High-Level Dietary Vitamin A Enhances T-Helper Type 2 Cytokine Production and Secretory Immunoglobulin A Response to Influenza A Virus Infection in BALB/c Mice

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ABSTRACT Vitamin A supplementation during acute pneumonia has not improved recovery in most human clinical trials. We hypothesize that high vitamin A intake may decrease the production of T-helper type-1 (Th1) cytokines and thereby inhibit antiviral responses. Such decreases might impair recovery from viral respiratory infections. We thus examined the effect of three interventions on viral pneumonia: 1) a high level vitamin A [250,000 IU/kg diet or 75,000 retinol equivalents (RE)/kg], or 2) control diet (4000 IU/kg diet or 1200 RE/kg) given before and during infection, and 3) initiating the high level diet upon infection to simulate the adjuvant therapy used in clinical trials. No difference was seen among the interventions in severity of disease (weight loss, lung virus titers and survival). However, both the high level diet group and the group in which vitamin A was increased at the time of infection had greater salivary immunoglobulin (Ig)A responses (geometric means, 166 and 105 μg/L, respectively) than did the control group (59 μg/L) (P = 0.0019). In contrast, the serum IgG response was higher in the control group (324 ± 158 mg/L) than in the high level group (225 ± 95 mg/L) (P = 0.028), although it did not differ from the group in which the diet was changed upon infection (230 ± 163 mg/L) (P = 0.084). The production of interferon-γ (IFN-γ), a Th1 cytokine, was lower in the high level diet group (median, 0.153 μg/L) compared with the control group (median, 0.839 μg/L) (P = 0.014), whereas the production of interleukin-10 (IL-10), a Th2 cytokine, was higher with the high level diet (median, 0.304 μg/L) than with the control (median, 0.126 μg/L) (P = 0.022). This change in the Th1/Th2 pattern was not sufficient to affect recovery from viral pneumonia but may account for the increased IgA and decreased IgG responses seen with high level dietary vitamin A in this study. These data reinforce the lack of utility of vitamin A in treating acute pneumonia in children and suggest that high dose vitamin A supplements may enhance Th2-mediated immune responses, which are particularly beneficial in the case of extracellular bacterial and parasitic infections and IgA-mediated responses to mucosal infections. J. Nutr. 130: 1132–1139, 2000.

KEY WORDS: vitamin A • pneumonia • influenza A virus • Mus musculus • immunoglobulin A • interleukin-10 • interferon-γ

Vitamin A deficiency is a major contributor to the high infectious disease mortality rates seen during early childhood in many areas of the developing world (Fawzi et al. 1993, Glasziou and Mackerras 1993). Mortality from diarrhea and measles is reduced by vitamin A supplementation, but pneumonia mortality is not affected (Glasziou and Mackerras 1993). This finding was surprising initially because children with xerophthalmia (clinical vitamin A deficiency) (Sommer et al. 1984) and biochemical evidence of vitamin A deficiency (serum retinol < 0.35 μmol/L) (Bloem et al. 1995) appear to be at increased risk of respiratory tract infection. However, in contrast to these observational studies on morbidity, placebo-controlled community intervention trials found that vitamin A supplements either had no effect on respiratory morbidity (Abdeljaber et al. 1991) or, in some cases, increased morbidity. For example, a trial in Indonesia found that supplements increased the prevalence of symptomatic respiratory infections, using cough plus rapid breathing as an indicator of disease (Dibley et al. 1996). Another community study showed a positive association between total dietary vitamin A intake and the incidence of cough (Fawzi et al. 1995), although these investigators hypothesized that cough alone might be viewed as a sign of a healthy respiratory epithelium (rather than increased morbidity) because higher vitamin A intake was associated with a lower prevalence of cough plus fever in the same subjects.

In addition to the community studies, many clinical trials have tested high level vitamin A supplements as adjuvant...
DIETARY VITAMIN A AND THE IMMUNOGLOBULIN A RESPONSE

therapy for pneumonia and other lower respiratory tract infections. Results from clinical trials have been mixed. Some studies have shown modest benefits (Nacul et al. 1997, Si et al. 1997), particularly among specific subgroups of children (e.g., those with underlying malnutrition) (Dowell et al. 1996, Si et al. 1997). Some have shown no effect (Fawzi et al. 1998, Kjolhede et al. 1995). Others have show some detrimental effects on recovery (Bresee et al. 1996, Stephensen et al. 1998). Plausibly, these differences may be due to a differential effect of vitamin A on the immune response to different respiratory pathogens. For example, the longer duration of hospitalization seen in U.S. infants with respiratory syncytial virus infection (Bresee et al. 1996) who received vitamin A suggests that high dose supplements may slow recovery from viral lower respiratory tract infections, at least in infants at low risk for underlying vitamin A deficiency.

