

## **Impact of Micronutrient Deficiencies on Immune Function**

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Micronutrients such as vitamin A and zinc play a major role in immunity to infectious diseases during the weaning period and the first years of life. In the last two decades, clinical trials have shown that vitamin A or zinc supplementation reduces morbidity and mortality from infectious diseases among infants and children in developing countries [1, 2]. The underlying assumption from these clinical observations is that vitamin A and zinc improve immunity to certain infectious diseases, an observation that is corroborated by extensive observations from experimental animal models and in vitro studies [3, 4]. Iron supplementation has been shown to reduce anemia and improve cognitive development among infants and young children, but less is known about the role of iron in immunity to infectious diseases in humans [5]. The immunologic consequences of micronutrient malnutrition among infants and young children are far-reaching and include greater morbidity and mortality from diarrheal disease, pneumonia, measles, and human immunodeficiency virus infection, reduced vaccine responses, and impaired growth and development. The purpose of this article is to highlight the role of three micronutrients, vitamin A, zinc, and iron, in immune function among infants and young children and to identify major gaps in knowledge that remain to be addressed regarding the role of these micronutrients and others in immune function.

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### **Historical Background**

Early empirical observations on the use of vitamin A in individuals with tuberculosis, puerperal sepsis, and measles, led to the idea that vitamin A was the 'anti-infective vitamin' in the 1920s and 1930s [6]. In London Joseph Bramhall Ellison made the seminal discovery in 1932 that vitamin A supplementation reduced mortality in young children with acute complicated measles [3, 6]. In the first half of the 20th century, studies showed that vitamin A deficiency affected the growth and survival of experimental animals, and a large series of clinical trials was conducted using vitamin A as prophylactic or therapeutic treatment for a variety of infections [6]. Vitamin A was thought to be essential for the development of the lymphoid system and for the maintenance of mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts. By the 1960s, a great deal of evidence had accumulated that vitamin A was essential for normal immune function [7], and further large clinical trials of vitamin A supplementation were conducted in the last two decades which showed that vitamin A supplementation or fortification could reduce diarrheal disease morbidity and mortality in preschool children [1, 3].

Zinc was used empirically for treating diseases, including diarrhea, during the 19th century. By the 1930s, zinc was considered to be essential for the growth of animals and low zinc levels were described among adults in China, but a syndrome of human zinc deficiency was not described until the 1960s by Prasad and colleagues. The recommended dietary allowance for zinc was not established by the National Academy of Sciences until 1974, which belies the relatively recent recognition of zinc as an essential nutrient. Zinc supplementation was evaluated as a therapeutic intervention for diarrheal disease by Sachdev and colleagues in 1988, and during the 1990s, many studies were conducted to evaluate zinc supplementation for diarrheal disease, pneumonia, malaria, and child growth and development [8]. Experimental animal studies conducted in the last two decades have shown that zinc-deficient animals are more susceptible to a wide variety of infections [4] and that zinc is required for many aspects of immune function [4, 9].

Iron has long been recognized to play a role in anemia, but the role of iron in immunity to infectious diseases has been less clear. Since the 1920s, the data have been inconsistent: there have been studies that suggest that iron supplementation reduces the incidence of diarrheal and respiratory diseases among infants, and others that suggest that iron supplementation may worsen the morbidity from infections. Side effects of parenteral iron were reported, and studies from developing countries suggested that there might be a deleterious effect of iron on malaria and on respiratory infections [10]. There is still much speculation about iron supplementation being a 'double-edged sword' in iron deficiency, but harmful effects of iron supplementation may be more relevant to the situation of excess iron stores or in situations

where iron supplementation is given to children with malaria without any concomitant treatment for malaria.

### **Immune Function in Infants and Young Children**

The immune system is often functionally divided into innate and adaptive immunity, the former consisting of nonspecific immune defenses such as phagocytosis, natural killer (NK) cells, antimicrobial substances in mucosal secretions, etc., with the latter consisting of specific immune responses to certain pathogens that involve immunologic memory and rapid expansion of immune effector cells. The mucosal surfaces of the body include the respiratory, gastrointestinal, and genitourinary tracts as well as the cornea and conjunctiva. NK cells play a role in antiviral and antitumor immunity that is not major histocompatibility complex (MHC)-restricted, and NK cells are involved in the regulation of immune responses. Neutrophils play an important role in nonspecific immunity because they phagocytize and kill bacteria, parasites, virus-infected cells, and tumor cells. Macrophages are involved in the inflammatory response and in the phagocytosis of viruses, bacteria, protozoa, fungi, and tumor cells.

