The Effect of Vitamin Deficiencies (E and A) and Supplementation on Infection and Immune Response

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Nutritional deficiencies contribute to the high incidence of morbidity and mortality from infectious diseases among children in developing countries. Infectious diseases (respiratory and diarrheal) are among the leading causes of death in children around the world. Deficiencies of vitamins E and A have been shown, in animal models and in humans, to impair the immune response and to decrease host resistance to infectious diseases. Furthermore, supplementation with higher than recommended levels of these nutrients in some, but not all cases, was associated with improved immune response and resistance to certain bacterial and viral infections. Several comprehensive reviews have been written on the role of vitamins E and A in the regulation of the immune response. This chapter, therefore, is not intended to be comprehensive; rather, it is a brief summary of past work, with emphasis on recent findings.

VITAMIN A AND IMMUNE RESPONSE

The existence of a relation between vitamin A deficiency and infectious diseases has been known since early in the 20th Century. These earlier studies gained vitamin A the reputation of an antiinfection vitamin. As immune function is an important determinant of host resistance to infection, a regulatory role for vitamin A in maintaining the immune response was suggested. Animal and human studies confirmed this speculation, although it is clear that nonimmune-related functions of vitamin A (e.g., maintaining the integrity and differentiation of mucosal epithelial cells and keratinocytes) also contribute to the overall vitamin A-induced resistance to infectious diseases.
Vitamin A Deficiency and the Immune Response

Atrophy of lymphoid organs, including spleen, thymus, and lymph nodes, has been reported in animals deficient in vitamin A (1) (Table 1). However, some of these effects may be caused by loss of appetite and decreased food intake. In addition, changes in spleen cell number are observed in the early stages of vitamin A deficiency and might be a more sensitive indicator of vitamin A deficiency (2). Controversy, however, exists over the effect of vitamin A deficiency on subpopulations of T and B lymphocytes. Some investigators have reported effects of vitamin A deficiency on distribution of T and B lymphocytes and their subpopulations in animal models (1,2), whereas others have reported a selective loss of CD4+ T cells from lymph nodes (3). These latter investigators further demonstrated decreases in the Th2 frequency, resulting in an imbalance in the Th1:Th2 ratio in vitamin A-deficient mice. This was associated with decreased interleukin (IL)-4 and IL-5, and increase in γ-interferon production (4). Further work showed that retinoic acid, through these cytokine changes, has a regulatory role in regulation of B cells and immunoglobulin isotype switching (4,5).

Abnormal T-cell subpopulations were also reported by Semba et al. in children with vitamin A deficiency (6). In that study, children with xerophthalmia had a lower CD4+:CD8+ ratio and lower proportions of CD4+ naive T cells and a higher proportion of CD8+ memory cells. Supplementation with vitamin A (60 mg retinol equivalent, RE) for 5 weeks significantly increased the percentage of CD4+ naive cells and the CD4+:CD8+ ratio, and reduced the percentage of CD8+ memory cells compared with the placebo treatment.

Vitamin A deficiency can also affect the function of different cells of the immune system (Table 1). Neutrophils, through their phagocytic function and production of...
cytotoxic metabolites, are important in providing the first line of defense against infection. Twining et al. (7) reported defects in chemotaxis, adhesion, phagocytosis, and the ability to generate reactive oxygen metabolites in neutrophils from vitamin A-deficient rats. Impairment of T- and B-cell function has been reported (2,8). Human leukocytes contain retinol and retinoic acid, as well as other metabolites (9). Retinol has been shown to be necessary as a cofactor in T-cell activation (10,11). Further, retinol through its metabolite, retinol, 14-hydroxy-4,14-retroretinol, is reported to be required for B-cell growth (12).

Delayed type hypersensitivity (DTH) skin response, a measure of T-cell-mediated function, was shown to be impaired (2,13) or increased (14) in vitamin A-deficient rats. The difference might be a result of the method of evaluating the DTH. Results from studies evaluating the DTH response in humans have also been inconclusive, as protein-energy malnutrition often coexists with vitamin A deficiency. Furthermore, as can be seen below, vitamin A repletion has not always been successful in restoring DTH in vitamin A-deficient subjects.

Another measure of T-cell function, mitogenic proliferation, has been shown to be reduced when T cells are derived from the spleen, but not from other anatomic locations (1). In general, it appears that when other nutrient deficiencies are controlled, T cells from vitamin A-deficient animals do not show a marked defect in proliferation. In fact, an increase in T-cell proliferation in response to concanavalin A (14) and Staphylococcus aureus (15) has been reported. This hyperactivity existed despite lower macrophage phagocytosis and complement activation and a higher incidence of arthritis induced by S. aureus. These observations have led Wiedermann et al. to propose a proinflammatory role for vitamin A deficiency (15).

Several studies have shown that vitamin A deficiency impairs B-cell function, as indicated by reduced production of both T-cell dependent and independent antibodies (1,2,8). The effect of vitamin A deficiency on antibody production against different antigens has been studied in both experimental animal models and humans. Several investigators have shown impaired primary antibody response to tetanus toxoid in rats (2). The secondary response to this antigen, however, does not seem to be impaired in vitamin A-deficient rats (16). The deficiency in antigen-specific antibody levels exists despite normal to higher serum immunoglobulin levels. Impairment in the production of other T-cell-dependent antibodies, such as sheep red blood cells, keyhole limpet hemocyanin, bovine serum albumin, and Salmonella pullorum (13,17,18) has also been reported in rats, mice, and chickens.

In a study by Smith and Hays (13), the primary IgM response was normal, whereas the greatest reduction occurred in the IgG class, particularly IgG1. These investigators attributed the effect of vitamin A to a change in the balance of Th1/Th2 cells and greater production of interferon. Others, however, have reported lower interferon production in vitamin A-deficient rats (19). The disagreement between these reports could reflect the differences in cell populations used in the two different studies.

The antibody response to T-cell-independent antigens (e.g., Streptococcus pneumoniae, type III, or meningococcal polysaccharides) has also been shown to be
depressed in vitamin A-deficient rats (20). These defects were corrected following vitamin A repletion. Information related to vitamin A deficiency and antibody production in humans, independent of other nutritional deficiencies, is not available.

Impaired antibody response to viral and parasitic antigens has also been reported in vitamin A deficiency (2). Studies also indicated impaired intestinal IgA production, which is attributed to impairment of gut-associated immune response (21–24).

Vitamin A Supplementation and the Immune Response

As vitamin A deficiency, both in experimental animals and in humans, is associated with an impaired immune response and increased morbidity and mortality from infectious diseases, several investigators have attempted to improve the immune response and, thus, resistance to infection by vitamin A supplementation in vitamin A-deficient subjects. The outcomes of these studies have varied, depending on the vitamin A status of the host, the type of infectious agent, and the immune response evaluated. In general, improvement in immune function and increased resistance to infection is observed if the host was deficient in vitamin A before supplementation.

Several animal experiments have shown that acute, high dose administration of retinoids improves specific and nonspecific immunity, including macrophage phagocytosis, bacterial clearance, cytotoxicity, and complement activation, natural killer (NK) cell activity, antibody production, and in vitro measures of T-cell–mediated function (2). Chronic high dose supplementation, however, was shown to decrease antibody production, lymphocyte proliferation, and resistance to infection (25). In that study, diets containing both deficient levels (0 μg/kg) and excess levels (1,000 μg/kg) of vitamin A resulted in higher mortality of chicks from Escherichia coli infection compared with adequate vitamin A levels (0.85 μg/kg). Fumarulo et al. (26) showed that incubation of human neutrophils with retinoic acid or retinyl acetate (1–100 μM) inhibited in a dose-dependent manner, oxygen-free radical production, chemiluminescence, and degranulation induced by phorbol myristate acetate, N-formyl-methionyl-leucyl-phenylalanine, zymosan, or ionophore A23187 in these cells. These studies might explain the apparently increased risk of respiratory infection reported in children supplemented with vitamin A (see below). A study by Gardner and Ross (16) showed that vitamin A supplementation (137.5 or 150 μg RE) given to vitamin A-sufficient nursling rats at the time of tetanus vaccination had no effect on antibody response.

