MINIREVIEW

Vitamin A Status: Relationship to Immunity and the Antibody Response (43436A)

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Vitamin A (retinol and its derivatives) is essential for a variety of biologic processes, many of which are related to growth, cellular differentiation, and cell-cell or cell-substrate interactions. Within the last 5 years, there has been a renaissance of interest in vitamin A and immunity that is based largely on three important advances. First, a number of controlled, field-based or hospital-based, studies have demonstrated that vitamin A supplementation can decrease mortality in preschool children in populations at high risk for vitamin A deficiency. Although a mechanistic link between child survival and improved immunity has yet to be established, it seems quite probable that at least some of the benefit of vitamin A is due to restoration of epithelial barriers and improved resistance to respiratory and gastrointestinal infections. Second, vitamin A, either in the form of retinol or its metabolite, retinoic acid, has been shown to stimulate the rejection of certain immunogenic tumors; this effect may be due to enhanced immunologic surveillance. Third, there has been a swell of interest in vitamin A's mechanism of action in nearly all organ systems as the result of the identification of nuclear receptors for retinoic acid and, consequently, advances in understanding the role of retinoic acid in gene regulation.

Vitamin A deficiency, even in relatively early stages, is associated with impairment of linear growth, cartilage, and bone development and changes in epithelial cell differentiation and function (1). If vitamin A deficiency is allowed to persist, animals either succumb or, if they survive, develop progressive xerophthalmia leading to blindness. In parts of the developing world, particularly Southeast Asia and sub-Saharan Africa, xerophthalmia remains a significant, and preventable, public health problem (2).

Both clinical and experimental evidence has shown that vitamin A deficiency is associated with decreased resistance to infection. Vitamin A deficiency could affect immunity through a number of mechanisms including (i) changes in lymphopoiesis and lymphocyte maturation, (ii) abnormal cytokine production, (iii) altered membrane structure affecting receptors for antigens, accessory molecules, or cytokines, (iv) increased penetration of bacteria, vires, and parasites through epithelial barriers, and (v) impaired clearance of pathogens by cytotoxic and phagocytic mechanisms.

The principal focus of this review will be on an examination of the evidence that vitamin A status, ranging from frank vitamin A deficiency to the highly supplemented state, has an important influence on the immune system and its various modes of response. Aspects of cellular immunity and nonspecific immunity will be considered, but the major emphasis will be on the humoral antibody response. From this review, it should be possible to identify some of the critical gaps in knowledge as well as some of the ways that new information may be used in future experiments and to improve intervention strategies in human populations.

Historical Background, Previous Reviews, and Overview

The potential relationship between vitamin A status and infectious disease has been appreciated for decades. The discovery of vitamin A in 1913 by McCollum and Davis (3) was followed in the 1920s by Bloch's (4) description of an association of vitamin A deficiency with malnutrition and by the histologic stud-

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ies of Mori (5) and Wolbach and Howe (6), who demonstrated that vitamin A is required for maintenance of normal epithelial morphology. Soon after the identification of vitamin A as an essential nutrient, investigators began to study experimental and natural infections in animals and to correlate these observations to human pathology (see Table I). Werkman (see Ref. 7) reported in 1923 that rats fed a diet of natural components, described as vitamin A deficient, were less resistant to typhoid or anthrax bacilli, although neither the serum agglutination response nor opsonic activity appeared to be decreased. Mortality to mouse typhoid was reported to be greater in vitamin A-deficient mice than in mice fed an adequate diet (7). In the late 1920s, Green and Mellanby (8, 9) showed that animals fed diets deficient in vitamin A and carotene often died with histopathologic evidence of infections, largely of the tongue, eyes, and bladder. Such spontaneous infections were seen very rarely in rats fed the same diet plus vitamin A. Lassen (7) reported decreased resistance to a specific infection in vitamin A-deficient rats. In contrast to control rats, which recovered after infection with paratyphoid bacilli, nearly all vitamin A-deficient rats died; similarly, whereas few bacteriologic cultures of normal rat tissues were positive, many of the cultures from vitamin A-deficient rats were positive, including those of the mesenteric lymph glands and submaxillary glands. Lassen (7) commented that infection in vitamin A-deficient animals did not seem to differ qualitatively from that in normal rats, but rather that infection persisted in the vitamin A-deficient state. Thus, by 1930, experimental studies had convincingly documented the association of infection and vitamin A deficiency.

A large number of subsequent studies have led to or supported the conclusion that marginal vitamin A status is often worsened by infectious diseases (of bacterial, viral, or parasitic origins) and that, reciprocally, poor vitamin A status is likely to prolong or exacerbate the course of illness. In an important review in 1968 of over 350 experimental studies on nutrition and infection disease, Scrimshaw *et al.* (10) wrote that "no nutritional deficiency is more consistently synergistic with infectious disease than that of vitamin A." Subsequently, Nauss (11) reviewed the literature through 1985 on the influence of vitamin A status on immune functions in humans and animals. This report has been taken as a point of departure for this review, which focuses primarily on more recent investigations.

Table I. Observations on Resistance to Infection in Vitamin A-Deficient Animals[a]

	Species	Reference
↓ Resistance to typhoid, anthrax, and paratyphoid bacilli	Rat	7
↓ Resistance to endogenous infection	Rat	120
↓ Resistance to *Escherichia coli* infection	Chick	49
Histopathologic evidence of infection	Rat	8
Longer survival of vitamin A-deficient animals when germ-free or given antibiotics	Rat Chick	21, 22, 24 23
↑ Corneal inflammation, ulceration, and necrosis after herpes virus infection	Rat	34
↑ Leukocyte infiltration and corneal ulceration after *Pseudomonas aeruginosa* infection	Rabbit	79
Impaired clearance of parasites and ↑ worm burden	Rat, mouse	81
Intestinal villus destruction in vitamin A deficiency combined with rotovirus infection	Mouse	78
↓ Clearance of *E. coli* and ↓ *in vitro* phagocytic activity	Rat	120
↓ Antibody response to *Schistosoma mansoni* infection	Rat	81
↓ Antibody response to Newcastle disease virus or *Salmonella pullorum* antigen	Chick	44, 75

[a] See also earlier reviews (10, 11).

Animal Models for Studies of Vitamin A Deficiency

Most investigators have used either the rat, mouse, or chick to examine the relationship between vitamin A status and the immune response. Each species has certain advantages: for example, the metabolism of vitamin A has been studied most extensively in the rat, and, thus, the nutritional requirements, rates of tissue exchange of retinol, transport proteins, and cellular retinoid metabolism are best characterized for this species (1, 12). The number of inbred strains of rat is fewer than for the mouse, but this species still provides opportunities to dissect interactions that are restricted by histocompatibility loci. Vitamin A deficiency can also be induced in the mouse (11, 13, 14), for which a wide range of immunologic reagents are available. The rabbit has been used for a smaller number of studies on ocular infection. Unlike most rodents similar to man, the chick absorbs certain carotenoids intact (15). Therefore, the chick may serve a role in studies of the potential effects of carotenoids (only a few of which are vitamin A precursors) on the immune system.

In animals fed a diet containing adequate vitamin A, reserves of retinol (as retinyl ester) accumulate in the liver, the major storage organ for vitamin A, and in other tissues during the suckling and postweaning periods (13, 16). Retinol is well conserved: it is eliminated from the body only after several passages between liver and peripheral tissues (17); therefore, depletion of established vitamin A reserves occurs very slowly. Because chronic vitamin A deficiency is associated with loss of appetite and inanition, the later stages of vitamin

A deficiency are usually compounded by general malnutrition. Two nutritional paradigms have been utilized to produce vitamin A deficiency in a timely fashion and to attempt to balance of overcome the concomitant effects of protein-energy malnutrition (11, 14). One strategy is to eliminate all forms of vitamin A from the diet of young, growing animals to induce deficiency and to pair-feed control animals with vitamin A-deficient animals so as to maintain nearly equal rates of growth. This strategy works well during the early stages of vitamin A deficiency, when anorexia is not severe. Vitamin A depletion may be achieved even more rapidly if nursing dams are fed a vitamin A-free diet to limit transfer to the young via milk, or if pups are weaned from their dams onto a vitamin A-free diet a few days earlier than usual (e.g., when rats are 16–18 days old) (13, 18, 19). A second strategy, retinoic acid cycling, developed by Lamb and colleagues (20), makes use of the observations that (i) retinol-deficient animals grow well if they are given supplementary retinoic acid, and (ii) very little retinoic acid is stored. If retinoic acid is removed from the diet of retinol-deficient animals, a rapid and nearly synchronous vitamin A deficiency ensues. This strategy minimizes the effects of anorexia and malnutrition; however, it probably is not so characteristic of the gradual onset of vitamin A deficiency that occurs in humans. As has been pointed out by Nauss *et al.* (14), it has not yet been demonstrated that the two methods of vitamin A depletion have comparable effects on the immune system.