The adaptive immune system consists of antigen-specific responses that are mediated by T and B cells, and this includes humoral and cell-mediated immunity. Immunoglobulins, or antibodies, are secreted by after B-cell proliferation into plasma cells. Immunoglobulins contain antigen-binding sites that bind to specific antigenic sites on microorganisms or toxins. The binding of antibodies can neutralize pathogens by inhibiting them from binding to cell surfaces or by promoting phagocytosis. T lymphocytes can be divided into two major sublineages, CD4+ and CD8+ T cells. CD4+ T cells are major regulatory cells that provide help for B-cell activation through MHC class-II-restricted responses. CD8+ T cells can develop into cytotoxic T cells that play an important role in defense against viral infections through recognition of virus-infected cells that express MHC class-I molecules.

Infants are also a special case in that they are protected by maternal antibodies that cross the placenta during pregnancy, and by immunologically active substances in breast milk, such as antibodies, lactoferrin, lysozyme, cytokines, and chemokines that may protect the gut from pathogens and play a role in gut development.

Micronutrient malnutrition can affect various aspects of the immune response, and there is a great challenge in linking specific micronutrient deficiencies with certain immune compartments. Multiple micronutrient deficiencies may occur together, making it difficult to conclude from cross-sectional studies that particular defects in immunity are associated with specific micronutrient deficiencies. Clinical trials with single micronutrients may provide more definitive data regarding specific immune compartments, but investigators are

usually limited to studies of peripheral blood, mucosal secretions, breast milk, and skin testing. Much more data have been derived from experimental animal models in which the immune system is more accessible for investigation.

### **Micronutrient Deficiencies and Immune Function**

#### *Vitamin A*

Vitamin A modulates both innate and adaptive immunity [3]. Some immune compartments are not affected by vitamin A deficiency. Vitamin A deficiency is associated with loss of cilia in the respiratory tract, loss of microvilli in the gastrointestinal tract, loss of mucin and goblet cells in the respiratory, gastrointestinal, and genitourinary tracts, squamous metaplasia with abnormal keratinization in the respiratory tract, alterations in antigen-specific secretory IgA concentrations, impairment of alveolar monocyte/macrophage function, and decreased integrity of the gut. Vitamin A is involved in the expression of both mucins and keratins, and lactoferrin, an iron-binding glycoprotein involved in immunity to bacteria, viruses, and fungi, appears to be modulated in mucosal secretions.

In the respiratory tract, pathogens are constantly trapped and removed by the mucociliary elevator in the normal tracheobronchial tree, but the loss of ciliated epithelial cells and mucus and replacement by stratified, keratinized epithelium in vitamin A deficiency may increase susceptibility to respiratory infections [3]. Vitamin A deficiency also appears to affect the linings of the inner ear and eustachian tube, making children more susceptible to otitis media.

Vitamin A deficiency reduces the number of NK cells and impairs their activity [11]. The function of neutrophils appears to be impaired during vitamin A deficiency [12]. Impaired phagocytosis and decreased complement lysis activity may occur during vitamin A deficiency. During vitamin A deficiency, the hematopoiesis of some lineages, such as CD4+ lymphocytes, NK cells, and erythrocytes, appears to be impaired. In humans, clinical vitamin A deficiency has been characterized by lower total lymphocyte counts and decreased CD4+ lymphocytes in peripheral blood, and CD4+ lymphocyte counts or percentage increased after vitamin A supplementation [13, 14]. Vitamin A supplementation does not appear to have any long-term effect on CD4+ or CD8+ lymphocyte subsets among infants without clinical vitamin A deficiency [15].

Vitamin A deficiency has been linked with reduced phagocytic function by macrophages. Vitamin A deficiency may influence T-lymphocyte-related immunocompetence through modulation of numbers or distribution of T cells, changes in phenotype, alterations in cytokine production, or decreased expression or function of cell surface molecules involved in T-cell signaling [3]. In a trial in Bangladesh, vitamin A supplementation improved responses to delayed-type hypersensitivity skin testing among infants who were supplemented to higher vitamin A levels [16]. In experimental animals, vitamin A

appears to modulate the balance between T-helper type-1-like responses and T-helper type-2-like responses, but there is less evidence to support this model for human vitamin A deficiency [3]. Observations from clinical trials are not consistent with this model, as vitamin A supplementation has been shown to enhance immunity to a wide variety of infections such as tuberculosis, measles, malaria, HIV infection, and diarrheal diseases, where the specific immune protective immune responses have been characterized as either T-helper-1-like or T-helper-2-like responses.