In West Java, Indonesia, Semba et al. (27) randomly assigned clinically normal children 3 to 6 years of age or those with mild xerophthalmia to a placebo group or a vitamin A (60,000 μg RE) group for 2 weeks. The children were then vaccinated with intramuscular diphtheria-pertussis-tetanus vaccine, trivalent oral polio vaccine, and trivalent inactivated intranasal influenza vaccine. Children with weight and height less than 80% of median National Center for Health Statistics values were excluded. Serum vitamin A analysis indicated that even some of the
clinically normal children had low serum vitamin A levels (< 0.7 μmol/l). Vitamin A-supplemented children in both groups had a greater antibody response to tetanus vaccine. The effect of the other two vaccines was not reported. Three other studies, however, reported no effect of vitamin A supplementation on antibody response to tetanus vaccine (28–30). The lack of the effect in these studies could reflect the use of a single injection of vitamin A at the time of vaccination (28) or the small sample size used (29,30). Rosales and Kjolhede (31) evaluated the effect of vitamin A on antibody response to measles vaccination and on reversing measles-induced suppression of DTH. In Ndola, Zambia, 200 children with acute measles, ranging in age from 5 months to 17 years, were randomly assigned a single dose of placebo or vitamin A (210 μmol retinol or retinyl palmitate and 92 μmol all-rac-α-tocopherol). Antibody titers were evaluated at baseline and 2 weeks after enrollment. DTH was also determined at 1 and 2 weeks after enrollment using Multi-test CMI (Merieux Institute, Miami, FL). Antibody titer increased in both groups and no significant difference was seen between placebo and treatment groups. No difference in DTH response was seen between the two groups, except for a prolongation of DTH unresponsiveness in the treatment group. The results from this study are difficult to interpret or to compare with other studies, as treatment contained tocopherol in addition to retinol, whereas plasma retinol concentrations also increased in both groups.

Semba et al. (32) determined the effect on antibody response of 100,000 IU of vitamin A given simultaneously with live measles vaccine in 336 infants aged 6 months in West Java, Indonesia, using a double-blind, placebo-controlled design. More than 50% of the infants had serum retinol levels less than 0.7 μmol/l. The vaccine was standard titer Schwarz measles. A higher percentage of children (33.7%) in the group treated with vitamin A did not seroconvert compared with the placebo group (20.7%). Furthermore, the vitamin A group had a higher percentage of children who did not reach protective serum antibody levels (> 120%). Those treated with vitamin A also developed fewer rashes after immunization. These results indicate that vitamin A supplementation interferes with the establishment of subclinical infection following vaccination and, therefore, reduces host antibody production. A subsequent study by Benn et al. (33) showed no adverse effect of vitamin A supplementation on antibody titer against measles vaccine at 6 and 9 months or 9 years of age. Similarly, in a second study in Indonesia by Semba et al., vitamin A supplementation had no effect on seroconversion to measles on any children immunized at age 9 months (34). Thus, recommendations to give vitamin A at the same time as childhood vaccinations need to be further evaluated.

Rahman et al. (35) showed that infants given 15 mg of vitamin A when receiving diphtheria, pertussis, and tetanus (DPT) or oral polio vaccine at monthly intervals had a similar DTH response to those treated with the placebo. However, the response was better in well-nourished than in malnourished children. Kramer et al. (36) also reported that supplementation with vitamin A (1,500 mg for 6 months) had a modest effect on improving lymphocyte proliferation in response to tetanus and diphtheria. These children had higher serum vitamin A concentrations than those reported in other studies (0.99 μmol/l).
Vitamin A and Infection

In case-control and prospective studies, xerophthalmia and low serum vitamin A concentrations have been associated with increased risk of child mortality and morbidity (37). These observational studies have had limitations, including small sample size and lack of adjustment for confounding variables such as socioeconomic and nutritional status. Here, we focus our discussion on controlled trials examining the efficacy of vitamin A supplements on mortality and morbidity. First, we present community-based trials that examined the effect of supplementation on total mortality. Next, we review hospital-based and community-based studies examining the effect of supplements on measles. Finally, we look at hospital-based and community-based trials that assessed the effect of supplements on the incidence and severity of acute respiratory and diarrheal infections.

Community-Based Mortality Trials

In community-based studies on children aged more than 6 months, the protective effect of vitamin A varied (Table 2). In two studies from the Sudan (38) and Hyderabad, India (39), vitamin A supplements had no effect on total mortality. In other trials, the supplements resulted in a significant reduction in mortality, but the effect ranged from 19% in Ghana (40) to 54% in Tamil Nadu, India (41). Pooling the results from nine studies, vitamin A supplements reduced total mortality by 30% (42). In a study from Tanzania that was completed after the publication of this meta-analysis, a 50% reduction in mortality was noted among children who received vitamin A, with protective effects observed among both human immunodeficiency virus (HIV)-infected and uninfected children (43).

The variability in effect between trials may be explained by various factors (44). The vitamin A status of the study population is important and the supplements are expected to have greater efficacy in geographical areas in which vitamin A deficiency is prevalent. However, the vitamin A status of the children participating in these trials was not measured; consequently, this variable was not examined. The bioavailability of the supplements can also differ among populations: absorption of the lipid

<table>
<thead>
<tr>
<th>Community</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamil Nadu, India</td>
<td>8,333 IU/wk</td>
<td>54% ↓</td>
<td>Rahmathullah et al., 1990 (41)</td>
</tr>
<tr>
<td>Hyderabad, India</td>
<td>200,000 IU/every 6 mo</td>
<td>No effect</td>
<td>Vijayaraghavan et al., 1990 (39)</td>
</tr>
<tr>
<td>Sudan</td>
<td>200,000 IU/every 6 mo</td>
<td>No effect</td>
<td>Herrera et al., 1992 (38)</td>
</tr>
<tr>
<td>Ghana</td>
<td>200,000 IU/every 4 mo</td>
<td>19% ↓</td>
<td>Ghana VAST Study Team, 1993 (40)</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>200,000 IU (&lt;yearly) or</td>
<td>30% ↓</td>
<td>Fawzi et al., 1993 (42)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>400,000 IU (&gt;yearly) × (2 at baseline, 2 at 4,8 mo)</td>
<td>49% ↓</td>
<td>Fawzi et al., 1999 (43)</td>
</tr>
</tbody>
</table>
TABLE 3. Community-based vitamin A trials and mortality in children (<6 months old)

<table>
<thead>
<tr>
<th>Community</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nepal</td>
<td>50,000 IU (&lt;1 mo)</td>
<td>No effect</td>
<td>West et al., 1995, (46)</td>
</tr>
<tr>
<td></td>
<td>100,000 IU (1–5 mo)</td>
<td></td>
<td>E/Pneumonia Working Group, 1995 (47)</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td></td>
<td>Humphrey et al., 1996 (45)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>50,000 IU (at birth)</td>
<td>64% infant mortality rate</td>
<td>WHO/CHO Study Group, 1998 (48)</td>
</tr>
<tr>
<td>Multicenter trial in Peru, Ghana, and India</td>
<td>25,000 IU (at 6,10,14, weeks)</td>
<td>No effect</td>
<td></td>
</tr>
</tbody>
</table>

soluble supplements is impaired when dietary fat intake is limited. In areas where nutritional deficits are common, deficiency of nutrients essential for the bioavailability of the vitamin (e.g., protein and zinc) can limit the beneficial effects of the supplements. Smaller and more frequent doses of vitamin A appear to protect against mortality more effectively than large periodic doses (42). Thus, small weekly doses in the Tamil Nadu trial, and vitamin A-fortified monosodium glutamate, resulted in a greater reduction in mortality than in trials in which large doses of vitamin A were given every 4 to 6 months. The prevalence of infections at the time of supplementation and the incidence of infections in the period after supplementation are also important factors. Infection at the time of supplementation reduces the absorption of the supplement; a new infection increases the use of vitamin stores and is associated with increased loss of vitamin A, which results in a diminished protective period of the large dose supplement.