One possible explanation for these findings is that high level vitamin A may decrease the production of T-helper type-1 (Th1) cytokines such as interferon-γ (IFN-γ). Such decreases might impair recovery from viral infections because Th1-mediated responses comprise the principal host defenses against intracellular pathogens, whereas Th2-mediated responses protect against extracellular pathogens (Abbas et al. 1996). Increased production of IFN-γ is seen during vitamin A deficiency in mice (Cantorna et al. 1994 and 1995, Carman and Hayes 1991). Conversely, treatment of mice with all-trans retinoic acid can increase the production of Th2 cytokines (Racke et al. 1995). Th1 and Th2 cells are cross-regulatory, in that promotion of a Th1 response against a virus, for example, will not stimulate and may inhibit effector mechanisms that are not part of the antiviral response [e.g., production of murine immunoglobulin (Ig)G1 or IgE]. Thus, if high level dietary vitamin A were to promote the production of Th2 cytokines, Th1-mediated antiviral responses could be diminished and thus might impair recovery from viral infections.

We previously found that vitamin A deficiency impairs both some aspects of recovery from influenza infection in mice (Stephensen et al. 1993) and the salivary IgA response, whereas it enhances the serum IgG response (Stephensen et al. 1996). The IgA response plays a crucial role in protecting the respiratory tract from infectious virus, causing upper respiratory infection as well as pneumonia. In this study, we examined the effect of high level dietary vitamin A on recovery from influenza A viral pneumonia in BALB/c mice. Our specific aims were to determine whether high level dietary vitamin A, sufficient to increase serum retinol concentration and liver stores of retinyl esters, would improve recovery from infection (as measured by survival, weight loss and lung virus titers), increase the IgA response and IL-10 production by lymphocytes from draining lymph nodes restimulated in vitro with influenza antigen, and diminish the serum IgG responses and IFN-γ production.

MATERIALS AND METHODS

Mice. Specific pathogen–free BALB/c breeders with litters <1 wk of age were purchased from Charles River (Raleigh, NC) and were kept in filter-top cages (Lab Products, Frederick, MD). Litters were balanced in size, and male mice were used in most experiments. One sex was used to eliminate sex-related differences in body weight comparison (used as a measure of severity of infection) and because hormone-mediated differences in the immune response might obscure differences in the immune response due to dietary manipulation by increasing the level of variation within the diet groups. However, cytokine production was examined in both male and female mice and significant differences were not seen between the sexes. Although female mice tended to have higher concentrations of both IFN-γ and IL-10, both male and female mice responded identically to the dietary treatments. Previous work from our laboratory has not found consistent differences in the IgA response to influenza A virus infection (Stephensen et al. 1993 and 1996). This work was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Diets. The breeders were fed normal, nonpurified diets (Purina, St. Louis, MO) and maintained with free access to water. Pups were weaned at 21 d of age to the same diet that was fed to the dams. Upon weaning, the pups were separated into three groups as follows: 1) one group was fed a diet containing retinyl palmitate at 250,000 RE/kg diet; 750,000 RE/kg diet beginning 4 wk before infection and continuing through the end of the experiment. This group was termed the high dose vitamin A group, or 250k group. The second group was fed a diet containing retinyl palmitate 4000 RE/kg (1200 RE/kg) for 4 wk before infection and continuing through the end of the experiment. This group is called the control group, or the 4k group. The control diet contained the amount of vitamin A recommended for growth or maintenance of rodents (Reeves 1997). 3) The third group was fed a diet containing retinyl palmitate 4000 RE/kg (1200 RE/kg) beginning 4 wk before infection. On the day of inoculation with influenza A virus, these mice were switched to the 250k diet for the remainder of the experiment. This group is referred to as the 4k/250k group. The basic composition of our diet has been reported previously (Stephensen et al. 1993). Mice in this study were given free access to the diets at all times.