Vitamin A deficiency impairs the growth, activation, and function of B lymphocytes. The hallmark of vitamin A deficiency is an impaired capacity to generate an antibody response to T-cell-dependent antigens [17, 18] including tetanus toxoid [19] and diphtheria antigens in humans [20], and T-cell-independent type-2 antigens such as pneumococcal polysaccharide [21]. These findings suggest that vitamin A deficiency may compromise immunity to many types of infections where the main immune defense is dependent upon antibody responses.

Controlled studies of vitamin A supplementation and aspects of immunity in infants and young children are summarized in table 1. Several trials have addressed the issue of vitamin A supplementation with measles vaccine at either 6 [22, 23], or 9 months of age [23–27]. When vitamin A supplementation is given simultaneously with live measles vaccine in 6-month-old infants who have maternal antibodies present, there appears to be an inhibitory effect upon antibody titers to measles [22]. In 9-month-old infants who have lower levels of maternal antibodies, vitamin A supplementation has been reported to have either no overall effect on antibody titers [24, 26] or to enhance antibody titers [23, 25]. Long-term follow-up among children who were previously immunized at 9 months of age shows that those who received vitamin A supplementation had higher geometric mean antibody concentrations against measles at age 6–8 years [27].

Vitamin A supplementation appears to improve gut integrity among hospitalized infants [28] and among infants whose mothers were supplemented with vitamin A/ $\beta$ -carotene during pregnancy [29]. Vitamin A supplementation, when integrated with oral poliovirus vaccination at 6, 10, and 14 weeks, did not influence seroconversion rates or geometric mean antibody titers to poliovirus types 1, 2, and 3 [30], although a study in India suggested that a slightly higher proportion of infants had protective antibody to poliovirus type 1 with no differences in antibody titers to types 2 and 3 [31]. A large trial from India recently showed that infants who received vitamin A at birth had reduced nasopharyngeal colonization by pneumococcus [32].

### *Zinc*

Zinc plays a role in both innate and adaptive immunity. Zinc deficiency impairs the function of neutrophils [33], NK cells [34], and chemotactic responses of monocyte/macrophages [4]. In preschool children, zinc

**Table 1.** Controlled studies of vitamin A and aspects of immunity in infants and young children

Location	n	Subjects	Intervention	Effects of vitamin A supplementation	Reference
Indonesia	236	Preschool children	30 mg RE vs. placebo	Enhanced IgG response to tetanus toxoid 3 weeks later; increase in circulating CD4%	13, 19
Indonesia	336	6-month-old infants	15 mg RE vs. placebo with measles vaccine	Reduced antibody titers to measles at 7 and 12 months; fewer infants with vaccine-related rash in vitamin A group	22
Indonesia	394	9-month-old infants	15 mg RE vs. placebo with measles vaccine	No impact of vitamin A on antibody titers to measles; antibody titers to measles at 10 and 15 months	24
Guinea Bissau	312	9-month-old infants	15 mg RE vs. placebo with measles vaccine	Enhanced antibody titers to measles at 18 months; higher antibody titers at 6–8 years	23, 27
Guinea Bissau	150	6-month-old infants	15 mg RE vs. placebo with measles vaccine	Higher antibody titers to measles after repeat vaccination at 9 months	23
India	100	9-month-old infants	15 mg RE vs. placebo with measles vaccine	Enhanced antibody titers to measles at 10 months	25
India	618	9-month-old infants	15 mg RE vs. placebo with measles vaccine	No impact on antibody titers to measles at 12 months; enhancement of antibody response in subgroup of malnourished infants	26
India	56	Infants	15 mg RE vs. placebo with DPT vaccine	Enhanced IgG response to diphtheria toxoid	20