A few studies examined the efficacy of vitamin A supplements among children under the age of 6 months (Table 3). In a study from Indonesia, a single dose of vitamin A given to newborns on the day of birth resulted in a significant reduction in the risk of death in infancy (45). In contrast, no effect was observed in a separate trial done in Nepal (46). A meta-analysis examining the effect of the supplements on morbidity and mortality associated with respiratory infections in this age group showed no effect of the supplements (47). Similar findings were noted from a large multicenter trial that was carried out in Peru, Ghana, and India. No differences in mortality or morbidity were observed between children who received placebo or vitamin A at the time of each of the first three doses of DPT or poliomyelitis immunization at 6, 10, and 14 weeks (48).

Hospital- and Community-Based Trials of Vitamin A and Measles

Measles is responsible for nearly 1.5 million deaths every year. Although prevention of measles through immunization is optimal, difficulties in procurement and distribution of the vaccine render millions of children unprotected against the virus.

The efficacy of vitamin A supplements on measles-associated morbidity and mortality has been examined in several hospital-based trials. In a meta-analysis of four
TABLE 4. Community-and hospital-based trials of vitamin A and morbidity and mortality associated with measles

<table>
<thead>
<tr>
<th>Community</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meta-analysis of community-based studies</td>
<td>Periodic supplement</td>
<td>39% ↓ in measles-related deaths</td>
<td>Fawzi et al., 1993 (42)</td>
</tr>
<tr>
<td>Meta-analysis of four hospital-based studies</td>
<td>Large dose at admission</td>
<td>60% ↓ in death risk</td>
<td>Fawzi et al., 1993 (42)</td>
</tr>
<tr>
<td>Kenya</td>
<td>50,000 IU (&lt;6 mo)</td>
<td>No effect on RI/DI</td>
<td>Ogaro et al., 1993 (49)</td>
</tr>
<tr>
<td></td>
<td>100,000 IU (6–12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200,000 IU (&gt;12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zambia</td>
<td>200,000 IU</td>
<td>↓ Risk of pneumonia</td>
<td>Rosales et al., 1996 (50)</td>
</tr>
</tbody>
</table>

RI, respiratory infection incidence; DI, diarrhea incidence.

studies carried out among children in South Africa, Tanzania, and the United Kingdom, large doses of vitamin A given on admission resulted in a reduction of about 60% in the risk of death overall, and with about 90% reduction among infants (42) (Table 4). In these trials, administration of vitamin A to children who developed pneumonia before or during hospital stays, reduced mortality by nearly 70% compared with control children. The protective effects of the supplements appeared to be greater among infants than older children.

In a later trial from Kenya, vitamin A supplements had no effect on mortality. However, the study had limited power to examine this question (49). Among those with diarrhea on admission to the hospital, vitamin A supplementation resulted in a significantly faster recovery than did the placebo. A trial was carried out among children from Zambia who had measles but whose infection was not severe enough to warrant hospital admission (50). Among children who did not have measles at baseline, there appeared to be a decreased risk of developing pneumonia, but also a decreased risk of recovery from pneumonia among children who had measles at baseline. The sample size of this study was also too small to allow for precise estimation of the associations of interest.

Periodic vitamin A supplements given to apparently healthy children are associated with protective effects against measles. Results pooled from community-based trials with periodic supplementation of vitamin A showed a 39% reduction in measles-related mortality, as well as a decrease in overall mortality (42).

Hospital-Based Studies of Diarrheal and Respiratory Infections

Given the protective effects of vitamin A noted among measles patients, it was natural to examine whether the supplements were similarly beneficial in other serious childhood infections such as pneumonia and diarrhea. In some of the measles trials, vitamin A resulted in a significant reduction in the occurrence and severity of respiratory and diarrheal complications. However, the application of the findings to non-measles infective episodes was not possible.
Four vitamin A efficacy trials were carried out among patients admitted to the hospital with diarrhea (Table 5). In a placebo-controlled study among children from Bangladesh with noncholera watery diarrhea, no differences were found in the duration of illness or the stool output between the two treatment arms (51). Diarrhea was mainly caused by rotavirus and enterotoxigenic E. coli. However, in another study from Bangladesh, protective effects of vitamin A supplements were noted among children admitted to the hospital with acute shigellosis (52). A significantly higher proportion of children receiving supplements achieved clinical cure by day 5 of the trial, although no difference was found in bacteriologic cure between the vitamin A and placebo groups. The difference in efficacy between the two studies from Bangladesh could have resulted from differences in the pathogenesis of intestinal infections, reflecting the different etiologic factors. Shigella infection is a serious intestinal disorder associated with mucosal breeches and protein-losing enteropathy, and it is likely to carry an increased risk of complications. Furthermore, in the pathogenesis of shigella, induction of an inflammatory response is important in the passage of the microbe from lateral to basal membrane. The anti-inflammatory role of vitamin A proposed by Wiedermann et al. (14) and the well-known role of the vitamin in maintaining endothelial cell integrity might make vitamin A supplementation more effective against this particular infection than against less severe enteric infections.

Vitamin A supplements had no effect on the duration of diarrhea among children in India (53), or malnourished children from the Congo (54), although the cause of diarrhea was not examined in either study. In subgroup analyses in the Indian trial, a significant reduction in the duration of diarrhea was observed among children with severe vitamin A deficiency, as defined by conjunctival impression cytology.

The efficacy of vitamin A supplements on the severity of disease among children admitted to the hospital with pneumonia was examined in several placebo-controlled trials (Table 6). In Guatemala, the supplements had no effect on the length of hospital stay, or on the duration of various signs of respiratory disease, including hypoxia, fever, and rapid respiratory rate (55). In Tanzania, vitamin A-treated and placebo groups were similar in the mean number of days of hospital stay and mean number of days of fever, rapid respiratory rate, and hypoxia (56). In trials from Brazil (57) and Vietnam (58), vitamin A supplements had no effect on the course of pneumonia. In Brazil, an apparently protective effect was seen among a subgroup of children who

<table>
<thead>
<tr>
<th>Community</th>
<th>Pathogen</th>
<th>Dose/period</th>
<th>Result</th>
<th>Reference</th>
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</thead>
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<tr>
<td>Bangladesh</td>
<td>Rotavirus</td>
<td>200,000 IU</td>
<td>No effect</td>
<td>Henning et al., 1992 (51)</td>
</tr>
<tr>
<td></td>
<td>Escherichid Coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>Not known</td>
<td>100,000 IU (&lt;yearly)</td>
<td>No effect</td>
<td>Dewan et al., 1995 (53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200,000 IU (&gt;yearly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congo</td>
<td>Not known</td>
<td>100,000 IU (&lt;yearly)</td>
<td>No effect</td>
<td>Donnen et al., 1998 (54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200,000 IU (&gt;yearly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Shigella</td>
<td>200,000 IU</td>
<td>Protective</td>
<td>Hossain et al., 1998 (52)</td>
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</table>
TABLE 6. Vitamin A and respiratory diseases: hospital-based trials

