supplements administered on day 0 alone (or on days 0, 3, and 7) were sufficient to support significant recovery of IgG and IgA responses, and (ii) greater improvements in IgA and IgG responses were observed with increasing doses of vitamins A and D. Supplements may ultimately improve responses to influenza virus vaccines in children and adults who are vitamin deficient and may ultimately improve defenses against respiratory viral disease.

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REFERENCES

1. Sommer A, Tarwojto I, Hussaini G, Susanto D. 1983. Increased mortality in children with mild vitamin A deficiency. Lancet ii:585–588.
2. Merewood A, Mehta SD, Grossman X, Chen TC, Mathieu J, Holick MF, Sommer A, Tarwojto I, Hussaini G, Susanto D. 2013. Vitamin A and vitamin D. Supplements may ultimately improve defenses against respiratory viral disease.
3. Stephens D, Jackson PL, Gutierrez Y. 2010. Vitamin D and The Magic Mountain: the anti-infectious role of the vitamin. J Pediatr Infect Dis 31:331–333.
4. Clements ML, Betts RF, Tierney EL, Murphy BR. 1986. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J Clin Microbiol 24:157–160.
5. Sealy R, Jones BG, Sealy RE, Hurwitz JL. 2014. Intranasal administration of retinol palmitate with a respiratory virus vaccine corrects impaired mucosal IgA response in vitamin A-deficient mice. Vaccine 32:2521–2524. http://dx.doi.org/10.1016/j.vaccine.2014.03.025.
6. Surman SL, Jones BG, Sealy RE, Hurwitz JL. 2012. Vitamin A deficiency: a potentially unrecognized problem in the United States. Pediatr Infect Dis J 31:1003–1007. http://dx.doi.org/10.1093/pidj/djs641.
7. Block SL, Falloon J, Hirschfeld JA, Krilov LR, Dubovsky F, Yi T, Belshe RB. 2011. Immunogenicity and safety of a quadrivalent live attenuated influenza virus vaccine in vitamin A deficient mice. Vaccine 29:2521–2524. http://dx.doi.org/10.1016/j.vaccine.2014.03.025.
8. Barria MJ, Garrido JL, Stein C, Scher E, Ge Y, Engel SM, Kraus TA, Banach D, Moran TM. 2013. Localized mucosal response to intranasal live attenuated influenza vaccine in adults. J Infect Dis 207:115–124. http://dx.doi.org/10.1093/infdis/jits570.
9. Barria MJ, Garrido JL, Stein C, Scher E, Ge Y, Engel SM, Kraus TA, Banach D, Moran TM. 2013. Localized mucosal response to intranasal live attenuated influenza vaccine in adults. J Infect Dis 207:115–124. http://dx.doi.org/10.1093/infdis/jits570.
10. Sommer A, Katz J, Tarwojto I. 1984. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 40:1090–1095.
11. Almekinder J, Manda W, Soko D, Lan Y, Hoover DR, Semba RD. 2000. Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations. scand J Clin Lab Invest 60:199–203. http://dx.doi.org/10.1080/003655150700344848.
12. Kane MA. 2012. Analysis, occurrence, and function of 9-cis-retinoid acid. Biochim Biophys Acta 1821:10–20. http://dx.doi.org/10.1016/j.bbalip.2011.09.012.
13. Huang P, Chandra V, Rastinejad F. 2014. Retinoid acid actions through mammalian nuclear receptors. Chem Rev 114:233–254. http://dx.doi.org/10.1021/cr400161b.
14. Al Tanoury Z, Piskunov A, Rochele-Egly C. 2013. Vitamin A and retinoid signaling: genomic and nongenomic effects. J Lipid Res 54:1761–1775. http://dx.doi.org/10.1194/jlr.R030833.
15. Boergesen M, Pedersen TA, Gross B, van Heeringen SJ, Hagenbek D, Bindessboll C, Caron S, Lallooy F, Steffensen KR, Neby HH, Gustafson JA, Stunkenberg HG, Staels B, Mandrup S. 2012. Genome-wide profiling of liver X receptor, retinoid X receptor, and peroxisome proliferator-activated receptor alpha in mouse liver reveals extensive sharing of binding sites. Mol Cell Biol 32:852–867. http://dx.doi.org/10.1128/MCB.01675-11.
16. Norman AW. 2008. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 88:5491–5499.
17. Holick MF. 2007. Vitamin D deficiency. N Engl J Med 357:266–281. http://dx.doi.org/10.1056/NEJMra070533.
18. Carlson C, Bendik J, Wysa A, Meier E, Sturzenbecker LJ, Grippo JF, Hunziker W. 1993. Two nuclear signalling pathways for vitamin D. Nature 361:657–660. http://dx.doi.org/10.1038/361657a0.
19. Bastie JN, Baltritard N, Guidiez F, Guillomet I, Larghero J, Calabresse C, Chomienne C, Delva L. 2004. 1 alpha,25-dihydroxyvitamin D3 transrepresses retinoic acid transcriptional activity via vitamin D receptor in myeloid cells. Mol Endocrinol 18:2685–2699. http://dx.doi.org/10.1210/me.2003-0412.
20. Allenby G, Janocha R, Kazmer S, Speck J, Grippo JF, Levin AA. 1994. Binding of 9-cis-retinoic acid and all-trans-retinoic acid to retinoic acid receptors alpha, beta, and gamma. Retinoic acid receptor gamma binds all-trans-retinoic acid preferentially over 9-cis-retinoic acid. J Biol Chem 269:16689–16695.
21. Surman SL, Jones BG, Sealy RE, Hurwitz JL. 2012. Oral retinol palmitate or retinoic acid corrects mucosal IgA responses toward an intranasal influenza virus vaccine in vitamin A-deficient mice. Vaccine 30:2521–2524. http://dx.doi.org/10.1016/j.vaccine.2011.10.017.
22. Burd and evidence for subclinical deficiency. Curr Opin Gastroenterol 1984. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 40:1090–1095.
23. Sommer A, Katz J, Tarwojto I. 1984. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 40:1090–1095.
24. Almekinder J, Manda W, Soko D, Lan Y, Hoover DR, Semba RD. 2000. Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations. scand J Clin Lab Invest 60:199–203. http://dx.doi.org/10.1080/003655150700344848.
25. Kane MA. 2012. Analysis, occurrence, and function of 9-cis-retinoid acid. Biochim Biophys Acta 1821:10–20. http://dx.doi.org/10.1016/j.bbalip.2011.09.012.
26. Huang P, Chandra V, Rastinejad F. 2014. Retinoid acid actions through mammalian nuclear receptors. Chem Rev 114:233–254. http://dx.doi.org/10.1021/cr400161b.
27. Al Tanoury Z, Piskunov A, Rochele-Egly C. 2013. Vitamin A and retinoid signaling: genomic and nongenomic effects. J Lipid Res 54:1761–1775. http://dx.doi.org/10.1194/jlr.R030833.
vitamin D in normal Biozzi and C57BL/6 mice and during the course of chronic relapsing experimental autoimmune encephalomyelitis (CR EAE). Inflamm Res 62:569–667. http://dx.doi.org/10.1007/s00218-008-0161-4.