India	120	Infants	15 mg RE/month with DPT vaccine	Increased skin test responses among subgroup of those supplemented to higher vitamin A levels	16
India	144	Hospitalized infants	(1) 60 mg RE at admission, (2) 60 mg RE at discharge, or (3) placebo at discharge	Improved gut integrity in groups 1 and 2 vs. group 3	28
India	80	Infants	16,700 IU/week vs. placebo	No difference in gut integrity	28
South Africa	238	Infants	1.5 mg RE and 30 mg $\beta$ -carotene/day to mothers during pregnancy	Improved gut integrity	29
Indonesia	467	Infants	7.5 mg or 15 mg RE, vs. placebo at 6, 10, 14 weeks with oral poliovirus vaccine	Seroconversion rates of 98–100% in all three treatment groups, no differences in mean antibody titers to types 1, 2, or 3 between treatment groups	30
India	399	Infants	7.5 mg RE vs. placebo at 6, 10, and 14 weeks with oral poliovirus vaccine	Higher proportion with protective antibody titer to poliovirus type 1; no differences in antibody titers to poliovirus types 2 and 3	31
India	464	Infants	7 mg RE vs. placebo at birth	Reduced nasopharyngeal colonization by pneumococcus	32

supplementation was associated with an increase in delayed-type hypersensitivity skin responses to multiple antigens [35]. Another trial among low birth weight infants showed that zinc supplementation had no effect on delayed-type hypersensitivity skin responses to phytohemagglutinin [36]. The investigators noted that phytohemagglutinin is a strong antigen, and the test may have been insensitive to detect more subtle differences in immunity. Another study that used a multiple antigen skin test to several antigens (tetanus, diphtheria, tuberculin, *Candida*, *Trichophyton*, and *Proteus*) showed that zinc supplementation reduced anergy by skin testing [37].

The number of circulating CD4+ and CD8+ lymphocytes decreases during zinc deficiency [4, 34, 38]. The number of circulating CD8+ CD73+ T cells, which are mostly precursors for cytotoxic T lymphocytes, are reduced during zinc deficiency [38]. A decrease in CD8+ CD73+ T cells could possibly increase the susceptibility of zinc-deficient individuals to viral, parasitic, and bacterial infections [38]. Zinc deficiency plays a role in thymic atrophy and may modulate maturation of T lymphocytes [4, 39].

Zinc deficiency is associated with impaired antibody responses to T-cell-dependent and T-cell-independent antigens [4]. In experimental human zinc deficiency, peripheral blood mononuclear cells showed a decrease in interferon- $\gamma$  and interleukin (IL)-2 production, but no change in IL-4, IL-6, and IL-10 production, suggesting that there may be a depression of Th1-like responses during zinc deficiency [40].

Many studies have shown that zinc supplementation reduces the morbidity from infectious diseases [8]. Among the more recent studies are investigations among infants in Ethiopia [41], infants in Bangladesh [42], and infants and young children in India [43], which show that zinc supplementation is associated with reductions in infectious disease morbidity. A few controlled trials have addressed the effect of zinc supplementation on aspects of immunity in infants and young children, and these are summarized in table 2. A recent trial among preschool children shows that oral zinc supplementation increases the antibody response to killed oral cholera vaccine [44]. Among infants and young children, zinc supplementation was associated with increases in CD3+ and CD4+ T cells, and an increase in the CD4/CD8 ratio [38].

### *Iron*

In contrast to vitamin A deficiency and zinc deficiency, less is known about the role of iron in immune function in humans, and much of the available data are inconsistent [5] (table 3). Iron deficiency appears to affect neutrophil function [45], impair NK cell function, and reduce delayed-type hypersensitivity skin testing [5, 46]. It is not clear whether iron deficiency affects lymphocyte proliferation responses to mitogens or the composition of T-cell subsets in peripheral blood, as results from various studies have been inconsistent [5, 10]. Iron deficiency does not appear to impair antibody responses following immunization in children [46, 47]. There are few solid data

**Table 2.** Controlled studies of zinc and aspects of immunity in infants and young children

Location	n	Subjects	Intervention	Effects of zinc supplementation	Reference
Ecuador	50	Children 12–59 months	10 mg/day vs. placebo	Higher delayed type hypersensitivity responses at day 60	35
India	66	Children 6–35 months	10 mg/day vs. placebo	Higher delayed type hypersensitivity responses at day 120; increase in CD3, CD4, and CD4/CD8 ratio	37
Brazil	134	Low birth weight infants	5 mg/day vs. placebo birth to 8 weeks	No differences in phytohemagglutinin skin tests at 8 weeks; reduction in diarrhea and cough	36
Bangladesh	256	Children	20 mg/day vs. placebo; 60 mg RE vitamin A in 2 × 2 factorial design	Improved seroconversion to vibriocidal antibody of killed oral cholera vaccine	44