<table>
<thead>
<tr>
<th>Community</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guatemala</td>
<td>100,000 IU (&lt;yearly)</td>
<td>No effect</td>
<td>Kjolhede et al., 1995 (55)</td>
</tr>
<tr>
<td></td>
<td>200,000 IU (&gt;yearly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>50,000 IU (1–5 mo)</td>
<td>No adverse effect</td>
<td>Bresee et al., 1996 (61)</td>
</tr>
<tr>
<td>multicenter</td>
<td>100,000 IU (6–11 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200,000 IU (&gt;12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>50,000 IU (1–5 mo)</td>
<td>More rapid recovery</td>
<td>Dowell et al., 1996 (62)</td>
</tr>
<tr>
<td></td>
<td>100,000 IU (6–11 mo)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>200,000 IU (&gt;12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>100,000 IU</td>
<td>No effect</td>
<td>Quinlan and Hayani, 1996 (60)</td>
</tr>
<tr>
<td>Brazil</td>
<td>100,000 IU/d for 2 days (&lt;1 y)</td>
<td>No effect</td>
<td>Nacul et al., 1997 (57)</td>
</tr>
<tr>
<td></td>
<td>200,000 IU/d for 2 days (1–4 y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietnam</td>
<td>100,000 IU/d for 2 days (&lt;1 y)</td>
<td>No effect</td>
<td>Si et al., 1997 (58)</td>
</tr>
<tr>
<td></td>
<td>200,000 IU/d for 2 days (1–4 y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanzania</td>
<td>100,000 IU/d for 2 days (&lt;1 y)</td>
<td>No effect</td>
<td>Fawzi et al., 1998 (56)</td>
</tr>
<tr>
<td></td>
<td>200,000 IU/d for 2 days (&gt;1 y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peru</td>
<td>150,000 IU/2 d (&lt;yearly)</td>
<td>Adverse effect</td>
<td>Stephensen et al., 1998 (59)</td>
</tr>
<tr>
<td></td>
<td>300,000 IU/2 d (&gt;yearly)</td>
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had pneumonia severe enough to warrant hospital admission. In Vietnam, the duration of hospital stay was shorter in the vitamin A group among children who were moderately malnourished. In the Tanzania trial, all subjects enrolled were hospital inpatients. No differences in the effect of the supplements were observed among children who had a more severe condition at baseline or among varying categories of age, breast-feeding status, anthropometric status at baseline, or category of dietary vitamin A intake in the 4 months before admission to the hospital. In the same study, no differences were found when the endpoints were examined among a subset with more severe clinical condition at baseline.

Evidence suggests, in some cases, vitamin A supplements can have adverse events when given to children with pneumonia. In a well-designed, placebo-controlled study from Peru, vitamin A supplements resulted in longer duration of clinical signs, including auscultatory evidence of consolidation, a higher prevalence of retractions, and lower oxygen saturation (59) (Table 6). Also, a greater need was seen for supplemental oxygen in the vitamin A group. In the Tanzania study, of the 346 children in the vitamin A group, 13 died in the hospital, in contrast to eight deaths among the 341 children in the placebo group (63% higher mortality in the vitamin A group) (56). This last finding was not statistically significant; however, the study was not designed with statistical power adequate to examine the effect of the supplements on case fatality.

Three trials were carried out to examine specifically the efficacy of vitamin A supplements on children admitted to the hospital with pneumonia caused by the respira-
tory syncytial virus (RSV). RSV is a paramyxovirus similar to measles, and is also an important cause of bronchiolitis and pneumonia among infants and children. In an American study, children in the supplemented group had longer duration of oxygen treatment and intensive care, although these differences were not statistically significant (60). In a larger, multicenter trial also from the United States, no differences were found in the mean number of days with rapid respiratory rate, nor in the need for supplemental oxygen or intensive care, between the vitamin A and placebo groups (61); however, children who received vitamin A had longer hospital stays than those given a placebo. In contrast, in a third trial done in Santiago, Chile, as a companion to the multicenter trial, vitamin A appeared to result in a more rapid recovery from tachypnea, but this finding was limited to children who had the most significant hypoxemia at baseline (PO2 < 12 kPa; 90 mm Hg) (62). Differences in other environmental factors (e.g., pollution and exposure to other pathogens affecting vitamin A status) might have contributed to differences observed in these two trials.

**Community-Based Studies of Diarrheal and Respiratory Infections**

Given the protective effects of vitamin A supplements on mortality, it was presumed that the vitamin would have beneficial effects on morbidity. However, in various community trials, vitamin A supplementation had no effect on morbidity despite having a strong protective effect against mortality in the same studies (63) (Table 7). In

### TABLE 7. Vitamin A and diarrheal or respiratory diseases: community-based trials

<table>
<thead>
<tr>
<th>Community</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamil Nadu, Southern India</td>
<td>8,333 IU/wk</td>
<td>No effect on Dl or Rl</td>
<td>Rahmathullah et al., 1991 (66)</td>
</tr>
<tr>
<td>Ghana</td>
<td>200,000 IU/every 4 mo</td>
<td>No effect on Dl or Rl</td>
<td>Ghana VAST Study Team, 1993 (40)</td>
</tr>
<tr>
<td>China</td>
<td>100,000 IU A, 40 IU E (2 doses at 3 mo, 9 mo after baseline)</td>
<td>(\uparrow) RI and Dl</td>
<td>Lie et al., 1993 (77)</td>
</tr>
<tr>
<td>Haiti</td>
<td>200,000 IU/every 4 mo</td>
<td>(\uparrow) RI symptoms</td>
<td>Stansfield et al., 1993 (76)</td>
</tr>
<tr>
<td>Brazil</td>
<td>100,000 IU (&lt;12 mo)</td>
<td>Significant (\downarrow) Dl; no effect on ALRI</td>
<td>Barreto et al., 1994 (67)</td>
</tr>
<tr>
<td></td>
<td>200,000 IU (&gt;12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Delhi, India</td>
<td>200,000 IU</td>
<td>Improvement in Dl</td>
<td>Bhandari et al., 1994 (68)</td>
</tr>
<tr>
<td>South Africa (HIV+ mothers)</td>
<td>50,000 IU (at 1, 3 mo)</td>
<td>Improvement in Dl and RI</td>
<td>Coutouvidis et al., 1995 (69)</td>
</tr>
<tr>
<td></td>
<td>100,000 IU (at 6, 9 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200,000 IU (at 12 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>200,000 IU/every 4 mo</td>
<td>No effect on RI or Dl duration</td>
<td>Ramakrishnan et al., 1995 (73)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>103,000 IU (&lt;12 mo)</td>
<td>39%(\uparrow) in ALRI</td>
<td>Dibley et al., 1996 (72)</td>
</tr>
<tr>
<td></td>
<td>206,000 IU (&gt;12 mo)</td>
<td>8% (\uparrow) in ARI</td>
<td>Pinnock et al., 1988 (75)</td>
</tr>
</tbody>
</table>

Dl, diarrhea incidence; Rl, respiratory infection incidence; ALRI, acute respiratory illness; ARI, acute lower respiratory illness; HIV, human immunodeficiency virus.
two trials that found an effect on mortality, in Aceh, Indonesia (64,65) and Tamil Nadu, India (41,66), no effect was seen on diarrheal or respiratory infections. In Aceh, morbidity was assessed in the week preceding a 6-month visit, whereas in Tamil Nadu, children were visited on a weekly basis. In these trials, however, mortality was the primary endpoint, and data sufficient to examine fully the effect of the supplements on morbidity were not collected.

Several studies were carried out specifically to examine the effect of the supplements on the incidence and severity of infections. A detailed morbidity profile was assessed on all children using passive and active surveillance of the study populations. In a trial in Ghana in which children were examined on an ongoing weekly basis to determine the rate of morbidity, no significant differences were found between the two treatment arms with respect to the prevalence of diarrheal or respiratory conditions (40). However, children who received vitamin A had fewer visits to clinics and fewer admissions to the hospital. In addition, the supplements resulted in a significant reduction in overall and diarrhea-specific mortality, whereas no effect on respiratory-related deaths was noted. These findings suggest that the protective effect of vitamin A is in reducing the severity rather than the incidence of infections. Among children from Brazil who were visited at home three times a week as part of a vitamin A versus placebo trial, no significant differences were found in the incidence of pneumonia or the frequency of hospital admission between the two groups (67). However, vitamin A resulted in a reduction in the mean daily prevalence as well as in the mean number of episodes of diarrhea, particularly severe episodes, both in this study and in another placebo-controlled study from New Delhi, India (68). In a third trial from South Africa among children born to women infected with HIV, vitamin A supplements resulted in an apparent reduction in the incidence of diarrhea overall, severe diarrhea, and lower respiratory tract infection (69). The protective effects were somewhat stronger among those infected with HIV and presumably more undernourished children than among those children not infected with HIV. However, the statistical power of the study to examine the effect of the supplements on various morbidity endpoints within HIV subgroups was limited. In trials from Brazil (70) and Indonesia (71), children given vitamin A supplement had similar risk and severity of respiratory infection compared with children given a placebo.