Resident intestinal bacteria may influence nutrient availability and affect the immune response through the agency of bacterial products (e.g., endotoxins and enterotoxins). Most studies have been conducted with conventionally housed animals having normal microflora. Vitamin A-deficient, germ-free rats and chicks have been reported to survive longer than conventionally housed controls (21–24), as have vitamin A-deficient rats supplemented with antibiotics (24). The reasons for prolonged survival have not been determined, but the absence of infection would seem an obvious factor. The rate of retinol utilization might also be significantly reduced.

Changes in Lymphoid Cells and Organs during Vitamin A Deficiency

Changes in lymphoid organ mass, cell distribution, histology, and lymphocyte characteristics have often been reported to accompany the vitamin A deficiency state, but the consistency of these reports is not striking. Vitamin A-deficient rats (25) and chicks (26) have been noted to develop a leukopenia characterized by an increase in neutrophils and a decrease in the number of circulating lymphocytes (25). Some of the earliest reports of vitamin A deficiency noted marked changes in epithelia and lymphoid organs (6). The exact pathological picture, however, seems to be dependent upon the duration of vitamin A deficiency and the animal species. In the rat, the weights of the spleen and thymus were unchanged early during vitamin A depletion (25, 27, 28) or somewhat decreased late in the course of vitamin A deficiency (19, 27, 29). Spleen cell number appeared to be more sensitive to vitamin A deficiency than spleen weight. A decrease in spleen cellularity was observed in rats without external signs of vitamin A deficiency; this difference reached statistical significance as deficiency progressed and vitamin A deficiency became clinically apparent (19, 25, 29). Some investigators have reported small germinal centers in the spleens of vitamin A-deficient rats (30), whereas others (31) did not observe any marked differences. This discrepancy may be due to differences in the severity of vitamin A deficiency in these studies. Thymus weight has also been reported to decrease, with marked atrophy occurring late in vitamin A deficiency in some animals. Thymic atrophy has long been known to be associated with protein-energy malnutrition in children (reviewed in [32]). A different picture has been reported for the mouse: vitamin A deficiency alone had no effect on lymphoid organ weight or cell number, but vitamin A deficiency combined with inanition resulted in significant enlargement of the spleen (33). Enlargement of regional lymph nodes has also been observed in both rats and mice (6, 18, 33–35) and is thought to result from accumulation of cell debris and altered cellular composition.

Analysis of the major T and B cell populations of the spleens of vitamin A-deficient and normal rats has been performed using fluorescent antibodies specific for total T cells, T cell subsets, or B lymphocytes (18, 36). Surprisingly, vitamin A deficiency did not result in significant shifts in the percentage of total T cells or the distribution of T cell subsets (helper and suppressor/cytotoxic) or of IgM- or IgD-positive B lymphocytes (18, 36). This picture contrasts with that reported for human protein-energy malnutrition, in which the fraction of helper ($CD4^+$) T cells was reported to be decreased (37).

The recent cell culture experiments of Buck *et al.* (38) suggest a role for retinol as a specific growth factor for B lymphocytes. When human Epstein-Barr virus-transformed B lymphocytes were cultured at low density in serumfree medium, growth ceased and most cells died within 24 hr. The addition of serum or delipidated serum protein recombined with the lipid fraction was sufficient to restore growth, as was the complex of retinol with serum retinol-binding protein. Studies with activated B cell blasts from spleen revealed a similar requirement for serum or retinol. The investigators were able to identify a new metabolite of retinol, 14-hydroxy-4, 14-*retro*-retinol, as an active component of the culture medium (39). In contrast, the addition of

all-*trans*-retinoic acid did not support growth of these B cells. These intriguing studies suggest a selective role of retinol as a factor, or cofactor, in B cell proliferation.

Vitamin A Deficiency and Cell-Mediated Immunity

The term cell-mediated immunity (CMI) was originally used to describe localized reactions to pathogens, mediated by lymphocytes and macrophages, and is now more generally used to describe cellular responses in which antibody plays a subordinate role (40). The effector cells in CMI include cytotoxic T cells, macrophages, and natural killer cells, which destroy infected or foreign cells through some combination of direct contact, secretion of soluble factors, and recruitment of other inflammatory cells such as neutrophils. Delayed-type hypersensitivity (DTH) has been used to assess CMI in human and animal studies (41). In vitamin A-deficient mice, the DTH response to dinitrofluorobenzene (33) or picryl chloride (42) was significantly reduced. However, in a study of Bangladeshi children, there was no difference in DTH response before and after vitamin A supplementation (43). Reports on DTH responses in humans have not been consistent, perhaps due to confounding of protein-energy malnutrition (14), which generally leads to impaired DTH.

A recent study has provided evidence that the function of cytotoxic T lymphocytes is also reduced during vitamin A deficiency. When vitamin A-deficient chicks were challenged with Newcastle disease virus, the cytotoxic activity of spleen cells was lower during the primary antiviral response (44). After reinfection, cytotoxic activity was detected in the peripheral blood lymphocytes of normal, but not of vitamin A-deficient, chicks. If the human cytoxic T lymphocyte response is similarly depressed, these experimental results would appear to have implications for the recovery from viral infections of young children with marginal vitamin A status. As noted by Thurnham (45) and others, humoral immunity develops slowly in young children, and their reliance on cell-mediated immunity is relatively greater than is that of older humans.

The proliferative response of lymphocytes after stimulation with mitogens has frequently been used to assess CMI *in vitro* or to demonstrate the activation state or proliferative potential of cells *ex vivo*. This method has been applied to determine whether vitamin A deficiency, or repletion with retinol, alters cellular responses to mitogens which specifically stimulate T or B lymphocytes (see Nauss *et al.* (14) for methodological considerations). There is good agreement that the proliferative response of splenic lymphocytes is decreased in vitamin A deficiency (11, 18, 25, 27, 28, 46–48). However, in contrast, the response of cells from other lymphoid tissues has not been consistent. In studies with good nutritional control, Nauss *et al.* (18, 25) determined lymphocyte proliferation at various stages of vitamin A deficiency after spleen, cervical, and mesenteric lymph node cells were stimulated with concanavalin A (con A) or phytohemagglutinin (T cell mitogens), pokeweed mitogen (a stimulator of T cell-dependent B cell proliferation), or lipopolysaccharide (a B cell mitogen). During the later stages of vitamin A deficiency, the response to other mitogens other than con A was either reduced, unchanged, or even increased, depending upon the anatomical site from which lymphocytes were obtained (18, 25, 48). Thus, no simple pattern of cell proliferation correlated well with vitamin A status. Some experiments have suggested that the kinetics of cell proliferation may differ between vitamin A-sufficient and vitamin A-deficient animals. Friedman *et al.* (49) reported a delayed proliferation of leukocytes from vitamin A-deficient chicks. In our laboratory, the proliferation of splenic lymphocytes from vitamin A-deficient rats after stimulation with con A was late as compared with cells from control rats or retinol-repleted rats, but the total area under the time-response curve for cells from vitamin A-deficient rats was only sightly reduced (S. Sri Kantha and A. C. Ross, unpublished results). These results suggest that the kinetics of signaling may be disturbed, but that the cell's basic ability to proliferate is not necessarily defective.

Vitamin A Deficiency and Natural Killer Cell Function

Natural killer (NK) cells mediate "natural cytotoxicity" and are thought to be especially important in the surveillance of virus-infected cells. These cells also secrete a number of soluble factors and play a regulatory role in hematopoiesis and antibody formation (50). NK cells are able to bind to and lyse certain tumor cells without prior antibody sensitization and without restriction by major histocompatibility type. NK cells also have characteristic surface receptors for the Fc portion of IgG or IgE through which they participate in antibody-dependent recognition and lysis of target cells. After stimulation by IgG or lymphokines such as interleukin (IL)2, or following viral infection, NK cells produce and release a number of cytokines, of which γ-interferon (IFN) is prominent. The interferon so produced can further increase the cytolytic activity of NK cells, enable binding to a broader spectrum of target cells, and regulate the production of certain classes of immunoglobulin (51).

Vitamin A deficiency has been associated with significantly decreased NK cell cytotoxic activity in rat spleen cell preparations (35, 52), but not in cells from the cervical lymph nodes (35). After vitamin A-deficient rats were repleted orally with retinol, NK cell cytolytic activity of spleen returned to normal values. A possible relationship between the decreased NK cell activity during vitamin A deficiency and IFN production was suggested by the observation that IFN release after

stimulation of spleen cells *in vitro* with con A was also reduced significantly (52). As observed for NK cell activity, improvement of vitamin A status also restored the ability of these cells to produce total IFN activity.

It may be relevant that low NK activity was found in the peripheral blood mononuclear cells of young children with acute measles (53). Although a connection to vitamin A status was not established in this work, serum retinol concentrations have been shown to be reduced during acute infection, and vitamin A therapy has been effective in reducing measles-related morbidity and mortality (see below). Despite this low basal activity, the NK cells from children with measles or other infections could be activated by the addition of IL-2 *in vitro*, which indicates that the potential for lytic activity was retained. It would be of interest to learn whether supplemental vitamin A improves NK cell activity in children with measles and whether this response has any role in their clinical recovery.