Influenza infection. Mice were infected at 7 wk of age with either 12 or 25 plaque-forming units (PFU) influenza A virus by intranasal inoculation. The influenza virus strain, A/Philippines/2/82 (H3N2), was adapted to mice (Chen et al. 1987) and produces viral pneumonia with a low inoculum. It was provided by Dr. Ker-Seng Chen, U.S. Food and Drug Administration. Mice were lightly anesthetized with ketamine-xylazine (2 mg of ketamine and 0.3 mg xylazine/20 g body weight) and inoculated intranasally with influenza A virus diluted in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY) containing 1% fetal bovine serum (FBS) and 25 mmol/L HEPES buffer in a total volume of 25 μL. This method of inoculation exposes both the upper and lower respiratory tract to infectious virus, causing upper respiratory infection as well as pneumonia.

Experimental design. In Experiment 1, 48 mice (4k group, n = 16; 4k/250k group, n = 16; 250k group, n = 16) were inoculated with 12 PFU of influenza virus and were monitored for 28 d to examine survival and changes in body weight, serum retinol, salivary IgA and serum IgG. In Experiment 2, 28 mice (4k group, n = 8; 4k/250k group, n = 8; 250k group, n = 12) were inoculated with 25 PFU of influenza virus and were monitored for 28 d to examine survival and changes in body weight, serum retinol, salivary IgA and serum IgG. In Experiment 3, 48 mice (n = 16 in each of the three diet groups) were inoculated with 25 PFU of influenza virus; four mice from each of the three diet groups were killed on d 3, 6, 9 and 12 after infection to measure lung and trachea virus titers, serum retinol, liver retinyl esters, salivary IgA and serum IgG. In Experiments 4 and 5, 7-wk-old mice (n = 53; 26 fed a 250k diet and 27 fed a 4k diet for 4 wk) were infected with 12 PFU influenza A virus. Mice were killed on...
Cellular debris was pelleted by centrifugation at 1000 rpm for 10 min at 4°C. DMEM containing 1% FBS, 25 mmol/L HEPES and antibiotics was used to resuspend the debris. Lungs were dissected free and homogenized in 10 mL ice-cold PBS (carbachol, Sigma Chemical, St. Louis, MO). Blood was collected after subcutaneous stimulation with 250k diet) before infection (at 7 wk of age). Retinol concentrations were measured in the two initial diet groups (4k and 250k) 3 d after the 4k/250k mice were switched from the 4k diet to the 250k diet. The corresponding recombinant mouse cytokines were used to produce the standard curves. Flat-bottomed microtiter plates were coated with 50 μL (2 mg/mL coating buffer) of rat monoclonal antibody to murine IFN-γ or IL-10 and stored at 4°C overnight; 200 μL PBS with 10% fetal calf serum was added to each well and the plates were allowed to incubate for 2 h at room temperature to block nonspecific binding. Then, 100 μL of culture supernatant or 100 μL of the recombinant IFN-γ or IL-10 standard that had been diluted in twofold steps in PBS with 10% calf serum was added to each well and the plates were incubated overnight at 4°C. This was followed by the addition of 100 μL biotinylated anti-mouse IFN-γ (1 mg/L) or IL-10 (1 mg/L), which was allowed to stand for 1 h at room temperature; 100 μL of streptavidin-horseradish peroxidase (1:4000) was added and the mixture was allowed to stand for another hour. Plates were washed three to eight times with PBS containing 0.05% Tween 20 between each incubation. Finally, 100 μL of 3,3′,5,5′-tetramethylbenzidine substrate was added to each well and the reaction was stopped by adding 20 μL of 2 mol/L H2SO4. The plates were read at 450 nm. The levels of IFN-γ and IL-10 were determined from the standard curve and were expressed as micrograms per liter. The dynamic range of the standard curve for both cytokines was typically from 12.5 to 1600 ng/L. Within-assay CV in this portion of the curve ranged from 4% to 8%. Between-day variation for samples in this concentration range was typically 30%. All samples for a given experiment were measured on the same day.

Serum retinol. Serum retinol was measured by mixing 0.05 mL serum with 0.05 mL ethanol, extracting with 0.5 mL hexane, evaporating the hexane and reabsorbing in 0.05 mL ethanol. HPLC analysis was performed using a Nova-Pak C18 column (Waters, Rochester, MN) and a 50% acetonitrile, 5% methylene chloride mobile phase (Stacewicz-Sapuntzakis et al. 1987). Retinol peaks were detected by measuring absorbency at 325 nm.