Findings from placebo-controlled trials in Indonesia (72) and India (73) suggest that large doses of vitamin A can be harmful when given to well-nourished children. In Indonesia was found a 39% increase in the risk of acute lower respiratory tract infection (ALRI) in the group receiving the vitamin A supplement. This negative effect was limited to children who were not stunted, among whom an 83% increase in ALRI was seen; among stunted children, a 29% reduction in ALRI was observed. Overall, the supplements had no effect on the risk of diarrhea, but vitamin A supplementation resulted in a significantly greater risk of diarrhea among children less than 30 months of age and in a significantly lower risk among older children. However, the effect of the supplements on the risk of pneumonia was not modified by the occurrence of wasting, nor did the effect on diarrhea differ between wasted and stunted children.
Moreover, no differences were seen in the mean duration of ALRI or diarrhea between the vitamin A and placebo arms. In a randomized trial from India, vitamin A supplements did not have a significant effect on either the percentage of time ill or the number of episodes of respiratory infection. Children who received vitamin A had an increased mean duration of diarrheal episodes. Compared with other trials, this population of Indian children had relatively better healthcare, including high coverage of immunization, awareness among mothers about health and nutrition, and routine deworming. These factors may have reduced the chances of finding a protective effect from the supplements.

Results from two trials in Australia and one in Haiti support the hypothesis that vitamin A supplements can increase signs of infection, particularly respiratory signs. In a study of Australian children who received either small doses of vitamin A or a placebo three times a week, children receiving supplement experienced a 12% increase in the mean number of days of cough. However, they also experienced a 9% and a 12% decrease in the number of days with chest and nose or throat symptoms, respectively (74). In a second Australian study of similar design, the group supplemented with vitamin A experienced a 17% increase in the median number of cough days as well as a 9% and a 43% increase in the median number of days with runny nose and sore throat, respectively (75). In a study from Haiti, vitamin A supplementation resulted in a significant increase in the 2-week prevalence of all symptoms of morbidity, including cough, rapid respiration, and diarrhea (76).

In contrast with the above, studies from China (77) and Thailand (78) showed reductions in the incidence of both respiratory infection and diarrhea in children receiving vitamin A supplement compared with control children. An important limitation of both studies was that the control group did not receive a placebo. Therefore, the investigators were not blinded with respect to the treatment arm, raising the possibility of bias by the research staff in ascertaining the outcomes.

Comment

Clear evidence is found of the importance of vitamin A supplementation in cases of measles and malnutrition, including vitamin A deficiency. Vitamin A supplementation may also be beneficial in reducing the severity of disease in some cases of diarrheal pneumonia. From hospital-based trials among children with pneumonia, no evidence indicates that vitamin A is protective. In some cases, the results suggest a possible increase in risk to patients with pneumonia, as indicated by the increased need for supplemental oxygen in the study from Peru (58) and the apparent increased mortality among Tanzanian children admitted to the hospital with pneumonia (55). In light of these findings, vitamin A supplements should not be given during nonmeasles episodes of pneumonia unless evidence is seen of vitamin A deficiency. The conditions under which vitamin A supplements can be harmful need to be examined further. Given that vitamin A supplementation may reduce diarrheal disease in the period after discharge from the hospital, supplements could be given after recovery from pneumonia and at the time of leaving the hospital.
Large doses of vitamin A provide a potentially quick solution in areas of the world where deficiency is a public health problem. In these communities, vitamin A supplements can reduce total and diarrhea-specific mortality. When periodic dosing is chosen as an intervention strategy, we suggest that these doses be given every 4 months (42). Given the varying degree of protection afforded in different studies, more research is needed on factors that affect the bioavailability of these large doses.

Although periodic large doses of vitamin A are beneficial in the short run, their use as the only approach to the problem of vitamin A deficiency has limitations. Vitamin A deficiency coexists with other nutrient deficits that are not addressed by the supplementation program. In addition, the effectiveness of this approach is limited to the duration of the program, and children who live in distant places and may need the supplement most would be difficult to reach. Furthermore, large dosing programs can put financial and logistic strains on healthcare systems in developing countries. Toxicity caused by ingestion of multiple large doses over a short period of time is a real possibility that needs to be guarded against.

A more sustainable solution to the problem of vitamin A deficiency lies in programs aimed at increasing consumption of vitamin A in the diet. Small, frequent doses (in amounts corresponding to those in the diet) may be more protective against mortality and morbidity than large, periodic doses. Most communities in which vitamin A deficiency is a serious problem have abundant supplies of fruits and vegetables rich in carotenoids with provitamin A activity. Dietary vitamin A intake has been associated with significant reductions in mortality (79), diarrheal and respiratory infection (80), and risks of stunting or wasting (81). Nutrition education programs in these communities should be undertaken in addition to the administration of supplements if the latter strategy is being implemented. In areas of the world where vitamin A foods are not abundant, horticultural approaches should be considered. Vitamin A intervention strategies should be integrated into existing community programs that focus on other health problems rather than implemented as a vertical program. Additional operational research on possible methods of integration is needed.

More studies are needed to determine the mechanism of the effect of vitamin A on morbidity and mortality. Such studies should include both the immune- and nonimmune-related functions of vitamin A, as well as the interaction of vitamin A with the pathogen. Results from these studies would help determine host conditions or infection types for which vitamin A supplementation could be beneficial and those for which it would be harmful.

An important mechanism through which vitamin A exerts both its beneficial and its harmful effects is through modulation of cellular and humoral immunity (see above). Furthermore, vitamin A deficiency can adversely affect the epithelial lining of the gastrointestinal tract (82), leading to decreased secretion of mucus and weakened local barriers to infection. Vitamin A-deficient mice had more severe mucosal changes than normal mice when infected with rotavirus (83), which is a major cause of diarrhea in children.

The positive relationship between vitamin A intake and cough that has been shown in a few studies has three possible explanations (which do not apply to studies that
showed other signs of respiratory infection, such as rapid respiration). First, the occurrence of cough may indicate a more competent respiratory epithelium and, hence, these results would suggest that vitamin A intake was protective against respiratory disease. Cough is a defense mechanism by which the body prevents the entry of harmful material into the respiratory system. Although the physiology of cough is not fully understood, it is probably initiated when airway receptors embedded in the tracheobronchial epithelium are stimulated by foreign substances (84). As vitamin A deficiency is associated with keratinization and squamous metaplasia of respiratory epithelium (82), this may adversely affect the cough receptors, leading to the decreased occurrence of cough among children with low levels of vitamin A intake. Alternatively, cough may occur more among vitamin A-deficient subjects, who may be at a higher risk of respiratory infection, in which case the overall association with dietary vitamin A intake indicates that vitamin A is harmful.

**VITAMIN E AND THE IMMUNE RESPONSE**

Vitamin E is the major chain-breaking antioxidant in membranes. Membrane phospholipids of cells of the immune system have a high content of polyunsaturated fatty acids and are prime targets for free radical reactions. Release of reactive oxygen species by phagocytes on encountering pathogens and rapid lymphocyte proliferation following antigenic stimulation expose the immune cells to high levels of oxidative stress. Thus, it is not surprising that cells of the immune system have a higher vitamin E content than other cells of the body (85).

Both deficiency and supplementation of vitamin E have been shown to affect the immune response and resistance against infection. The influence of vitamin E on immune function has been reported in a variety of species including rodents, chickens, calves, and humans, and has been shown to affect different aspects of immune function including T-cell response, antibody production, NK-cell activity, phagocytic activity, and the production of immunoregulatory molecules. Here, effects of vitamin E deficiency and supplementation on immune functions and their clinical significance will be discussed.