Vitamin A Deficiency and Antibody Response

The antibody response, including the production of antibody-secreting plasma cells and memory B and T cells, is the mechanism by which the immune system provides highly specific protection of long duration against many pathogens and molecules recognized as non-self. For most soluble proteins, antigen processing precedes presentation of peptide fragments to appropriate T cell receptor-class II complexes that initiate the production of IL-2 and other factors that drive B cell growth. In the early phase of the primary response, plasma cells secrete mainly IgM. This is followed by selection of high affinity clones and recombination within the immunoblobulin gene heavy chain region to produce the class switch from IgM to IgG. In the secondary response, re-exposure of memory B and T cells to antigen leads to a rapid and much greater production of high-affinity IgG antibodies. The response to some other types of antigen, for instance, bacterial polysaccharides, is typically of the primary type and is discussed below.

The relationship of vitamin A status to antibody production has been investigated for a number of antigens, some of which are relevant to human vaccination programs while others are mainly of experimental interest. This section first reviews experiments with antigens that are part of the World Health Organization's Expanded Program on Immunization, a program that has succeeded dramatically in delivering vaccine protection to children in developing countries (54). This is followed by a discussion of other antigens that are of clinical interest but are not yet part of broad immunization programs, and, finally, of antigens mainly of experimental interest. Both animal and human studies have been included together so that, when data are available for both, comparisons and contrasts in the immune response can be appreciated.

Tetanus Toxoid. Neonatal tetanus due to infection with *Clostridium tetani* has been reduced significantly as a result of vaccination with tetanus toxoid. Even now, however, it is estimated to be the cause of over 750,000 deaths each year (54). In contrast to the other vaccines of the Expanded Program on Immunization which are directed to young children, the main target population for immunization with tetanus toxoid is pregnant women. Antitoxin titers have been shown to increase with each subsequent immunization. Three immunizations induce high and durable antitoxin levels (55). Of the vaccines used in this and other immunization programs (those against tetanus, tuberculosis, diphtheria, measles virus, whooping cough, and polio), most reports on vitamin A and immunity have concerned tetanus toxoid. Table II provides a summary of human and experimental investigations with this antigen.

The role of vitamin A status in the response to tetanus toxoid has been investigated in both humans and animals. Brown *et al.* (43) conducted a field study in Bangladesh in the late 1970s to determine whether a large dose of vitamin A could be used to enhance the antibody response to tetanus toxoid. This hypothesis was based on the work of Dresser (56), discussed below, which had demonstrated adjuvant properties of retinol in the mouse. Ninety-five young children were matched by age and sex and assigned randomly to receive either a 60 mg dose of water-miscible vitamin A, delivered intramuscularly at the time of immunization with tetanus toxoid, or of tetanus toxoid only. A second dose of tetanus toxoid, but no additional vitamin A, was administered 4 weeks later. Baseline serum vitamin A concentration equaled 0.5 μmol/liter,[2] indicative of low vitamin A status, and baseline anti-tetanus toxoid antibodies were undetectable. Although antitoxin titers were measurable, there was no difference in the mean titers between children treated with vitamin A and the control group. Skin testing to Monilia also revealed no difference. Unfortunately, these negative results are difficult to interpret because there was no follow-up to demonstrate that vitamin A administration increased plasma retinol, nor was information on infections provided.

Semba *et al.* (57) recently reported on a randomized placebo-controlled clinical trial with 236 Indonesian children, ages 3–6, designed to determine whether the immune response in mild vitamin A deficiency is

[2] Concentration data originally presented in units of μg/dl have been converted to molar (SI) units (28.6 μg/dl equals 1 μmol/liter). For uniformity in comparing the amounts of dietary vitamin A used in various studies, data originally presented in International Units (IU) have been converted to mg of vitamin A (retinol), or mg of retinyl palmitate when specified. One IU equals 0.3 μg of retinol or 0.55 μg of retinyl palmitate.

Table II. Field-Based and Experimental Studies of Vitamin A Status on the Antibody Response to Tetanus Toxoid[a]

Situation/model	Vitamin A treatment	Immunization	Outcome	Reference
Children (mean 39 months) in Bangladesh	Half received 60 mg of VA along with first immunization with TT	Immunized with TT at time of VA; reimmunized with TT 4 weeks later	Immunization resulted in increased anti-TT titers, but no difference due to VA	43
Children, 3–6 years in Indonesia, divided into two groups based on ocular status (mild xerophthalmia vs normal eyes)	Half of each group received 60 mg of VA orally, or placebo	DPT vaccine given 2 weeks after treatment with VA	Anti-TT titers 3 weeks after immunization were higher in children given VA regardless of previous ocular status	57
Rat, VA-deficient, compared with pair-fed and *ad libitum*-fed controls	No supplementation	DPT vaccine, im	Anti-TT titers were less for VA-deficient than for control rats fed *ad libitum*, but were not different from pair-fed controls	27
Rat, VA-deficient, compared with pair-fed and VA-repleted rats	0.03 mg/day for 7 days before immunization	TT, iv	Anti-TT titers of VA-deficient rats were lower than those of either control group; most supplemented rats had a normal anti-TT titer	58
Rat, VA-deficient, compared with pair-fed and VA-repleted rats	1.5 mg orally divided into two doses given 4 days before and on the day of immunization	TT, ip	Primary anti-TT IgM concentration lower in VA-deficient rats; normal response after VA repletion	29
Rat, VA-deficient, compared with pair-fed controls and VA-repleted rats	1.5 mg orally as single dose either 1 day after first immunization or just before secondary immunization	TT, ip	Primary and secondary IgM and IgG concentrations lower in VA-deficient rats; repletion at time of primary immunization led to normal primary and secondary responses. Repletion before secondary immunization restored a normal secondary response; total IgM and IgG not reduced	59
Mouse, normal diet	1 mg im 3 times	Combined immunization with TT, BCG, and bovine γ-globulin, ip	VA increased the response to TT and bovine γ-globulin and counteracted the suppressive effects of steroids and cyclophosphamide	91
Mouse, normal diet	0.9–4.5 mg VA im at time of first immunization	TT, im	Secondary antibody response greater in VA-treated group; primary response not consistent	43

[a] VA, vitamin A; TT, tetanus toxoid.

responsive to vitamin A supplementation. One hundred eighteen children with mild xerophthalmia and an equal number of children with normal eyes were randomly assigned to receive either 60 mg of vitamin A or a placebo. Two weeks after treatment with vitamin A, children were immunized with diphtheria-pertussis-tetanus vaccine. The IgG response was determined at baseline and 3 weeks after immunization. After correction for previous immunization, there was a significant difference in tetanus toxoid titers between the vitamin A-supplemented and control groups. However, there was no difference in response between those children with pre-existing signs of vitamin A deficiency and those without such signs. It may be that significant vitamin A depletion existed even in the absence of ocular signs, or that the differences were due in some manner to the adjuvant properties of retinol discussed below. In either case, this study raises the possibility that the antibody response in some young children can be enhanced by retinol administration. Further defini-

tion of the kinetics and duration of this enhanced response would be desirable.

At least three different investigators have used the rat as a model to investigate the primary antibody response to tetanus toxoid. Consistent findings of reduced antibody production have been reported (27, 29, 58). Krishnan *et al.* (27) compared the response to tetanus toxoid of vitamin A-deficient rats and rats fed a vitamin A-adequate diet *ad libitum*; thus, it is likely that there was some confounding of vitamin A deficiency and protein-energy deficiency. In these studies, the primary antibody response of vitamin A-deficient rats to tetanus toxoid, as well as to diphtheria toxoid and to sheep red blood cells, was reduced significantly. Lavasa *et al.* (58) conducted a well-controlled study of the primary antibody response to tetanus toxoid of the vitamin A-deficient rat in comparison to both pair-fed and *ad libitum*-fed normal rats and to previously vitamin A-deficient rats after repletion for 7 days with 0.03 mg of vitamin A. The response of rats with vitamin A deficiency, confirmed histologically and by very low serum and liver vitamin A concentrations, was consistently low, whereas all other treatment groups responded well to this antigen.

The antibody response to tetanus toxoid has also been studied recently in the vitamin A-depleted rat before and after oral repletion with retinol (29). The primary anti-tetanus toxoid IgM response was measured in three groups of rats: a vitamin A-depleted group that had low plasma and tissue vitamin A reserves, but did not yet show symptoms of vitamin A deficiency; a group repleted with 1.5 mg of retinol (as retinyl palmitate) given by mouth near the time of immunization; and a pair-fed control group that was maintained on a vitamin A-sufficient diet. Plasma anti-tetanus toxoid IgM concentrations were significantly reduced (18% of control) in the vitamin A-depleted group. In contrast, both antibody production and tissue vitamin A concentrations were equal to the control group after vitamin A repletion.