33. Jin H, Zhou H, Lu B, Kemble G. 2004. Imparting temperature sensitivity and attenuation in ferrets to A/Puerto Rico/8/34 influenza virus by transferring the genetic signature for temperature sensitivity from cold-adapted A/Ann Arbor/6/60. J Virol 78:995–998. http://dx.doi.org/10.1128/JVI.78.2.995-998.2004.

34. Huber VC, Thomas PG, McCullers JA. 2009. A multi-valent vaccine approach that elicits broad immunity within an influenza subtype. Vaccine 27:1192–1200. http://dx.doi.org/10.1016/j.vaccine.2008.12.023.

35. Lefrancois L, Lycke N. 2001. Isolation of mouse small intestinal intraepithelial lymphocytes, Peyer’s patch, and lamina propria cells. Curr Protoc Immunol 17:3.19.1–3.19.16.

36. Zhang J, Dong Z, Zhou R, Luo D, Wei H, Tian Z. 2005. Isolation of lymphocytes and their innate immune characterizations from liver, intestine, lung and uterus. Cell Mol Immunol 2:271–280.

37. Kuper CF, Koonstra PJ, Hameleers DM, Biewenga J, Sminia T. 1992. The role of nasopharyngeal lymphoid tissue. Immunol Today 13:219–222. http://dx.doi.org/10.1016/1044-7313(92)90158-4.

38. Liang B, Hyland I, Hou S. 2001. Nasal-associated lymphoid tissue is a site of long-term virus-specific antibody production following respiratory virus infection of mice. J Virol 75:5416–5420. http://dx.doi.org/10.1128/JVI.75.11.5416-5420.2001.

39. Palmer AC, West KP, Jr, Dalmiya N, Schultink W. 2012. The use and interpretation of serum retinol distributions in evaluating the public health impact of vitamin A programmes. Public Health Nutr 15:1201–1215. http://dx.doi.org/10.1017/S136894621000560.