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**Table 3.** General influence of selected micronutrient deficiencies on immunity

Immune component	Vitamin A	Zinc	Iron
Phagocyte function	↓	↓	↓
Natural killer cell function	↓	↓	↓
T-cell subsets (CD4+, CD8+), circulating	↓	↓	↔
Lymphocyte proliferation	↓	↓	↔
Delayed-type hypersensitivity	↓	↓	↓
Cytotoxic T-cell function	↓	↓	↔
Antibody responses	↓	↓	↔
Monocyte function	↓	↓	?
Mucosal surfaces	↓	?	↔
Apoptosis	?	↓	?
Breast milk immune factors	?	?	?

↓ = Decreased; ↑ = increased; ↔ = no effect; ? = unknown or not well characterized.

from observational studies that show that iron deficiency increases the morbidity and mortality of infectious diseases [10], and it appears to be fairly clear that increased infectious disease morbidity is not part of the syndrome of iron deficiency [46]. Oral iron supplementation has not been associated with a reduction in the morbidity of infectious diseases [10], and a recent systematic review of 28 controlled clinical trials shows that iron supplementation has no apparent effect on the incidence of infectious diseases, including malaria, except for a slightly increased risk of diarrhea [48].

### *Other Micronutrients*

Iodine deficiency is highly prevalent in certain areas of the world among infants and young children, yet iodine deficiency remains one of the most promising, yet neglected areas for investigation in nutritional immunology. Iodine supplementation has been shown to reduce infant mortality [49], but little has been done to examine the relationship of iodine to immune function in humans. Vitamin D deficiency has been associated with immune abnormalities and may influence macrophage function [50], and further studies are needed to characterize the relationship between vitamin D status and immune function in infants and children.

### **Future Directions**

There are several general areas that merit attention in future studies of the effects of micronutrient status on immune function in humans. Controlled clinical trials of single micronutrient supplementation may provide the most

valid data for assessing the effects of single micronutrients on specific immune compartments, provided that there is sufficient statistical power to examine the immunological endpoint being addressed and that there is evidence that individuals with marginal or deficiency micronutrient status are being supplemented to a replete state. Of all the micronutrients, the relationship of vitamin A to immune function is the best characterized, but there are still notable gaps in knowledge relevant to infants and children, such as the possible effects of vitamin A deficiency on immunological modulators in breast milk and on transfer of maternal antibodies from mother to infant. Much work remains to be done with regard to zinc deficiency, especially on the relationship between zinc deficiency to antibody responses following immunization, and the effect of zinc deficiency on mucosal immunity in humans. Studies using newer methods such as fluorescence in situ hybridization (flow FISH), more complex flow cytometry, single cell gel electrophoresis, and microarrays could be used to study the effects of micronutrient deficiencies upon telomere shortening, clonal expansion of T cells, DNA damage in lymphocytes, and cytokine and chemokine expression by specific immune effector cells. Such new data could provide insights into the overall role of micronutrients in the maturation of the immune system in infants and children.

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## Discussion

*Dr. Tolboom:* Thank you for a very clear and comprehensive presentation. I have a question related to gut integrity. You mentioned that vitamin A supplementation improves gut integrity. How was that checked? Were permeability studies done? How could, e.g., effects on villous atrophy be differentiated?

*Dr. Semba:* The lactose/mannose ratio was analyzed. So these are permeability studies.

*Dr. Albar:* Nephrotic syndrome, especially minimal chain nephrotic syndrome, is thought to be caused by T-cell dysfunction. You mentioned that zinc plays a role in T-cell function. If zinc deficiency is repaired in the neonate, can this syndrome be prevented in infants and children? Some treatments, for example levamisol, have been tried to stimulate T-cell functions in children with minimal change nephrotic syndrome as well as prednisone therapy. So if zinc is routinely given to neonates, perhaps we can also prevent children from developing minimal chain nephrotic syndrome. Any comment?

*Dr. Semba:* I missed the first part of the question. What kind of syndrome?

*Dr. Albar:* Minimal chain nephrotic syndrome, is one of the renal diseases which mostly occurs in children presenting with generalized edema, massive albuminuria, and heavy albuminemia.