None of the animal studies cited above investigated the secondary response to tetanus toxoid. A recent study by Kinoshita *et al.* (59) was designed to determine whether immunologic memory to tetanus toxoid can be established and maintained in the vitamin A-deficient rat. The design included two vitamin A repletion groups: one group was repleted with a single oral dose of 1.5 mg of retinol given 1 day after primary immunization, whereas a second group remained vitamin A-deficient throughout the primary antibody response, but was repleted with retinol 2 days before the second immunization. Whereas vitamin A-depleted rats had a low primary and a low secondary response (for IgM as well as IgG), the kinetics of antibody production were normal. Additionally, vitamin A-deficient rats had a normal amplification of anti-tetanus toxoid IgM and IgG in the secondary response, as well as a normal ratio of IgG to IgM antibodies in each response (59). In rats that were deficient in vitamin A throughout the primary response but were repleted with retinyl ester 2 days before reimmunization, the secondary IgM and IgG responses were equal in magnitude to the response of the control group. The inference drawn from these data was that immunologic memory and the class switch from IgM to IgG developed normally during retinol deficiency, despite a low primary production of antibodies, and that memory cells could be activated after repletion with vitamin A.

In these studies, it was also observed that repleting vitamin A-deficient rats with a single oral dose of retinol at the time of the first immunization resulted in plasma anti-tetanus toxoid concentrations that were, on average, 1.5–2-fold higher than that of the control group. Of note, this increase was maintained during the secondary IgM and IgG responses without additional vitamin A. It appears that either the rapid change in vitamin A status at the time of immunization or adjuvant properties of oral vitamin A had both immediate and longer term effects on specific antibody production.

The decreased levels of anti-tetanus toxoid antibodies observed in this study were not simply a reflection of a generally low level of antibody production. Indeed, the concentration of plasma total IgG, as compared with anti-tetanus toxoid IgG, was elevated significantly in vitamin A-depleted rats (59). A similar mild hypergammaglobulinemia was noted by Gershwin *et al.* (60) in mice fed a vitamin A-deficient diet. It may be noteworthy that circulating immunoglobulins are also elevated in children with protein-energy malnutrition (32), despite the poor response to some antigens.

Thus, the results from several animal studies are quite consistent in demonstrating that vitamin A deficiency is associated with a marked decrease in the antibody response to tetanus toxoid. The human studies, which are fewer in number and less controlled in design, lead to a mixed assessment of the importance of vitamin A status. It is possible that the effects of low vitamin A status, or supplementation with vitamin A, may be quantitatively smaller in humans or may be easily masked by confounding variables. Seasonal variation in nutrient availability and the vitamin A status of children is well known in areas where vitamin A deficiency is most prevalent. Therefore, it is also possible that only the continuous form of vitamin A deficiency, as produced experimentally, compromises the antibody response toward this antigen. Some of the apparent differences in outcome might be resolved by exploring the antibody response in animals with more marginal forms of vitamin A deficiency and by use in the future of more reliable estimates of vitamin A status in human studies.

Tuberculosis. McMurray *et al.* (61) have recently

reviewed the literature on micronutrient status and immune function in tuberculosis. *Mycobacterium tuberculosis* infection remains a major cause of morbidity and mortality among malnourished children, and reactivation of disease later in life may be related to, or a cause of, poor nutritional status. It is known that malnourished individuals vaccinated with Bacille Calmette-Guérin vaccine do not respond normally to skin tests with purified protein derivative, but the mechanisms that decrease host response to vaccination or to tubercular infection are unknown. McMurray *et al.* (61) identified four studies in the early literature (1923 through 1961) in which vitamin A deficiency in humans or animals exacerbated tubercular disease. However, no recent studies, particularly on antibody production, have added to this literature.

Diphtheria and Pertussis. Despite the long-standing use of these toxoids as vaccines, they have received little attention in vitamin A studies. Diphtheria toxoid was used to immunize vitamin A-deficient rats in the study of Krishnan *et al.* (27) described above. These investigators reported a reduction by half in the hemagglutination titers of vitamin A-deficient rats. However, the number of animals was small and concomitant protein-energy malnutrition probably confounded this study; thus, confirmation of this result is needed.

Measles Virus Infection. Measles kills ~2 million children annually and has no specific therapy (62). Vitamin A status is now recognized to be one of the critical determinants of the outcome of measles infection in many developing countries (63). Acute measles infection may cause a decompensation of vitamin A status, precipitating corneal lesions in children with pre-existing hypovitaminosis A (63). The importance of vaccination against measles to prevent the rapid deterioration of body vitamin A reserves leading to corneal lesions was highlighted in a 1988 report of the Expanded Program on Immunization's Global Advisory Group (63).

Of the infectious diseases for which vitamin A status is thought to be important, the relationship between measles virus infection and vitamin A status has been investigated most extensively. However, few, if any, of these studies have investigated the antibody response per se. The subject has mainly been approached by determining whether measles infection (or other infections) directly alters vitamin A status or by asking whether the pathogenesis of measles-related xerophthalmia is due to an abrupt change in vitamin A status or to corneal involvement by the measles virus directly or by secondary infections. Clinical studies have addressed whether vitamin A therapy is effective in reducing the high rate of mortality due to measles. A recent culmination of these research efforts was the report of successful intervention with vitamin A treatment in a randomized clinical trial in South African children with measles (62). Treatment with vitamin A significantly reduced mortality (10 of 97 among the control group vs two of 92 among vitamin A-treated children [62]) and resulted in significant differences for duration of pneumonia, duration of diarrhea, and number of days of hospital stay. As a result of these and other observations, WHO/UNICEF (64) has jointly recommended that all children diagnosed as having measles in countries where the fatality rate is 1% or more should immediately be given 30–60 mg of vitamin A, depending on age. Other investigators have recommended up to 120 mg of vitamin A for children of all ages (62, 65).

Responses to Bacterial Polysaccharide and Lipopolysaccharide Antigens. Vitamin A deficiency is associated with a poorer prognosis and longer duration for many respiratory and diarrheal infections. In an earlier study, Arroyave and Calcaño (66) observed that a variety of upper respiratory tract infections in children and adults was associated with decreases in serum retinol and retinol-binding protein, as well as carotene and serum proteins. Infections were associated with marked reduction (as great as 0.5–1 μmol/liter) in serum retinol; decreases were greater when fever accompanied infectious episodes.

Even in the face of antibiotic therapy, pneumonia, meningitis, and diverse intestinal infections remain major causes of morbidity and mortality in both the young and, in the case of pneumonia, the elderly. The antibody response to polysaccharides, such as those from pneumococcal and miningococcal bacteria, are generally classified as T cell independent (TI) based on the ability of these antigens to elicit a response in athymic animals. The antibody response is largely a primary, IgM response, although some IgG is also produced (67). In comparison to soluble proteins which are T cell-dependent (TD) antigens, there is little formation of immunologic memory to the polysaccharide antigens. Generally, the antibody response to bacterial polysaccharides is very weak in young children or animals. The effect of vitamin A status in humans on the response to these antigens has not been reported, but a number of recent experimental studies have provided new information.

To investigate the effects of vitamin A deficiency on the antibody response to bacterial antigens, Pasatiempo *et al.* (29) conducted a study with five bacterial antigens in the vitamin A-deficient rat. The antigens used were pneumococcal polysaccharide (from *Streptococcus pneumoniae*, type III, one of the more pathogenic strains of pneumococci), meningococcal polysaccharide (from *Neisseria meningitidis*, type C), lipopolysaccharide (from *Pseudomonas aeruginosa*, an opportunistic pathogen associated with respiratory and ocular infections, and from *Serratia marcescens*), and tetanus toxoid, a TD protein antigen. In vitamin A-

deficient rats immunized with pneumococcal polysaccharide, the antibody response was very low (≤20% of pair-fed control rats). Decreased antibody production was apparent before outward signs of vitamin A deficiency were manifest (29). In all experiments, repletion with vitamin A (equal to 1.5 mg of retinol given orally) completely restored a normal level of antibody production, even in rats with symptoms of vitamin A deficiency, showing that this impairment is reversible and causally related to a dificiency of vitamin A. In studies that examined the ontogeny of antibody production, a significant reduction in the IgM response specific for pneumococcal polysaccharide was detectable as early as 35 days of age (36).

Similarly, vitamin A-deficient rats (either with our without symptoms of retinol deficiency) had almost no response after immunization with meningococcal polysaccharide (29). As was also observed for pneumococcal polysaccharide, the response to meningococcal polysaccharide was normal after repletion with retinol. Although the contribution of meningococcal infection to childhood morbidity or mortality in studies of vitamin A deficiency has not been determined, it is interesting that Keusch (68) has commented that, for some of the convulsions reported in children with low vitamin A status (69), the first logical association would be with meningitis.

In contrast, when rats with the same low vitamin A status were immunized with lipopolysaccharide from either *P. aeruginosa* or *S. marcescens*, antibody production was quantitatively normal (29). These antigens are also TI antigens; however, an immunologic distinction within the class of TI antigens has been proposed. The polysaccharide antigens used above have the characteristics of TI type 2 antigens, to which the antibody response develops later during ontogeny, presumably because a late-maturing B cell population (designated $Ly5^+$ in the mouse) is required (70). In contrast, the lipopolysaccharides, which were clearly immunogenic in vitamin A-deficient animals, are generally classified as TI type 1 antigens. Type 1 antigens are immunogenic early in life (29, 70) and have less stringent requirements for mature B cells and cytokines. Thus, this study revealed a potentially relevant correlation between the immunologic type of antigen and whether or not vitamin A deficiency compromised antibody production: for the TI type 2 and TD antigens, antibody production was poor, whereas for TI type 1 antigens, antibody production was normal.