Statistical analysis. Statistical analysis was performed with the SigmaStat program (Jandel Scientific, San Rafael, CA). A P-value of 0.05 was used to determine significance. All variables (except cytokine values) were compared among the three diet groups using either one- or two-way ANOVA. Two-way ANOVA was used when data from experiments done at different times were analyzed together (i.e., data were analyzed by “experiment” and by “diet group”). Pairwise multiple comparisons among the three diet groups were routinely made (in conjunction with the ANOVA procedure) by the Student-Newman-Keuls method. Preplanned, two-group comparisons (e.g., 4k vs. 250k diet) were also made using Student’s t test or, when data were not normally distributed, using the Mann-Whitney rank-sum test (as was true for IL-10 and IFN-γ data). If the data were not normally distributed or the variances (sd) were not equal (at P < 0.05), then log10 transformed data were analyzed. If they still were not distributed normally or had unequal variance, the nonparametric Mann-Whitney rank-sum test was used to compare groups. IgG and IgA data from Experiments 1 and 2 were pooled for analysis because two-way ANOVA (analyzing by “experiment” and “diet group”) found no significant effect of the “experiment” variable on antibody titers. Survival rates were analyzed by chi-square test.

RESULTS

Body weight. No significant differences in body weight were seen among the three diet groups before or after infection (P > 0.05). Body weights decreased significantly in all diet groups after inoculation with 12 PFU of influenza A virus, with a mean loss of 15% by wk 1 and 20% by wk 2 (Fig. 1). By wk 4, body weights returned to preinoculation levels. Similar changes were seen when mice were inoculated with 25 PFU of virus, i.e., the percentage of weight loss was greater with the larger inoculum but the differences among the diet groups again were not significant (P > 0.05; data not shown).

Vitamin A status and effect of infection on serum retinol. The high level vitamin A diet increased serum retinol concentrations and liver vitamin A stores. Serum retinol concentrations were measured in the two initial diet groups (4k and 250k) before infection (at 7 wk of age). Retinol concentrations were consistently higher in the 250k group than in the 4k group. For example, in Experiment 1, serum retinol (mean ± sd) in the 250k group was 1.02 ± 0.09 μmol/L (n = 5) vs. 0.85 ± 0.12 μmol/L (n = 4; P = 0.004) in the 4k group. In addition, liver vitamin A stores were measured in Experiment 1 on d 3 after infection in all three diet groups (4k, 4k/250k and 250k) and d 3 after the 4k/250k mice were switched from the control to the high level diet. The mean liver concentration of retinyl palmitate (mean ± sd) was lowest in the 4k group (0.48 ± 0.13 mmol/kg; n = 4), intermediate in the 4k/250k group (1.51 ± 0.10 mmol/kg; n = 4) and highest in the 250k group (11.0 ± 0.78 mmol/kg; n = 4). All three groups differed significantly from one another (P < 0.001).

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diet group after infection than in either the 4k or 4k/250k groups. Serum retinol concentrations decreased in all three diet groups (Fig. 2) by d 3 after infection, remained low through d 12 and returned to preinoculation levels by d 28. When the three diet groups were compared by two-way ANOVA (diet \( \times \) day) during the acute phase of infection (d 3, 6, 9 and 12 postinoculation), serum retinol concentrations in the 250k group were greater than in the 4k group (\( P = 0.010 \)) but were not different from the 4k/250k group. The serum retinol concentrations for the 4k and 4k/250 groups did not differ from one another.

**Virus replication.** Clearance of virus from the lungs of mice inoculated intranasally with influenza A did not differ among the three diet groups. Virus titers typically peaked between d 3 and 6 after inoculation and then decrease to undetectable levels by d 12 (Stephensen et al. 1993). In this study, virus titers (mean \( \pm \) sd) did not differ significantly among the 4k, 4k/250k and 250k diet groups on d 3 (6.0 \( \pm \) 3.2 \( \times \) 10^3, 5.4 \( \pm \) 3.1 \( \times \) 10^3, 5.0 \( \pm \) 1.2 \( \times \) 10^3 PFU/mouse, respectively; \( n = 4 \) /group) or d 6 after infection (7.8 \( \pm \) 1.3 \( \times \) 10^3, 7.8 \( \pm \) 2.8 \( \times \) 10^3, 7.2 \( \pm \) 1.2 \( \times \) 10^3 PFU/mouse). By d 9, virus titers were undetectable in all diet groups and remained undetectable at d 12. Similar results were obtained with the three diets in a second experiment in which mice were infected with 25 PFU of virus at 8 wk of age. Virus titers were again measured on d 3 and 6 postinoculation (\( n = 2 \) female and 2 male mice per diet) and no significant differences were seen (data not shown).