40. Penniston KL, Tanumihardjo SA. 2006. The acute and chronic toxic effects of vitamin A. Am J Clin Nutr 83:191–201.

41. Bendich A, Langseth L. 1989. Safety of vitamin A. Am J Clin Nutr 49: 358–371.

42. Rudraraju R, Surman SI, Jones BG, Sealy R, Woodland DL, Hurwitz JL. 2012. Reduced frequencies and heightened CD103 expression among virus-induced CD8 T cells in the respiratory tract airways of vitamin A-deficient mice. Clin Vaccine Immunol 19:757–765. http://dx.doi.org/10.1128/CVI.05576-11.

43. Prince GA, Horswood RL, Chanock RM. 1985. Quantitative aspects of passive immunity to respiratory syncytial virus infection in infant cotton rats. J Virol 55:517–520.

44. Renegar KB, Small PA, Jr, Boykins LG, Wright PF. 2004. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. J Immunol 173:1978–1986. http://dx.doi.org/10.4049/jimmunol.173.3.1978.

45. Rudraraju R, Jones BG, Surman SI, Sealy RE, Thomas PG, Hurwitz JL. 2014. Respiratory tract epithelial cells express retinaldehyde dehydrogenase ALDH1A1 and enhance IgA production by stimulated B cells in the presence of vitamin A. PLoS One 9(1):e86554. http://dx.doi.org/10.1371/journal.pone.0086554.

46. Tokuyama H, Tokuyama Y. 1993. Retinoids enhance IgA production by lipopolysaccharide-stimulated murine spleen cells. Cell Immunol 150:353–363. http://dx.doi.org/10.1006/cimm.1993.1203.

47. Hurwitz JL, Penkert RR, Xu B, Fan Y, Partridge JF, Maul RW, Gearhart PJ, J 7 January 2016. Hot spots for vitamin-steroid-thyroid hormone response elements within the switch regions of immunoglobulin heavy chain loci predict a direct influence of vitamins and hormones on B cell class switch recombination. Viral Immunol http://dx.doi.org/10.1089/vim.2015.0104.

48. Fisker AB, Bale C, Jorgensen MJ, Balde I, Hornshoj L, Bibby BM, Aaby P, Benn CS. 2013. High-dose vitamin A supplementation administered with vaccinations after 6 months of age: sex-differential adverse reactions and morbidity. Vaccine 31:3191–3198. http://dx.doi.org/10.1016/j.vaccine.2013.04.072.

49. Camargo CA, Jr, Gannaa D, Frazier AL, Kirchberg FF, Stuart JJ, Kleinman K, Sumberzul N, Rich-Edwards JW. 2012. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. Pediatrics 130:e561–e567. http://dx.doi.org/10.1542/peds.2011-3029.

50. Jones BG, Oshansky CM, Bajracharya R, Tang L, Sun Y, Wong SS, Webby R, Thomas PG, Hurwitz JL. 2016. Retinol binding protein and vitamin D associations with serum antibody isotypes, serum influenza virus-specific neutralizing activities and airway cytokine profiles. Clin Exp Immunol 183:239–247. http://dx.doi.org/10.1111/cei.12718.

51. Benn CS, Aaby P, Bale C, Olsen J, Michaelsen KF, George E, Whittle H. 1997. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, West Africa. Lancet 350:101–105. http://dx.doi.org/10.1016/S0140-6736(96)21009-6.

52. Ross AC. 2007. Vitamin A supplementation and retinoic acid treatment in the regulation of antibody responses in vivo. Vitam Horm 75:197–222. http://dx.doi.org/10.1016/S0083-6729(06)75008-7.

53. Benn CS, Fisker AB, Dines BR, Aaby P. 2006. Neonatal vitamin a supplementation: sex-differential effects on mortality? J Infect Dis 194: 719. http://dx.doi.org/10.1086/506457.

54. de Francisco A, Chakraborty J, Chowdhury HR, Yunus M, Baqui AH, Siddique AK, Sack RB. 1993. Acute toxicity of vitamin A given with vaccines in infancy. Lancet 342:526–527. http://dx.doi.org/10.1016/S0140-6736(93)91648-6.

55. Prince GA, Jenson AB, Hemming VG, Murphy BR, Walsh EE, Horswood RL, Chanock RM. 1986. Enhancement of respiratory syncytial virus pulmonary pathology in cotton rats by prior intramuscular inoculation of formalin-inactivated virus. J Virol 57:721–728.