Responses to Experimental Antigens. In addition to the serologic studies using natural antigens as described above, a number of investigations have been carried out with experimental antigens, often TD protein antigens or heterologous cells. Such studies may be helpful in understanding the immune response to natural antigens of similar immunologic type.

A decreased plaque-forming cell response to heterologous red blood cells has been reported for the rat (27–29). Red blood cells are TD antigens and, thus, the lack of response is consistent with the categorization suggested above.

Smith and Hayes (71) reported that vitamin A-deficient mice have an altered response to the protein keyhole limpet hemocyanin. The primary serum IgM response was either normal or reduced, but the greatest reduction was in the IgG class, particularly IgG1. By mixing T lymphocytes from vitamin A-deficient mice and B cells from normal mice, Carman *et al.* (72) provided evidence that helper T cells of vitamin A-deficient mice do not provide help for antibody production to protein antigens *in vitro*. The frequency of such helper T cells in the mouse popliteal lymph node was found to be reduced during vitamin A deficiency and restored after addition of retinyl acetate to cultures *in vitro*. Decreased IgG1 production may be linked to an overproduction of IFN-γ, as recently reported for T cells from spleens of vitamin A-deficient mice (73). It seems that the relationship of vitamin A status to IFN production requires further study, inasmuch as other work has indicated either a decrease in inducible IFN activity released from spleen cells of vitamin A-deficient rats (52) or an increase in secretion of inducible IFN-γ from spleen cells of mice supplemented with vitamin A (74). It is not clear what accounts for these differences; however, the species studied, the type of assay for IFN, and the end points examined have differed.

The effects of a broad range of vitamin A levels on antigen-specific immune responses have been studied in the chick. In 1963, Panda and Combs (75) reported that chicks fed a diet low in vitamin A had a reduced agglutination response following challenge with *Salmonella pullorum* antigen. Recently, Friedman and Sklan (76) reported impaired T lymphocyte responses after cells from vitamin A-deficient chicks or rats were restimulated *in vitro* with antigen (bovine serum albumin). Responses were normal soon after repletion with vitamin A ester. These same investigators also compared antibody production in chicks fed four diets ranging in vitamin A contents from deficient to highly supplemented (77). Direct measurement of liver and plasma vitamin A concentrations showed that the four dietary treatments did, indeed, produce a spectrum of plasma and liver vitamin A concentrations ranging from barely detectable to excessive (>8.7 μmol of vitamin A/g liver and >2.5 μmol/liter of plasma retinol). Both chronic deficiency and chronic excess of vitamin A were associated with low antibody production and reduced lymphocyte proliferation. By comparison, a large, bolus, oral dose of vitamin A given to vitamin A-deficient chicks restored lymphocyte proliferation to the control level and did not result in evident toxicity (76). In a subsequent study of similar design, Friedman

et al. (49) determined morbidity and mortality rates after *Escherichia coli* infection in chicks that were either vitamin A-deficient, -sufficient, or treated with excess dietary vitamin A. Those chicks that received an excess of vitamin A proved even more sensitive to *E. coli* infection than the vitamin A-depleted chicks.

Response to Viral Infections. The specific antibody response to viral antigens has been studied during vitamin A deficiency in several animal models. In chicks exposed to Newcastle disease virus (44), a difference in total versus specific antibody production was demonstrated. Whereas the total serum IgG and IgM concentrations were greater in chicks fed a vitamin A-deficient diet than in those fed diets adequate in retinol or supplemented with retinoic acid, the concentration of virus-specific antibody was reduced. The antibody response of chicks fed retinoic acid (2 mg/kg of diet) was greater than that of chicks fed the same amount of retinol. In contrast, Davis and Sell (26) concluded from an earlier study of the chick that serum antibody titers following immunization with bovine serum albumin were not different in chicks fed low or adequate levels of retinol or retinoic acid, but that lymphocyte transformation was reduced in vitamin A-deficient chicks. They also concluded that maintenance of lymphoid tissues, as judged by growth, was poorer in chicks fed diets containing retinoic acid than in those containing retinol.

The interaction of viral infection and vitamin A status on intestinal integrity was recently evaluated by Ahmed *et al.* (78) in weanling mice infected by the oral route with rotovirus. Vitamin A-deficient mice showed a moderate reduction in the T cell area of the spleen and a significant reduction in thymus mass. Vitamin A-deficient mice, whether infected or not, had a significantly reduced number of goblet cells per duodenal villus. In those mice with both vitamin A deficiency and rotovirus infection, there was marked destruction of the villus tips; neither vitamin A deficiency nor rotovirus infection alone produced such a marked effect. In a subsequent study (42), vitamin A-deficient mice infected with rotovirus produced significantly lower levels of specific antibody than mice pair-fed the control diet or fed *ad libitum*. Mice refed the vitamin A-sufficient diet for 1 week before infection showed a partial restoration of antibody production, but little improvement in the DTH response that was determined concurrently.

Nauss *et al.* (34) developed the vitamin A-deficient rat as a model to study ocular infection with type 1 herpes simplex virus (HSV). In this model, the corneal surface is lightly abraded and HSV is applied directly to the corneal epithelium. The onset of herpetic keratitis was more rapid and clinical disease was more severe in vitamin A-deficient rats than control rats, even in the beginning stages of vitamin A deficiency. Histopathologic examination revealed that the inflammatory response was significantly greater, as was the incidence of epithelial ulceration and necrosis. Similarly, in rabbits in which the conjunctiva had been inoculated with *P. aeruginosa*, there were sequelae of infiltration of polymorphonuclear leukocytes, corneal ulceration, and stromal melting in the vitamin A-deficiency rabbits, but not in the controls (79).

In a companion investigation, Nauss and Newberne (35) asked whether alterations in specific or nonspecific immune responses were responsible for the increased susceptibility of vitamin A-deficient rats to ocular HSV infection. Cell-mediated responses and natural killer cell activity (see below for further discussion) were monitored. The responses of spleen cells to con A decreased rapidly after HSV infection in both control and vitamin A-deficient rats. Similar to previous studies, the response of cervical lymph nodes to con A was greater in vitamin A-deficient rats than in normal rats, although both were decreased significantly 7 days after HSV infection. The NK cell activity of spleen cells was somewhat greater in normal rats than in vitamin A-deficient rats and both decreased during the postinfection period. Nauss and Newberne (35) commented that, despite reduced responses *in vitro*, the local ocular inflammatory response was strong in the vitamin A-deficient animals. They favored a multifactorial cause of the increased susceptibility to ocular infection, stating that "multiple, partial defects in both specific and nonspecific host defenses probably combine to increase incidence and severity of the disease in animals with hypovitaminosis A."

Responses to Parasitic Infection. In 1982, Beisel (80) discussed the concept that malnutrition and parasitic infections most often synergize, leading to an infectious process of greater-than-expected severity. Yet there are sufficient examples of either an antagonistic relationship, or no apparent influence of malnutrition, so that a categorical prediction cannot be made. In their earlier review, Scrimshaw *et al.* (10) noted that malnutrition worsened the severity of most (>80%) bacterial infections, reduced or increased the severity of viral infections in nearly equal numbers, and worsened over 60% of the parasitic infections that had been studied. In nearly all studies of vitamin A deficiency and parasitic infections, the interaction has been synergistic (80). Low plasma retinol levels are common in patients with parasitic diseases (10) and malabsorption of vitamin A has been demonstrated during a number of infections in humans (11).

An inverse relationship between plasma vitamin A levels and pathogenicity of parasitic infections has been observed in rodent models. For example, in rats infected with *Plasmodium berghei, Trypanosoma cruzi, Schistosoma mansoni*, or *Angiostrongylus cantonensis* infection and in mice infected with *T. musculi* (re-

viewed in [81] and [82]), low plasma retinol levels were associated with an inability to reject worm infestation.

Infection by a number of parasites elicits an IgE response. Parent *et al.* (81) correlated nutrition, parasitologic, and immunologic parameters in three groups of rats: a noninfected and normally nourished control group, a normally nourished group infected with *Sc. mansoni*, and a group of vitamin A-deficient rats also infected with *Sc. mansoni*. The worm burden and the number of eggs per gram of liver were significantly greater in the vitamin A-depleted animals. Additionally, IgE levels and anti-*Sc. mansoni* antibodies were much lower in the vitamin A-depleted rats. Because lymphocyte transformation after stimulation with con A did not differ and lymphocytes of vitamin A-deficient rats still responded to stimulation with *Sc. mansoni*, these authors concluded that the humoral immune response was markedly depressed during vitamin A deficiency, whereas the cellular immune response was not significantly altered.

Mucosal Immunity. The importance of mucosal immunity is well recognized, but this subject has received little experimental attention in relationship to vitamin A status. In malnourished children whose vitamin A status was not reported, Chandra (83) reported that the secretory immune response (IgA) to live attenuated measles and polio vaccines was reduced significantly.