**Survival analysis.** Dietary vitamin A did not affect survival in the two experiments in which mice were maintained through recovery from infection. The percentage of mice surviving at 28 d postinfection in the 4k, 4k/250k and 250k diet groups with an inoculum of 12 PFU was 88% (14 of 16), 88% (14 of 16) and 75% (12 of 16), respectively. With a 25PFU inoculum, the survival rates were 75% (6 of 8), 50% (4 of 8) and 58% (7 of 12), respectively. None of these rates differed significantly from one another.

**Influenza-specific salivary IgA and serum IgG.** High level dietary vitamin A significantly increased the salivary IgA response to influenza infection. On d 28 after infection, the mean log_{10} influenza-specific salivary IgA concentrations (\( \mu \)g/mL) differed significantly among the three diet groups (\( P = 0.0019 \)). The concentrations in the 250k diet group (log_{10} mean \( \pm \) sd = 2.22 \( \pm \) 0.38) and the 4k/250k group (2.02 \( \pm \) 0.25) did not differ significantly from one another, but both were significantly greater (\( P < 0.05 \)) than the mean of the 4k group (1.77 \( \pm \) 0.44). The geometric mean values in the 250k and 4k/250k groups were 180 and 80% greater, respectively, than the geometric mean in the 4k control group (Fig. 3, upper panel). When the influenza-specific IgA titers were expressed as a percentage of total salivary IgA, significant differences among the means again were found by ANOVA (\( P = 0.019 \)). The mean (\( \pm \) sd) of the 250k group (4.3 \( \pm \) 2.4%) was significantly greater (\( P < 0.05 \)) than the mean for either the 4k/250k group (2.8 \( \pm \) 2.3%) or the 4k group (2.2 \( \pm \) 2.1%), whereas the latter two means did not differ significantly from one another (Fig. 3, lower panel). Total salivary IgA did not differ among the groups (\( P = 0.47 \); data not shown).

In contrast to the salivary IgA response, high level dietary vitamin A significantly decreased the serum IgG response. The highest mean (\( \pm \) sd) serum IgG response was seen in the 4k diet group. It was 44% higher than that in the 250k group and 41% higher than that in the 4k/250k group (Fig. 4). Although means did not differ among the diet groups by ANOVA (\( P = 0.064 \)), direct comparison of the two extreme diet groups in accordance with our initial hypothesis that the high level vitamin A group (250k diet) would have lower IgG titers than the control (4k diet) revealed that the mean of the 4k group was significantly greater than that of the 250k group using a two-tailed \( t \) test (\( P = 0.028 \)). The 4k/250k group mean did not differ from that of either the 4k group (\( P = 0.084 \)) or the 250k group (\( P = 0.90 \)).
This study showed that high level dietary vitamin A initiated 4 wk before infection or at the time of infection to mimic vitamin A adjuvant therapy, which has been used in clinical trials with pneumonia, did not affect the clinical course of disease. Body weight, survival and virus clearance were not affected by these interventions. Because our interventions did increase serum retinol concentrations (250k group) and liver vitamin A stores (4k/250k group and 250k group), the failure to affect these endpoints was not due to a failure of the intervention to affect vitamin A status. These results are consistent with the majority of clinical trial results, which have shown no overall benefit or detriment to vitamin A supplements with regard to recovery from infection (Dowell et al. 1996, Fawzi et al. 1998, Kjolhede et al. 1995, Si et al. 1997).