*Dr. Semba:* You think zinc supplementation can correct that?

*Dr. Albar:* The supplementation of zinc may correct minimal change nephrotic syndrome in children if zinc has a similar T-cell-activating effect as levamisol. A clinical study is necessary to make any conclusions.

*Dr. Semba:* I am not aware of any data.

*Dr. Albar:* Levamisol, as a stimulator of T-cell function, has been tried based on the hypothesis that T-cell dysfunction is a cause of minimal change nephrotic syndrome

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in children [1–6]. If we can prevent zinc deficiency in neonates, this may stimulate T-cell function normally, and subsequently we could also prevent the development of nephrotic syndrome in children. But a zinc supplementation trial seems to be necessary to support the role of zinc as a T-cell stimulator in this syndrome. So zinc deficiency has not only an impact on infections but also on the syndrome.

*Dr. Semba:* This is an interesting idea. I think someone needs to address this.

*Dr. Pettifor:* A nice cover of the immune situation. If I quote Moore et al. [7] correctly from studies in the Gambia, they showed that babies with intrauterine growth retardation didn't have an increased mortality in childhood. But there seemed to be significant effects on adult mortality once they were into adult life. Do you have any comments on this? It looks as though early infant malnutrition might well influence long-term immune function, mortality and morbidity, and infections in adult life.

*Dr. Semba:* This is an important phenomenon that needs to be addressed, and the first way to address this will be to look at T-cell subsets to see whether some markers are being altered. For example, in older adults immune dysfunction is associated with loss of the CD28 receptor which occurs earlier on in life and it is linked to cytomegalovirus infection. So one must ask what happens earlier in life that could cause the loss of CD28, for example, from CD8 and CD4 T cells. Now whether this is something that happens earlier on and influences immunity later, we don't know for certain. I hope I have answered your question.

*Dr. Zlotkin:* As a pediatrician I have always been taken by the fact that in order to assess immunologic status a lot of blood is needed because white blood cells are being measured, and in young infants that precludes a lot of studies that perhaps could be done in adults. My question has to do with indirect markers of immune function, and let me just give an example from vitamin A and from zinc. We can define vitamin A status based on the serum level, or below a serum level we say someone may be deficient in vitamin A. For zinc it is a bit harder because we really don't have super markers which could distinguish between severe zinc deficiency because of the clinical science, but for moderate zinc deficiency it is often difficult to define that population. Are there any indirect markers with regard to zinc or vitamin A that would help us to find the immunologic risk in an individual subject?

*Dr. Semba:* I don't know of any good marker that can be used for that. One thing that might be useful for looking at comorbidity that we can't observe very well, and we discussed this yesterday, would be to look at different acute phase proteins that have different dynamics of response to an acute phase reaction. That might tell us a little bit about what is going on, but it is not to say that there is a certain CD4/CD8 ratio where you see something like moderate deficiency. I don't think we have anything.

*Dr. Zlotkin:* About vitamin A specifically: there is a definition of vitamin A deficiency based on the serum level. Is that level actually related to immunological dysfunction or function?

*Dr. Semba:* Again that is hard to sort out because there is an effect when studying this in children. There can be children with low vitamin A levels, but a high proportion of them have elevated C-reactive protein and  $\alpha_1$ -glycoprotein. It is hard to interpret. I don't know if anybody has actually done a dose-response study to find out at what vitamin A level T-cell subsets are altered.

*Dr. Villalpando-Carrión:* I am interested in the use of antioxidants not only for immunological purposes of this nutrient but also in fat metabolism, etc. What kind of dose and what type of vitamin A did you use for the HIV children, and did you have any antioxidant evidence or reactive oxygen species activity or levels in plasma or something like that?

*Dr. Semba:* The dose we used in the Uganda study was 60 mg RE, or 200,000 IU, which is the standard UNICEF capsule, and we gave that every 3 months. We don't have any indicators of antioxidant status.

*Dr. Villalpando-Carrión:* Do you have plasma?

*Dr. Semba:* Yes, we do.

*Dr. Tolboom:* I have a question on iron and malaria. You mentioned that there is no adverse effect of iron supplementation in a population of children living in malaria-endemic areas. But you gave a word of caution, you said that we have to be careful with untreated severe malaria. I would like you to elaborate a little bit on that in view of the fact that 80% of African children, in sub-Saharan Africa, if they die, die at home, and most probably die of malaria. So we don't know what the effect on severe illness is, and the mortality of supplementation programs. All the programs in which iron supplementation is used are connected to anti-malaria treatment or prophylactics, for example. Could you elaborate a little bit on that?