Sirisinha and co-workers (84, 85) used the model of retinoic acid cycling in the rat to investigate IgA levels and local, intestinal immune responses (IgA and IgG) to antigen injected directly into Peyer's patches. The IgA levels in intestinal fluid and bile were significantly reduced, as was the transport of labeled IgA into bile (85). After immunization with hapten-protein complexes, the intestinal IgA response of vitamin A-deficient rats was undetectable. Antigen-specific serum IgA did not seem to differ with vitamin A status, but the number of observations was small. Thus, these data are suggestive of impaired local immunity, but further experiments are needed. Vitamin A deficiency has also been associated with a decreased number of Peyer's patches and fewer immunoglobulin-bearing cells in the gut-associated lymphoid tissues of the guinea pig (86) and reduced proliferative response to mitogens (87).

Influence of Vitamin A Administration on Immune Responses

Although the consequences of vitamin A deficiency on immunocompetence have been the focus of most research, there has also been a strong interest in the ability of vitamin A or related retinoids to stimulate immune responses, even in the vitamin A-adequate host (11, 88). Thus, vitamin A administration has been used in two quite different settings, either to test the reversibility of immunocompromise due to vitamin A deficiency or to determine the efficacy of supplemental vitamin A as an immunomodulator. The subject of immunostimulation by retinoids was reviewed by Dennert (88) in 1984. In a number of studies, administration of either natural or synthetic retinoids has decreased the growth of immunogenic tumors or stimulated cell-mediated tumor immunity.

The levels of vitamin A that have been used in studies of immunostimulation have often been well above normal dietary requirements. It is difficult to distinguish effects that may be considered nutritional, i.e., which are mediated through normal pathways and occur within the range of safe intake of vitamin A, versus effects that most likely are due to pharmacologic actions of high levels of retinoids. Because large doses of preformed vitamin A, ingested chronically or acutely, are well known to produce systemic toxicity in both animals and humans,[3] studies of immunostimulation by retinoids should include, but frequently have not, clinical examination for toxic effects and measures of tissue vitamin A concentrations.

Adjuvant Properties of Retinol and Retinoic Acid. A number of studies have revealed that retinol or retinoic acid can function as an adjuvant to enhance the antibody response to specific antigens, even in healthy animals with adequate vitamin A reserves. The adjuvant properties of retinol were first reported in 1968 by Dresser (56), who showed that retinol-treated mice produced antibodies specific to soluble bovine γ-globulin, which is not immunogenic in the mouse. The response was dose dependent, but required large amounts of retinol (1–10 mg/mouse given intraperitoneally 1 day before immunization). Dresser speculated that macrophage activation might be responsible, in part, for the adjuvant properties of retinol, or that destabilization of cell membranes by retinol might stimulate lymphocytes to divide. Subsequently, Dennert *et al.* (89) reported that retinoic acid functioned as an adjuvant in mice immunized with syngeneic and allogeneic tumor cells. Brown *et al.* (43) tested whether high doses of retinol (0.9–9 mg, im) given at the time of primary immunization could increase the antibody response to tetanus toxoid in the normal mouse. The highest dose of vitamin A was reported to have been toxic. After the second of two doses of tetanus toxoid, mice treated with vitamin A had higher antitoxin titers, most likely due to enhanced production of IgG. Fried-

[3] The Recommended Dietary Allowance of vitamin A is 0.8 mg for women, 1 mg for men, and 0.375–0.7 mg for children depending on age (118). Diets that have been recommended for rodents contain ~1.2 mg of retinol/kg of diet and provide ~0.01–0.03 mg/day for the rat (119). Regarding excessive intake and the potential for retinol toxicity in humans, the National Research Council states that signs of toxicity usually appear only when sustained daily intakes, including preformed vitamin A in both foods and supplements, are more than 10 times higher than the Recommended Dietary Allowance. A single oral dose of 60 mg of retinol has been well tolerated by Asian prechildren in prophylactic programs. Carotenoids are not known to be toxic even when ingested in high amounts for an extended period (118).

man (90) has recently reported adjuvant effects of water-miscible forms of retinyl palmitate and retinoic acid admixed with protein antigens. There is not yet enough systematic information to know whether the adjuvant properties of vitamin A apply to nearly all antigens, or only to particular types of antigens. The few antigens that have been studied so far have been TD antigens, either proteins or intact cells.

In a study that examined a variety of immune responses, Nuwayri-Salti and Murad (91) asked whether administration of retinol to normal mice challenged with Bacille Calmette-Guérin would counteract the immunosuppressive effects of a glucocorticoid and cyclophosphamide. After challenge with Bacille Calmette-Guérin, mice were also challenged with bovine γ-globulin and tetanus toxoid. Groups of mice were treated with either an intramuscular injection of vitamin A, prednisolone, prednisolone plus vitamin A, cyclophosphamide, cyclophosphamide plus vitamin A, or the appropriate vehicle. Treatment with three 1-mg doses of vitamin A significantly increased the antibody titers to both tetenus toxoid and bovine γ-globulin, and also counteracted the significant depression in antibody production caused by prednisolone or cyclophosphamide. This outcome agreed well with an earlier report of Cohen and Cohen (92) that vitamin A treatment alone enhanced the antibody response of mice to a hapten-protein conjugate (trinitrophenylovalbumin) and to sheep red blood cells and also countered the immunosuppression caused by hydrocortisone. The serum retinol levels of retinol-treated mice were significantly elevated; however, signs of toxicity were not observed. This study confirmed and extended Dresser's (56) observation that retinol can increase the antibody response of normal mice. Increased cell-mediated immunity as judged by lymphocyte proliferation *in vitro* was also demonstrated (91). The authors speculated that vitamin A may enhance immune functions both by recruiting leukocytes and monocytes to the circulation and by altering membrane structure. The opposing effects of vitamin A, which generally makes membranes more labile (93), and prednisolone, which stabilizes membranes, are consistent with the latter hypothesis.

Although the mechanisms underlying adjuvant effects are not understood, a number of changes have been reported to follow administration of vitamin A. Activation of naive or quiescent lymphocytes is often accompanied by increased expression of cell surface receptors for cytokines or other factors that function in the further expansion or maintenance of the activated state. Among the lymphocyte surface receptors that are expressed early and appear critical to further differentiation are various forms of the IL-2 receptor expressed on activated T cells and NK cells and, on some cells, the transferrin receptor. Using monoclonal antibodies that specifically bind to these receptors, Sidell and Ramsdell (94) showed that retinoic acid *in vitro* could increase the expression of IL-2 receptors on human T lymphoblasts. Other investigators (95) tested a variety of retinoids and carotenoids *in vitro* on the expression of IL-2 and transferrin receptors on peripheral blood T lymphocytes and NK cells from healthy young volunteers. Retinoids seemed to primarily activate T cells, whereas carotenoids had a greater effect on NK cells. Although the concentrations used were near the physiologic range, all compounds were added in ethanol and, thus, the potential importance of lipoproteins or retinoid-protein associations in regulating the physiologic uptake of vitamin A was bypassed.

Sidell and co-workers (96) recently tested whether antigen-specific immune responses in humans can be modulated by synthetic retinoids *in vivo*. Patients with cystic acne undergoing therapy with 13-*cis*-retinoic acid for 4 months, and a similar group of patients not receiving retinoic acid, were reimmunized with tetanus toxoid and were immunized on two occasions with a small amount of keyhole limpet hemocyanin. Plasma levels of 13-*cis*-retinoic acid were measured and found to be within the range that had been reported previously to be immunomodulatory *in vitro*. There was no difference in the level of anti-tetanus toxoid IgG; however, the response to hemocyanin was significantly greater in patients treated with 13-*cis*-retinoic acid. Whether such responses are also regulated by biologic retinoids in the physiologic concentration range is unknown.

Stimulation of Cellular Immune Responses. Cytokine production, lymphocyte transformation, resistance to tumor cells, and CMI have all been reported to be greater in normal animals supplemented with high doses of vitamin A. Forni *et al.* (74) studied lymphocyte proliferation, IL-2 and IFN-γ production, and tumor growth in normal mice supplemented for 60, 90, or 150 days with 0.067–0.33 mg/day of retinol, as retinyl palmitate in drinking water. The response to mitogens and cytokine production were greater in supplemented mice. Resistance to challenge with three transplantable tumors was also improved. However, dose-dependent effects were seen after 2 and 3 months, but not after 5 months, raising the possibility that excessive accumulation of vitamin A was detrimental or that compensating mechanisms had come into play after this time. Lymphocyte transformation after stimulation with phytohemagglutinin or purified protein derivative was also reported to be greater in retinol-treated normal mice (91) and, as for antibody production, retinol administration counteracted the suppressive effect of prednisolone. In a rat model of sepsis (97), supplementation with vitamin A for 3 days prior to sepsis increased the survival rate. The number of white blood cells increased, with a greater percentage of lymphocytes and fewer neutrophils.