We had hypothesized that the mild detrimental effects seen in two studies (Bresee et al. 1996, Stephensen et al. 1998) might be related to an effect of high level dietary vitamin A on recovery from viral infection. In particular, the study of Bresee et al. (1996) found that vitamin A supplementation increased the duration of hospitalization of U.S. infants with respiratory syncytial virus infection. Our study design reflected the probable situation in subjects in the Bresee study in that we were providing supplements (the 250k diet) in addition to an already adequate level of vitamin A (the 4k diet). However, our study design also had limitations. For example, we used a mouse-adapted strain of human influenza A virus, and the effect of vitamin A on other viral infections may be different. In addition, our power to detect significant differences in survival of the magnitude seen in this study was not high (power = 14% with a P-value of 0.05 by χ² analysis of survival in the three groups from the 12 PFU experiment). Our power was sufficient (80%) to detect a 40–50% difference in survival between two groups (e.g., 88 vs. 42%). Smaller differences in survival were unlikely to be detected. In contrast, our power to detect what we consider biologically significant differences in viral titers (one log₁₀) was quite high (power = 0.96 in a three-group ANOVA). Thus, if vitamin A does adversely affect recovery from viral infections, which it may, the effect...
Dietary vitamin A and the Immunoglobulin A response

FIGURE 5 Interferon (IFN-γ) (lower panel) and interleukin (IL)-10 concentrations (upper panel) in supernatants of murine mediastinal lymph node cell cultures restimulated with influenza antigen in vitro 6 d after primary intranasal infection. Supernatants were collected and assayed by ELISA 48 h after stimulation. Data were not normally distributed and were compared using the Mann-Whitney rank-sum test. These box plots show median (horizontal line in middle of box), 25th and 75th percentiles (bottom and top of box), and 10th and 90th percentiles (indicated by error bars). The sample size was 7 in the 4k and 250k groups and 7.5 in the box plots. Each sample represents a pool of lymphocytes from 3 or 4 mice. Cytokine concentrations differed between the diet groups for both IFN-γ (P = 0.014) and IL-10 (P = 0.022). The 4k and 250k diets contained 4000 IU [1200 retinol equivalents (RE)] and 250,000 IU (75,000 RE) vitamin A/kg diet, respectively.

was not of sufficient magnitude to affect the clinical and virologic endpoints assessed in this study.

In contrast to the clinical and virologic endpoints, both the 250k and 4k/250k diets increased the IgA response above the level seen in the 4k diet, and the 250k diet diminished the IgG response in comparison with the 4k and 4k/250k diets. Although secretory IgA protects primarily against reinfection and thus may not affect recovery from a primary infection directly, serum IgG is thought to have a role in clearance of virus from the lungs during a primary infection (Ramphal et al. 1979). The modest increase in IgG seen in the 4k diet group may not have been sufficient to affect virus clearance or neutralization. Because we did not measure a functional antibody endpoint (e.g., virus neutralization), it is difficult to conclude whether such an increase in serum IgG would be functionally important. Thus we have shown that high level dietary vitamin A alters the immune response significantly, but the alterations in this experiment were not of sufficient magnitude, or did not affect other important innate host defense mechanisms (e.g., defenses or natural antibodies) or protective responses (e.g., cytotoxic T-lymphocytes), to affect recovery from infection. Given the substantial effect of vitamin A on the IgA response, it is plausible that resistance to a subsequent challenge infection might be altered by dietary vitamin A, even though resistance to a primary infection was not significantly altered in these experiments.

Although these changes in IgA and IgG in response to dietary vitamin A may not have had clinical ramifications in this study, the increase in IgA and decrease in IgG do indicate that high level dietary vitamin A is modulating the antibody response to infection. In considering these results, it should be noted that antibody titers were measured only for mice surviving 28 d beyond infection. It is thus plausible that the effect of diet on the IgA or IgG response might have been more or less substantial had survival not been a factor (e.g., as would be the case if a purified protein antigen had been used for immunization). However, in previous studies, vitamin A deficiency has had the same depressive effect on the secretory IgA response in mice surviving influenza A infection (Stephensen et al. 1996) as in rats immunized with a purified protein (cholera toxin) (Wiedermann et al. 1993). In addition, the percentage of survival did not differ significantly among the diet groups in this study. It is thus likely that analyzing antibody data from surviving animals did not alter the conclusions of this study.