**Vitamin E Deficiency and Immune Function**

Vitamin E deficiency has been shown to impair both humoral and cell-mediated immune functions in animals and humans.

The effect of vitamin E deficiency on humoral immune response in animals was demonstrated in experiments in which mice fed a diet deficient in vitamin E had fewer plaque-forming cells and a lower hemagglutination titer in response to injection of sheep red blood cells than mice fed a diet adequate in vitamin E (86). Depressed lymphocyte proliferation in response to T-cell mitogen concanavalin A in rats fed a vitamin E-deficient diet indicates that cell-mediated immune response is also impaired in vitamin E deficiency (87,88). Vitamin E deficiency has a significant impact on phagocytic functions. Harris et al. (89) reported that chemotactic and in-
gestive responses of neutrophils were impaired when rats were fed a vitamin E-deficient diet for 2 months. Warschauer et al. (90) also showed that vitamin E deficiency augmented the adverse effect of ozone-induced impairments in pulmonary bactericidal capacity following prolonged exposure of rats to low levels of ozone. These effects of vitamin E deficiency might result from increased free radical reactions, oxygen consumption, and hydrogen peroxide release by phagocytosing neutrophils from vitamin E-deficient animals (89).

In humans, primary severe deficiency of vitamin E rarely occurs, whereas secondary deficiency is observed as a consequence of certain diseases such as primary biliary cirrhosis (91), cholestatic liver disease (92), cystic fibrosis (93), and intestinal malabsorption disorders (94). Decreased plasma vitamin E concentrations have been observed in patients with severe viral hepatitis and in children infected with HIV-1 (95,96). In a case report by Kowdley et al. (94), in vivo and in vitro impairment of T-cell function, as well as polynuropathy, were observed in conjunction with vitamin E deficiency in a woman aged 59 years with progressive systemic sclerosis and malabsorption. Impaired mitogenic responses to concanavalin A and phytohemagglutinin, IL-2 production, and DTH were improved following vitamin E supplementation.

Most premature low birthweight infants have a true deficiency of vitamin E at birth that requires early treatment. Vitamin E deficiency in preterm infants is associated with hemolytic anemia, hyperbilirubinemia, intraventricular hemorrhage, and retinopathy of prematurity (97). Neutrophils from neonates have impaired phagocytic ability, depressed oxidative metabolic responses, and depressed bactericidal activity, as well as a reduced ability to move toward defined chemotactic stimuli, compared with cells from normal adults (98). In healthy children aged 3 years, lower serum vitamin E levels (< 10th centile) were associated with lower lymphocyte proliferation and serum IgM compared with those with higher vitamin E levels (> 90th centile) (99).

**Vitamin E Supplementation and the Immune Response**

Vitamin E has been shown to have immunostimulatory effects in various species, including humans, when given in quantities exceeding established dietary requirements. Vitamin E supplementation has been shown to affect both humoral- and cell-mediated immune responses.

Dietary supplementation with vitamin E increased lymphocyte proliferation in response to T-cell and B-cell mitogens in mice (100), rats (88), and calves (101). Meydani et al. (100) showed that dietary supplementation with 500 ppm vitamin E for 6 weeks increased lymphocyte proliferation and DTH response, and decreased prostaglandin (PG) E2 production in old mice. In addition, in vitro introduction of vitamin E increased the mitogenic response of splenic lymphocytes in mice (102,103). In a coculture study, Beharka et al. (103) showed that the in vitro addition of vitamin E increased concanavalin A-stimulated cell proliferation when macrophages from old mice were cocultured with purified T cells from either old
VITAMIN E AND A DEFICIENCIES AND SUPPLEMENTATIONS

or young mice, or when macrophages from young mice were cocultured with purified T cells from old mice. IL-2 production was also increased with vitamin E supplementation in cocultures composed of macrophages from old mice and purified T cells from either old or young mice. The immunostimulatory effects of vitamin E supplementation seem to be transferred to the offspring. Chicks hatched from breeders fed diets supplemented with vitamin E had significantly higher tetrahydrofuran-stimulated bursal lymphocyte proliferation and higher concanavalin A and phorbol 12-myristate 13 acetate (PMA) stimulated splenic lymphocyte proliferation compared with control chicks (104). It is suggested that the immunostimulatory effect of vitamin E is mediated by either reduced prostaglandin synthesis (100) or decreased free radical synthesis (105). PGE\textsubscript{2} has been shown to have a direct inhibitory effect on an early stage of T-cell activation, resulting in decreased IL-2 production, decreased IL-2 receptor expression, decreased responsiveness to exogenous IL-2, and decreased proliferation (106).

Vitamin E has been shown to increase antibody production by enhancing humoral immune response or by acting as an adjuvant. Dietary vitamin E supplementation increased the number of plaque-forming cells and hemagglutination titers following immunization with sheep red blood cells and tetanus toxoid in mice (107). Enhancement of humoral immune response to Venezuelan equine encephalomyelitis attenuated live virus vaccine was observed in guinea pigs given intramuscular injections of vitamin E before and after immunization (108). Oral supplementation of vitamin E, begun 2 weeks before vaccination and continued for 3 weeks afterward, did not have an effect on humoral response in this study. Vitamin E supplementation in lambs (20 mg/kg vitamin E diet for 10 weeks) stimulated secondary humoral immune response following parainfluenza 3 virus challenge (109). The adjuvant effect of vitamin E was reported by Francini et al. (110). Vitamin E, which partially replaced mineral oil in viral inactivated emulsified vaccines, increased the hemagglutination inhibition titers to the viral antigen (Newcastle disease virus) in chicks.

Several studies have shown that vitamin E supplementation affects immune response in humans. Baehner et al. (111) found that administration of 1,600 mg/d of vitamin E for 1 week increased the rate of neutrophil phagocytic activity, but decreased bactericidal activity, which correlated with a reduced level of H\textsubscript{2}O\textsubscript{2} release. Vitamin E supplementation has been shown to enhance immune response in the elderly. In a double-blind, placebo-controlled study, Meydani et al. (112) found that DTH scores, mitogenic response of peripheral blood mononuclear cells to concanavalin A, and IL-2 production were significantly higher in the elderly subjects (> 60 years of age) supplemented with 800 mg/d vitamin E for 30 days. Decreased PGE\textsubscript{2} production by peripheral blood mononuclear cells and decreased plasma lipid peroxide levels were also observed. In a more recent study, the effect of long-term vitamin E supplementation on \textit{in vivo} indices of immune response in healthy elderly subjects was evaluated (113). After 4 months of vitamin E supplementation at levels of 60, 200, or 800 mg/d, DTH and antibody titer to hepatitis B were significantly increased in the groups supplemented with 200 and 800 mg/d. The
largest increase was observed in the 200 mg/d group, which also showed a significant increase in antibody titer to tetanus vaccine. This long-term vitamin E supplementation did not adversely affect the elderly subjects (114). Vitamin E supplementation had no significant effects on plasma concentrations of other nutrients, serum autoantibodies (anti-DNA and antithyroglobulin), liver enzyme function (glutathione peroxidase and superoxide dismutase), or on cytotoxic activity of neutrophils against *Candida albicans*.