Medawar and Hunt (98) suggested that the ability

of high levels of dietary vitamin A to suppress cancer in rodent models may be attributable to immunopotentiation. T cell-mediated enhancement of the graft-versus-host reaction was increased in mice fed a high level of vitamin A (99). Mice chronically fed a diet very high in retinyl acetate had enlarged thymus and lymph nodes, a greater antibody response after sensitization with sheep red blood cells (100), and a more rapid rejection of skin allografts (98, 100). While these observations are provocative, it is not yet clear whether they represent strictly pharmacologic effects of chronic large-dose supplementation or whether they are indicative of normal immune responses governed by vitamin A. Unfortunately, the tissue vitamin A levels of these animals were not reported. Other investigations of the suppressive actions of vitamin A, as retinoic acid, on immunogenic tumors also support an indirect mechanism through thymus-dependent, immune-mediated effector cells (89, 101).

In humans, Cohen *et al.* (102) investigated the ability of large doses of vitamin A to increase the magnitude of the cellular immune response that is known to be transiently depressed following surgery. Patients scheduled for surgery were assigned to a control group (no treatment) or to treatment with a large daily dose (90–135 mg) of vitamin A (generally given orally) preoperatively and for approximately 7 days after surgery. The proliferation of lymphocytes from patients treated with vitamin A was not different from the control group 1 day after surgery, but the response of cells from vitamin A-treated patients was significantly greater after 7 days. Monocyte function, assessed by lysis of sensitized erythrocytes, did not change. The authors reported that no toxic or ill effects were observed, but they did not make direct assessment of serum vitamin A or of liver enzymes that might have revealed toxic effects at the tissue level. Thus, while this *in vitro* study supported immunoenhancement by retinol on cellular immunity, the vitamin A dose in this study was clearly in the pharmacologic range and evaluation of adverse consequences was limited to clinical impressions. However, Penn *et al.* (103) reported recently that elderly nursing home residents given supplemental vitamins, including but not limited to vitamin A, for a month also showed increased cell-mediated immune function as measured by a greater number of T cells, an increased ratio of CD4 to CD8 T cells, and an increased response to phytohemagglutinin.

Stimulation of Phagocytosis or Cell-Mediated Cytoxicity. There is considerable evidence from studies of animals, and some from humans, that administration of high doses of vitamin A affects the nonspecific arm of the immune system by stimulating phagocytosis or cell-mediated killing of pathogens. The results from a number of experimental investigations with supplemental vitamin A support enhanced phagocytosis of bacteria. In 1974, Cohen and Elin (104, 105) reported improved clearance of gram-negative (*P. aeruginosa*) and gram-positive (*Listeria monocytogenes*) bacteria or fungus (*Candida albicans*) from the blood of normal mice that had been treated for 4 days with a high dose (1.5 mg, ip) of water-miscible vitamin A palmitate. Mice treated with vitamin A had sterile blood 5 hr after challenge with *P. aeruginosa*, in comparison to vehicle-injected control mice, which developed a persistent bacteremia. Mortality differed significantly among these groups. Survival was also extended in animals infected with *L. monocytogenes* or *C. albicans*, although mortality was not prevented with these infections. Because vitamin A treatment provided protection to three unrelated organisms, Cohen and Elin (104, 105) inferred that the nonspecific arm of the immune system was activated by vitamin A. Similarly, hypervitaminosis A in mice has been reported to enhance host resistance to *Salmonella typhimurium* (106) and *L. monocytogenes* (107), presumably by activating mononuclear phagocytes. Large doses of retinoic acid and a synthetic derivative were also effective in the latter system. *In vitro*, replication of the tuberculli bacillus was reduced in human macrophages treated with retinoic acid (108).

More recently, Hatchigian *et al.* (109) evaluated semiweekly intraperitoneal administration of vitamin A palmitate, totaling 16 mg over 5 weeks, on the ability of normal Lewis rats to clear a sublethal dose of *Sa. typhimurium.* Serum retinol was approximately doubled, but the total number of white blood cells and their distribution changed very little. Bacteria were cleared at a significantly greater rate from blood as well as from the liver and spleen of vitamin A-treated rats. Kupffer cells and peritoneal and splenic macrophages of rats infected with *Sa. typhimurium* and treated with vitamin A had the greatest phagocytic activity, whereas phagocytosis was significantly depressed in the macrophages of infected rats not treated with vitamin A. Enhanced phagocytosis by peritoneal macrophages was also reported by Moriguchi *et al.* (110) for mice fed diets with high levels of retinyl palmitate (approximately 0.02–0.4 mg of retinyl palmitate/g of diet). This dietary treatment also increased the activation of macrophages and T lymphocytes, as assessed by IL-2 receptor expression.

Natural killer cell activity, antibody-dependent cell-mediated toxicity, and lymphocyte transformation have been studied in patients with chronic lymphocytic leukemia (111) or lupus erythematosus (112) who were treated with 30 mg of vitamin A/day for 2 weeks. Each of these activities was reported to increase after vitamin A treatment.

Gaps in Knowledge and Opportunities for Future Investigation

Correlation of the Response to Vaccines with Human Vitamin A Status. Despite the importance of the antibody response to successful immunization pro-

grams, the question of whether low vitamin A status in children or adults significantly impairs the serologic response is still an open one. Although most of the studies conducted with animals have shown a low response to natural and experimental antigens during vitamin A deficiency, there is still a need to document the kinetics as well as the magnitude of antigen-specific antibody production following immunization in human populations, with concomitant evaluation of vitamin A status.

There is essentially no information from human studies on whether vitamin A administration concurrent with immunization will effectively increase the frequency of children who respond, the magnitude of antibody concentration, or the longevity of the antibody response. Large single doses of retinol are of proven value in controlling xerophthalmia in populations in which vitamin A deficiency is prevalent (2). These doses seem to be safe as they have been used, but it needs to be kept in mind that long-term safety and efficacy studies have not examined the immune system as an outcome variable and, generally, have not focused on children less than 1 year of age. The question should be raised as to whether there is any evidence that infrequent high-dose supplementation with vitamin A would suppress, rather than stimulate or have no effect on, the immune system of the previously vitamin A-deficient child. Based on the literature reviewed here, it seems most likely that immune suppression would not result from such treatment. In animal studies, chronic high-dose supplementation decreased resistance to infection, suppressed the antibody response, and decreased lymphocyte proliferation (49), but an acute large dose of vitamin A restored the immune response in the previously vitamin A-deficient rat or chick (31, 49, 77). When vitamin A-deficient rats were given single large doses of retinol at the time of immunization with pneumococcal polysaccharide, the antibody response was restored to near-normal levels and no outward signs of toxicity were observed (36). The response to tetanus toxoid of rats given a single moderate dose of retinol 1 day after immunization with tetanus toxoid was slightly greater than the normal response (59). In adult surgical patients, cell-mediated immunity (lymphocyte transformation) appeared to be greater after short-term treatment with a large dose of vitamin A (102). Thus, these studies provide some reassurance. Nonetheless, vitamin A toxicity is a potential problem and only direct follow-up studies will be able to establish the long-term safety of high doses of vitamin A on the immune system. Such studies might include the recall response to specific antigens, lymphocyte counts, and cell typing using monoclonal antibodies, in conjunction with tracking of anthropometric measures. Based on sound nutritional practices and the positive outcome of field-based studies such as that of Ramathullah *et al.* (69), it would seem prudent to institute horticultural and educational programs to improve dietary intake or intervention programs with more frequent administration of smaller doses of supplemental retinol whenever feasible.

The area of mucosal immunity and antibody production as they are related to vitamin A status and vitamin A supplementation has received little attention. Future studies, both experimental and clinical, of local IgA production and serum IgA levels, particularly in response to known antigens, could help to clarify whether changes in intestinal differentiation associated with vitamin A deficiency also affect gut-related immunity.

Efficacy of Adjuvants in the Vitamin A-Deficient Host. An area deserving further attention is the role of adjuvants in modulating the immune response to antigens. Along with development of subunit antigens and other strategies designed to improve the antigen portion of vaccines, there has been progress in developing new adjuvants that enhance the response to weak antigens. Detoxified forms of bacterial lipopolysaccharide or lipid A are being tested as adjuvants to stimulate the antibody response to various antigens (113). Other agents such as block copolymers may also be useful in this regard (114). We have tested the ability of bacterial lipopolysaccharide, at doses that are nontoxic in the rat, to stimulate the antibody response to pneumococcal polysaccharide or tetanus toxoid in the vitamin A-deficient rat (115; M. Kinoshita and A. C. Ross, unpublished results). A remarkable enhancement to both antigens was observed in vitamin A-deficient animals whose response to these antigens is otherwise very poor. These studies provide evidence that the potential to produce antibody is intact during vitamin A deficiency and suggest that vaccines with an appropriate mix of antigen and adjuvant could elicit a good serologic response even in individuals with low vitamin A status.

The adjuvant properties of retinol itself may also be exploited in the future to modulate the antibody response. Recently, Friedman (90) evaluated water-miscible retinyl palmitate and retinoic acid as adjuvants with ovalbumin or hen egg lysozyme in mice and found increased lymphocyte numbers in draining lymph nodes, greater antibody titers, and greater proliferation following restimulation of cells *in vitro*. In recent years, increased interest has been focused on water-soluble forms of vitamin A, such as the glucuronide derivatives, because of their reduced retention and, hence, low toxicity (116). Tests of these and other forms of vitamin A as immune adjuvants may also prove to be of value.