The results of this study are consistent with previous findings that have shown that vitamin A deficiency diminishes the IgA response dramatically, whereas it increases the IgG response modestly (Stephensen et al. 1996). Taken together, these data show that the IgA and IgG responses to influenza infection are responding to dietary vitamin A across a wide spectrum of intake. There is a continuum from vitamin A deficiency through normal levels of intake (1200 – 6000 RE/kg) and into very high dietary levels (75,000 RE/kg, or 625 times greater than the current recommendation for rodents) along this continuum, increasing levels of dietary vitamin A increase the IgA response and decrease the IgG response. In addition, these results are reminiscent of the decreased serum antibody response to measles vaccine (Semba et al. 1992), which has occasionally, though not consistently (Benn et al. 1997, Semba et al. 1997) been seen in human studies using high doses of vitamin A.

Because the IgA response is promoted by Th2 cytokines and the IgG response to viral infections is promoted by Th1 cytokines, we predicted that high level dietary vitamin A might modulate these two cytokines in a manner consistent with the effects seen on the IgA and IgG responses. The hypothesis that above normal levels of dietary vitamin A could affect these responses was plausible because vitamin A deficiency increases IFN-γ production (a Th1 cytokine), whereas it diminishes production of Th2 cytokines (Cantorna et al. 1994 and 1995, Carman and Hayes 1991), and because retinoic acid treatment of mice has been shown to increase the production of Th2 cytokines (Racke et al. 1995). Indeed, we found that IL-10, a major Th2 cytokine produced during influenza infection (Sarawar and Doherty 1994), was increased by the 250k diet, whereas the production of IFN-γ was decreased. Decreased IFN-γ production could lead directly to decreased serum IgG levels because IFN-γ promotes the IgG2a response (Snapper and Paul, 1987), although other cytokines are also involved (Graham et al. 1993). It is also possible that IL-10 has a direct role in modulating the IgA response (Yamamoto et al. 1996), although IL-4 and IL-5 are presumably also involved in inducing and maintaining the IgA response (Mgcchee et al. 1992).

Because Th1 and Th2 cytokines are counterregulatory...
(Ramphal et al. 1979, Seder and Mosmann 1998), vitamin A could affect one, the other or both cell types and produce the effects seen here on the IgG and IgA responses. Promoter analysis of cis-regulatory elements upstream of the human IFN-γ gene indicates that retinoic acid decreases transcription from this gene, apparently via an unidentified regulatory protein (Cippitelli et al. 1996). Thus vitamin A could affect IFN-γ directly, and the modulating effect of vitamin A on Th2 cytokines could be secondary. However, we have found that the ability of vitamin A deficiency to diminish the IgA response remains unaltered in mice with targeted disruption of the IFN-γ gene (unpublished observations). This result demonstrates that vitamin A must act via another mechanism to modulate the IgA response.

The diets used in this study had vitamin A levels consistent with previous studies of vitamin A and immune function. The 4k diet contained 4000 IU/kg retinyl palmitate (1,200 RE/kg), the amount of vitamin A currently recommended for growth or maintenance of rodents (Reeves 1997). Control diets used in other laboratories examining the role of vitamin A deficiency on the immune response ranged from a high of 7200 IU/kg (Bresee et al. 1993) and 3960 RE/kg (Pasatiempo et al. 1989) to 1200 IU/kg retinyl palmitate (75,000 RE/kg) and did not produce any adverse effects, such as weight loss, which have been reported with other high level vitamin A diets. Studies in the literature examining the role of high dose vitamin A diets on rodents have used diets ranging from 75,000 RE/kg diet for 5 wk (Hatchigian et al. 1989) to 82,500 RE/kg diet for 60 –150 d (Forni et al. 1986), to 150,000 RE/kg diet for 3 wk (Fraker et al. 1986) to 510,000 RE/kg diet for “several” weeks (Malkovskay et al. 1983).

In summary, we have shown that high level dietary vitamin A did not alter the clinical or virologic outcome of viral pneumonia in this experiment, but did enhance the salivary IgA response and diminish the serum IgG response to influenza infection. These changes occurred in conjunction with increased production of a Th2 cytokine, IL-10 and decreased production of IFN-γ, an important Th1 cytokine. This altered pattern of cytokine production may have caused the changes in antibody response seen in this study. These data, in combination with previous work in vitamin A–deficient mice (Carman and Hayes 1991, Cantorna et al. 1994 and 1995), rats (Pasatiempo et al. 1990 and 1992) and humans (Semba et al. 1992) demonstrate that vitamin A modulates the immune response over a wide range of intakes in experimental animals and suggests that the same may be true in humans as well.

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