Premature infants are under severe oxidative stress as they are faced with an abrupt change to a relatively hyperoxic extrauterine environment and lower levels of antioxidant defenses. In current clinical practice, premature infants usually receive vitamin E in a multivitamin preparation that is added to parenteral formula at a level of 2 to 3 IU/kg body weight (97). Most of the vitamin E supplementation studies on premature infants focus on the outcomes related to retinopathy, bronchopulmonary dysplasia, necrotizing enterocolitis, and sepsis. The effect of vitamin E on the immune function of premature infants is not well documented. The use of vitamin E in premature infants has yielded conflicting results. Pharmacologic levels of α-tocopherol combined with cryotherapy was found to be more effective than cryotherapy alone in decreasing the severity of threshold retinopathy of prematurity in infants weighing less than 1,250 g (115). Although Fish *et al.* (116) reported that necrotizing enterocolitis and sepsis did not occur more frequently in the neonates treated with intramuscular injections of vitamin E, Johnson *et al.* (117) reported an increased incidence of necrotizing enterocolitis and neonatal sepsis associated with vitamin E treatment (oral, intravenous, or intramuscular application). Necrotizing enterocolitis is a condition that results in widespread intestinal necrosis and often leads to perforation of the bowel and peritonitis. The difference in outcomes of the studies by Fish *et al.* (116) and Johnson *et al.* (117) might reflect the difference in target serum vitamin E levels: 0.5 to 3.5 mg/dl in the study by Fish *et al.* and 5.0 mg/dl in the study by Johnson *et al.* In fact, nearly 43% of infants supplemented with vitamin E had serum vitamin E levels greater than 5.0 mg/dl in the study by Johnson *et al.* Sobel *et al.* (118) reported an increased incidence of necrotizing enterocolitis associated with serum levels of vitamin E greater than 3.5 mg/dl, whereas Finer *et al.* (119) reported an increased incidence in association with an oral dose (200 mg) of vitamin E, which may have been related to hyperosmolarity of the preparation. Mino (120) investigated the effect of oral vitamin E supplementation on leukocyte function of premature infants: no effect was seen on zymosan-induced superoxide anion formation by neutrophils following the administration of 40 mg/kg of all-rac-tocopherol nicotinate to the infants for 8 to 14 days. Chirico *et al.* (121) reported improved neutrophil function with intramuscular injections totalling 120 mg/kg vitamin E to healthy premature infants during the first 13 days after birth. Enhanced index and frequency of phagocytosis were observed at 5 days of age. As clinical outcomes of vitamin E treatment in premature infants vary with dose, the route of administration, and the preparation of vitamin E used, administration to premature infants should be done with caution, with dose and route of administration carefully examined.
Vitamin E and Infection

**Vitamin E Deficiency and Infection**

Beck *et al.* (122) showed that vitamin E deficiency can be detrimental to mice infected with coxsackievirus. Vitamin E deficiency in mice exacerbated the cardiac damage caused by a virulent myocarditic strain of the coxsackievirus and allowed the nonvirulent amyocarditic CVB3/0 strain to become myocarditic. Viral passage experiments showed that the increased virulence of both these viral strains was caused by phenotypic change in the viruses as a result of their replication in a vitamin E-deficient host. It is thought that the nonspecific effect of oxidative stress is responsible for these results as N,N'-diphenyl-p-phenylenediamine, a synthetic antioxidant structurally unrelated to vitamin E, was active in protecting vitamin E-deficient mice against the cardiotoxic effects of the coxsackievirus (123). On the other hand, prooxidant dietary conditions (menhaden fish oil-enriched and vitamin E-deficient) produce beneficial effects against lethal *Plasmodium yoelii* infection in mice that lack the ability to produce an acquired antimalarial immune response (124).

Whereas vitamin E deficiency has an effect on the outcome of infection, infection can alter vitamin E status of the host as well, leading to exacerbation. In animals, decreased vitamin E concentration in lung and liver was observed following influenza infection (125). Furthermore, retrovirus infection significantly reduced vitamin E levels in the liver, spleen, and thymus (126). These decreases in vitamin E levels may reflect increased oxidative stress that follows viral infection. In humans, plasma vitamin E concentrations were significantly lower in patients with acute or chronic viral hepatitis with high disease activity (95). Furthermore, significantly lower serum concentrations of α-tocopherol were observed in papillomavirus-positive patients with cervical intraepithelial neoplasia (127). Circulating α-tocopherol values were lower in patients infected with HIV; this decrease corresponded with increased malondialdehyde levels (128). Results from a study by Tang *et al.* (129) suggest that high serum levels of vitamin E can be associated with lower HIV-1 disease progression. Men in the highest quartile of serum vitamin E levels showed a 34% lower risk of progression to the acquired immunodeficiency syndrome compared with those in the lowest quartile.

**Vitamin E Supplementation and Infectious Diseases**

The immunostimulatory effect of vitamin E supplementation was shown to confer resistance against infection with different pathogens, in different species of animals. Lower mortality from *E. coli* infection in chicks (130), fewer clinical cases of mastitis in cows (131), faster recovery from chlamydia infection in lambs (132), higher survival from *Diplococcus pneumoniae* type I infection in mice (133), higher resistance to *Mycoplasma pulmonis* infection in rats (134), and lower influenza viral titers in mice (135) were observed with vitamin E supplementation. In a study by Hayek *et al.* (135), dietary supplementation with 500 ppm vitamin E significantly reduced lung viral titers in old mice infected with influenza virus. It was suggested that the effect
of vitamin E may be caused, in part, by preservation of antioxidant status and NK cell activity. Wang et al. (136) also reported that vitamin E supplementation prevented retrovirus-induced suppression of splenocyte proliferation and NK activity and partially restored production of IL-2 and γ-interferon by splenocytes. \textit{In vitro} addition of RRR-α-tocopheryl succinate to splenocytes from avian erythroblastosis virus-infected chickens resulted in normalization of T-cell response to concanavalin A and phytohemagglutinin, which is suppressed in infected animals (137).

Few studies have investigated the direct effect of vitamin E supplementation on the incidence of infectious diseases in humans. Harman and Miller (138) gave supplement (200 or 400 mg/d of vitamin E for 6 months) to 103 patients in a chronic care facility and determined the serum antibody titers to influenza virus vaccine and the number of cases of pulmonary, urinary tract, and other infections. No effect was seen of vitamin E on the serum titers or the incidence of infectious diseases. Unfortunately, because data on subjects' health status, medication use, and other relevant variables were not reported, it is hard to determine the effect of confounding factors. Meydani et al. (113) reported a nonsignificant ($p = 0.09$) 30% lower incidence of self-reported infection in elderly subjects receiving vitamin E supplement compared with a placebo group. Chandra (139) gave a multinutrient supplement formulation to 96 healthy elderly individuals for 12 months. The supplemented group had a greater antibody response to influenza vaccine and less infection-related illness than the placebo group. It is impossible to attribute the effect to a particular nutrient because the intervention included multinutrients. However, vitamin E was the only nutrient provided at greater than 400% of the recommended daily allowance (RDA); other nutrients were provided at 30% to 200% of the RDA. Results from the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) study showed no effect of 50 mg/d vitamin E supplementation on symptoms of chronic obstructive pulmonary disease (e.g., chronic cough, phlegm, or dyspnea) over a period of 5 to 8 years in male smokers (140).

Vitamin E supplementation has been used to improve the clinical outcomes of several infectious disease states, including viral hepatitis, chronic respiratory tract infection, and sepsis in humans and animals. In a randomized, double-blind, placebo-controlled study by von Herby et al. (141), treatment of patients with hepatitis C with 800 IU/d RRR—tocopherol for 12 weeks improved clinical indices indicative of liver damage. Alanine aminotransferase and aspartate aminotransferase levels were lowered after 12 weeks of vitamin E treatment. Andreone et al. (142) also reported improved biochemical and virologic outcomes in chronic hepatitis B with 3 months of vitamin E supplementation (600 mg/d). However, Yurdakök and Kanra (143) did not observe beneficial effects of vitamin E therapy (300 mg/d intramuscular injection for 7 days) in children with acute viral hepatitis. These conflicting results may reflect difference in the duration of treatment.

**Comment**

Vitamin E deficiency, both in animal models and in humans, impairs the immune response and renders the host (animal models) more susceptible to infectious diseases.
Severe primary vitamin E deficiency rarely occurs. However, marginal deficiency is observed in premature infants, in malnourished children, and following viral and bacterial infection. Further studies are needed to determine the role of vitamin E status in the resistance of premature or malnourished infants to infectious disease.

Clinical trials in the aged have shown significant improvement in immune response. This effect in animal models is associated with increased resistance to influenza infection. Such information is not yet available in humans. At least two clinical trials in the United States and Europe are currently addressing this question. Similarities exist between immunologic changes in the elderly and in malnourished children. The success of vitamin E supplementation in improving the immune response in elderly people suggests that vitamin E may produce similar results in malnourished children. Studies are needed to determine the role of vitamin E supplementation in maintaining the host defense of malnourished, low birthweight infants against pathogens.