*Dr. Semba:* There is an unpublished meta-analysis by Shankar showing that iron supplementation is associated with a slight increase in malaria morbidity, and Oppenheimer [8] has also looked at this and published it in the *Journal of Nutrition*. I made this cautionary note because I have heard that there is an ongoing study in which the iron arm was dropped because of some apparent effect on malaria, but I don't know the details.

*Dr. Endres:* In this workshop we are mainly talking about deficiencies which occur in many infants, and apparently it is somewhat difficult to study the consequences of clinical micronutrient deficiencies, e.g. the impact on immune function. I would like to ask you whether in some human models of severe micronutrient deficiencies such as zinc, copper and selenium, acrodermatitis enteropathica, Menkes disease and Keshan disease would be wonderful human models to study the impact on the immune system. Are you aware of any studies in these diseases?

*Dr. Semba:* I think acrodermatitis enteropathic disease has been studied. What else did you mention?

*Dr. Endres:* The next one would be Keshan disease, a cardiomyopathy due to selenium deficiency, which has practically disappeared from China.

*Dr. Semba:* I am not aware of any immune studies that have been conducted with Keshan disease.

*Dr. Guesry:* We have been studying selenium deficiency in animal models in collaboration with Dr. Beck in conjunction with influenza virus infection, and it was shown that it was not the immune defense of the animal which was impaired by selenium deficiency or vitamin E deficiency, which gave the same effect, but the virulence of the virus [9]. In the presence of selenium deficiency or vitamin E deficiency, influenza virus, becomes more virulent.

*Dr. Semba:* I think that it is a very interesting model that has been developed and perhaps it raises some questions that we might be seeing viral mutations coming from areas where a large part of the population is micronutrient-deficient. That is something that people have been extrapolating from these animal studies.

*Dr. Bloem:* You showed a slide of a market with a lot of vegetables and fruits, and I have a question about  $\beta$ -carotene or carotenoids. If you look at a study on maternal mortality, one of the Hopkins studies [10], it shows a reduction in maternal mortality actually more by  $\beta$ -carotene than by vitamin A. It was not significant and that is why it is a vitamin A-related mortality. We also know from some studies that the  $\beta$ -carotene is not very effective in vegetables. So could you elaborate a little bit more on whether  $\beta$ -carotene in itself or other carotenoids perhaps have an effect on the immune status besides the vitamin A effect, so that vegetables could potentially have an effect on mortality reduction?

*Dr. Semba:* There is an excellent review on this by Bendich [11], and I think as far as  $\beta$ -carotene is concerned it goes beyond the pro-vitamin-A quality, and some of the properties of  $\beta$ -carotene in immune response relate to its property as an antioxidant, which is of course much stronger than that for retinol. But there is a lot we don't know

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about the excentric cleavage of  $\beta$ -carotene. We don't know what happens with all these other excentric cleavage products. What role do they play in immunity, and I think that hasn't been well studied. But  $\beta$ -carotene has been shown to have similar effects on immune function as vitamin A.

*Dr. Bloem:* So why then don't they use  $\beta$ -carotene instead of vitamin A in supplements for example?

*Dr. Semba:* Cost has got to be the main thing.

*Dr. Delgado:* Pro- and anti-inflammatory cytokines can be released. Which specific supplementation could improve anti-inflammatory cytokines?

*Dr. Semba:* That was perhaps vitamin E, I think this has been shown in studies in Toronto [12].

*Dr. Horton:* I know the focus in the sessions here is on the weaning period in the first year of life, but another group where we might see the effects of micronutrient deficiencies on immune function would be in the elderly, and I wonder if you could comment on any of those studies.

*Dr. Semba:* There have been some studies looking at immune function in the elderly. A lot of these studies have come from Europe and we see some effects of micronutrient malnutrition on T-cell subsets. But I think that when we are dealing with older adults, the main deficiencies we are dealing with are vitamin D or low vitamin E and zinc. Some studies have been conducted by Bogden [13] in New Jersey looking at zinc and immune function in older adults. Meydani [14] showed that with lymphocyte proliferation, by supplementation you can increase these indicators of immune function.

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