Research is needed to determine the mechanisms by which vitamins E and A exert their effect, as well as those of supplementation on immune response and infection. Such information will help in developing more effective intervention strategies. In particular, such information is needed to determine the type of infection and host conditions most conducive to effective interventions.

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DISCUSSION

Dr. Cochran: It has been mentioned many times during this conference that it is uncommon clinically to see isolated nutrient deficiencies. This morning both you and Dr. Chandra have shown not only that nutrient deficiency adversely affects the immune system, but also that excess nutrients can have adverse effects too. I would like your opinion, in a case where an individual is deficient in both iron and vitamin E and only a therapeutic dose of iron is given without supplementing vitamin E, whether that will adversely affect the immune system?

Dr. Meydani: My guess would be yes. If there is vitamin E deficiency, reversing that will be beneficial. However, this has not been well studied.

Dr. Chandra: I think that, with the sole exceptions of iodine, iron, and vitamin A deficiencies, we often deal with multiple nutrient deficiencies as a result of a reduced dietary intake. This applies to all age groups in the elderly. Therefore, for arguments that I have made elsewhere (1–3), I do believe very strongly that supplements of a single nutrient, be it a trace element or a vitamin, sometimes cause more problem than benefit. You never know what other nutrients may become conditionally deficient if you give large amounts of a single nutrient. So, I would be very cautious, particularly in people who are already immunocompromised, of giving very large amounts of one nutrient that may induce problems of absorption, interaction, and requirements of another nutrient.

Dr. Meydani: I do not totally agree with Dr. Chandra’s statement because I believe we need to know what particular deficiencies exist, or what nutrients are a problem for specific situations, and deal with these. I think the idea of supplementing the whole range of nutrients for every situation will not really help us in understanding how we need to deal with each situation. So, I think we need to understand better what are the particular nutrient problems that exist in different situations and deal with them directly.

Dr. Zoppi: In a study published in 1982 in the *Journal of Pediatric Gastroenterology and Nutrition* (4), we studied infants fed from birth with soy milk. We showed that T-cell immunity was impaired in those children. We think that could have resulted from a deficiency of vitamin A or zinc because of the presence of phytates. I would like your opinion on that.

Dr. Meydani: I do not believe it has been shown that feeding soy-based formulas or soy protein to animal models causes vitamin A or zinc deficiency.

Dr. Chandra: Earlier soya-based formulas did have problems with zinc absorption, but with the additional amounts of zinc that are currently being used, that is no longer a problem.

Dr. Novelli: The National Institutes of Health is about to start a large vaccination program on elderly patients, giving varicella vaccine to prevent herpes zoster. I might have missed it in your talk, but does vitamin E supplementation in elderly patients prevent zoster or decrease its incidence?

Dr. Meydani: We have not looked at that. Currently, we are doing a large study with 600 nursing home residents, looking at the effect of vitamin E supplementation on infectious disease. Our emphasis is on respiratory infections but we certainly will be looking at other types of infection as well.

Dr. Woodward: With regard to the community-based vitamin A supplementation studies showing decreased childhood mortality, my impression has been that the programs are successful in populations in which protein-energy malnutrition manifests primarily as a stunting disease. Is that true?

Dr. Meydani: My understanding is that the effect was independent of protein-energy malnutrition; in other words, it was also effective in children who were not severely malnourished or who had stunting.
Dr. Kennedy: I think you have a unique opportunity to study a population that is at risk for T-cell immune defects. My comments are more from an immunologist’s point of view. We have begun to use our large T-cell model as a mechanism with which to screen micronutrients. Based on your work and that of Dr. Chandra and others, we picked three nutrients: vitamin E, zinc, and vitamin D₃. We have used different stimulants. We looked at the interleukin 2 gene and asked what specifically was going to activate IL-2 gene transcription in humans. We focused on T-cell antigen receptor ligation and whether or not co-stimulatory molecules such as a CD28 or possibly inhibitory molecules such as CTLA4 would be influenced by supplementation with these molecules. Looking at CD3 and CD28 stimulation, we found that vitamin E actually suppressed IL-2 and did not increase it, but when we applied stimulation, such as with phytohemagglutinin (PHA), there was an increase in comparison with controls. Thus, the IL-2 response generated by vitamin E plus PHA was augmented compared with control plus PHA. We found IL-2 levels consistent with yours, from 100 to 200 pg/ml per culture. When we looked at anti-CD3 cross-linking and anti-CD3 with co-stimulation with CD28, we found that vitamin E was actually inhibitory compared with control; we had IL-2 levels of about 900 pg/ml per culture, but when we added vitamin E, we got about 300 to 400 pg/ml. We hypothesized that vitamin E may be actually affecting the response by activating PI3 kinase. Therefore, we started looking at CTLA4 interactions, as PI3 kinase is clearly involved with CTLA4, maybe as a shut-off mechanism. When we used cross-linking with CTL4 along with anti-CD3, we found that vitamin E was probably having its effect by activating PI3 kinase, because we found an even more pronounced inhibition of IL-2. Do you think that in this situation vitamin E may be playing a role in augmenting immune response by attenuating some of these reactions?

Dr. Meydani: Certainly. I did not have time to go into the mechanism-related work that we have done. It is interesting that you see suppression in your large T-cell model. I wonder if that is something specific to the particular cell, because we have done studies with purified T cells from mice looking at anti-CD3 and anti-CD28 and certainly in that situation we see an enhancement similar to what we see with PHA. In purified T cells from both young and old animals, there was an enhancement with vitamin E, so I am curious to the reason for the difference. Like you, we hypothesized that vitamin E, as well as inhibiting prostaglandin formation by macrophages which is one way of enhancing T-cell function, has some direct effect on the T cells by affecting signal transduction or perhaps by affecting nuclear transcription factors. In some other cell lines, in fibroblasts for example, vitamin E has been shown to affect API activation.

Dr. Kennedy: I agree. Our cell lines do not express CTLA4 to any large extent, so we need to transfect them and get them to express CTLA4. They have already been exposed to a plasmid pathogen, so there may be some influences there. We have only begun to look at gene transcription. My next question: In your isolated cells do you see activation of transcriptional regulators?

Dr. Meydani: We have not got that far. So far, we have just demonstrated an effect occurs on purified T cells. That is the next step.

Dr. Kennedy: Have you stimulated your cells with things like anti-CD3?

Dr. Meydani: Yes, that is what we have done. We used anti-CD3 and then anti-CD3 in combination with anti-CD28, which is where we actually see an increase in proliferation. That is interesting, because in other transformed cell lines vitamin E does inhibit proliferation. If you look at fibroblasts, for example, it inhibits proliferation. I think it has to do with the type of cell. When we test purified T cells using the same stimuli, we see enhancement.

Dr. Coovadia: I want to comment on your reference to a study on the return of delayed hypersensitivity and vitamin A in measles (5–6). The point I want to make is that when you
choose the wrong test and do it at the wrong time you will get the wrong result. There is no question in my mind that vitamin A is useful in measles. So the question is, what tests do you do? That study tested delayed hypersensitivity, but delayed hypersensitivity correlates very poorly with outcome in measles. Furthermore, the test was done at 2 weeks, and it takes about 6 weeks for the immune response and its various manifestations to return to normal after measles.

Dr. Meydani: I totally agree. Thank you for pointing that out.

Dr. Keusch: In some of the vitamin A studies that showed no effect of supplementation, I wonder if that was because there was no effect of vitamin A, or because vitamin E had a significant effect and was used as the control.

Dr. Meydani: I was surprised at those results. It seems that in the vitamin A field nobody is aware of what vitamin E does to immune function! I was disturbed to see it being used as a placebo; although the levels were low, they were certainly high enough to have an effect. That is an excellent point and needs to be pursued.

REFERENCES