Vitamin A and Nonspecific Resistance to Infection. The effects of supplemental retinoids on nonspecific immunity in experimental studies are quite striking and consistent. Studies have generally shown enhanced bacterial clearance, macrophage function, and phagocytosis in vitamin A-supplemented rats and mice.

Complement activation and cytotoxicity also appear to be greater in some cases. Whether polymorphonuclear leukocytes and macrophage phagocytosis is normal or not in children with vitamin A deficiency is unknown. Vitamin A deficiency is often said to be associated with increased morbidity and/or mortality from acute respiratory disease and intestinal infections in children, but documentation of this relationship is still weak. Studies could be conducted to determine whether vitamin A supplementation programs change the rate of positive throat cultures for respiratory pathogens or shorten the course of acute infection. In a recent randomized, controlled trial of Australian children who had no evidence of vitamin A deficiency but who had histories of frequent respiratory illness, children who received 0.45 mg/day of vitamin A had significantly fewer episodes of illness than the placebo group (117). Hospital studies along the lines of those recently conducted to establish the benefit of vitamin A treatment for acute measles infection could be extended to studies of non-measles pneumonia or diarrheal infections.

Conclusion

Vitamin A deficiency in experiment animals has broad effects on the immune system, including changes in organ morphology, cell numbers, and the response to specific pathogens and antigens, as well as nonspecific protection through phagocytic and cytotoxic mechanisms. Repletion with retinol has effectively reversed these changes in nearly all studies. A number of investigations of antibody production and of phagocytosis also support a role of retinoids in immune stimulation in animals whose vitamin A nutritional status is normal. It is not yet clear whether the vitamin A status of children in human populations at risk of vitamin A deficiency is sufficiently low to compromise the response to vaccines or the ability to clear infectious microbes. This information would be highly significant to public health strategies aimed at decreasing childhood morbidity and mortality in the developing world.

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1. Underwood BA. Vitamin A in animal and human nutrition. In: Sporn MB, Roberts AB, Goodman DS, Eds. The Retinoids. Orlando, FL: Academic Press, Vol **2**, pp281–392, 1984.
2. West KP Jr, Sommer A. Delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness. A state-of-the-art review. In: FAO, Administrative Committee on Coordination, Subcommittee on Nutrition, Ed. ACC/SCN State-of-the-Art Series Paper **2**, 1987.
3. McCollum EV, Davis M. The necessity of certain lipins in the diet during growth. J Biol Chem **15**:167–175, 1913.
4. Bloch CE. Blindness and other diseases in children arising from deficient nutrition (lack of soluble A factor). Am J Dis Child **27**:139–148, 1924.
5. Mori S. The changes in the para-ocular glands which follow the administration of diets low in fat-soluble A, with notes of the effect of the same diets on the salivary glands and the mucosa of the larynx and treachea. Johns Hopkins Hosp Bull **33**:357–359, 1922.
6. Wolbach SB, Howe PR. Tissue changes following deprivation of fat-soluble A vitamin. J Exp Med **42**:753–777, 1925.
7. Lassen HCA. Vitamin A deficiency and resistence against a specific infection. A preliminary report. J Hygiene **30**:300–310, 1930.
8. Green HN, Mellanby E. Vitamin A as an anti-infective agent. Br Med J **2**:691–696, 1928.
9. Green HN, Mellanby E. Carotene and vitamin A: The anti-infective action of carotene. Br J Exp Pathol **11**:81–89, 1930.
10. Scrimshaw NS, Taylor CE, Gordon JE. Interactions of Nutrition and Infection. Geneva: World Health Organization, 1968.
11. Nauss KM. Influence of vitamin A status on the immune system. In: Bauernfeind CJ, Ed. Vitamin A Deficiency and Its Control. New York: Academic Press, pp207–243, 1986.
12. Goodman DS. Plasma retinol-binding protein. In: Sporn MB, Roberts AB, Goodman DS, Eds. The Retinoids. Orlando, FL: Academic Press, Vol **2**, pp42–88, 1984.
13. Smith JE. Preparation of vitamin A-deficient rats and mice. Meth Enz **190**:229–236, 1990.
14. Nauss KM, Ross AC, Twining S. Immunotrophic methodology. Methods Enzymol **190**:259–273, 1990.
15. Bondi A, Sklan D. Vitamin A and carotene in animal nutrition. Prog Food Nutr Sci **8**:165–191, 1984.
16. Davila ME, Norris L, Cleary MP, Ross AC. Vitamin A during lactation: Relationship of maternal diet to milk vitamin A content and to the vitamin A status of lactating rats and their pups. J Nutr **115**:1033–1041, 1985.
17. Green MH, Uhl L, Balmer Green J. A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores. J Lipid Res **26**:806–818, 1985.
18. Nauss KM, Phua C-C, Ambrogi L, Newberne PM. Immunological changes during progressive stages of vitamin A deficiency in the rat. J Nutr **115**:909–918, 1985.
19. Pasatiempo AMG, Bowman TA, Taylor CE, Ross AC. Vitamin A depletion and repletion: Effects on antibody response to the capsular polysaccharide of *Streptococcus pneumoniae*, type III (SSS-III). Am J Clin Nutr **49**:501–510, 1989.
20. Lamb AJ, Apiwatanaporn P, Olson JA. Induction of rapid, synchronous vitamin A deficiency in the rat. J Nutr **104**:1140–1148, 1974.
21. Bieri JG, McDaniel EG, Rogers WE Jr. Survival of germfree rats without vitamin A. Science **163**:574–575, 1968.
22. Raica N Jr, Stedham MA, Herman YF, Sauberlich HE. Vitamin A deficiency in germ-free rats. In: DeLuca HF, Suttie JW, Eds. The Fat Soluble Vitamins. Madison, WI: University of Wisconsin Press, pp283–289, 1970.
23. Rogers WE Jr, Bieri JG, McDaniel EG. Vitamin A deficiency in the germ-free state. In: DeLuca HF, Suttie JW, Eds. The Fat Soluble Vitamins. Madison, WI: University of Wisconsin Press, pp241–255, 1970.
24. Anzano MA, Lamb AJ, Olson JA. Growth, appetite, sequence of pathological signs and survival following the induction of rapid, synchronous vitamin A deficiency in the rat. J Nutr **109**:1419–1431, 1979.
25. Nauss KM, Mark DA, Suskind RM. The effect of vitamin A deficiency on the in vitro cellular immune response of rats. J Nutr **109**:1815–1823, 1979.
26. Davis CY, Sell JL. Effect of all-trans retinol and retinoic acid nutriture on the immune system of chicks. J Nutr **113**:1914–1919, 1983.
27. Krishnan S, Bhuyan UN, Talwar GP, Ramalingaswami V.

Effect of vitamin A and protein-calorie undernutrition on immune responses. Immunology **27**:383–392, 1974.
28. Chandra RK, Au B. Single nutrient deficiency and cell-mediated immune responses. III: Vitamin A. Nutr Res **1**:181–185, 1981.
29. Pasatiempo AMG, Kinoshita M, Taylor CE, Ross AC. Antibody production in vitamin A-depleted rats is impaired after immunization with bacterial polysaccharide or protein antigens. FASEB J **4**:2518–2527, 1990.
30. Yamamoto M, Kitano T, Oma M, Hiyama Y, Yumoto S, Matsui M. Supplemental effect of retinoids on immune function in vitamin A deficient rats. Nutr Res **8**:529–538, 1988.
31. Pasatiempo AMG, Abaza M, Taylor CE, Ross AC. Effects of timing and dose of vitamin A on tissue retinol concentrations and antibody production in the previously vitamin A-depleted rat. Am J Clin Nutr **55**:443–451, 1992.
32. Suskind R. The immune response in the malnourished child. In: Watson RR, Ed. Nutrition, Disease Resistance and Immune Function. New York: Marcel Dekkar, pp149–163, 1984.
33. Smith SM, Levy NL, Hayes CE. Impaired immunity in vitamin A deficient mice. J Nutr **117**:857–865, 1987.
34. Nauss KM, Anderson CA, Conner MW, Newberne PM. Ocular infection with herpes simplex virus (HSV-1) in vitamin-A deficient and control rats. J Nutr **115**:1300–1315, 1985.
35. Nauss KM, Newberne PM. Local and regional immune function of vitamin A-deficient rats with ocular herpes simplex virus (HSV) infections. J Nutr **115**:1316–1324, 1985.
36. Pasatiempo AMG, Taylor CE, Ross AC. Vitamin A status and the immune response to pneumococcal polysaccharide: Effects of age and early stages of retinol deficiency in the rat. J Nutr **121**:556–562, 1991.
37. Chandra RK. Cellular and molecular basis of nutrition-immunity interactions. Adv Exp Med Biol **262**:13–18, 1990.
38. Buck J, Ritter G, Dannecker L, Katta V, Cohen SL, Chait BT, Hämmerling U. Retinol is essential for growth of activated human B cells. J Exp Med **171**:1613–1624, 1990.
39. Buck J, Derguini F, Levi E, Nakanishi K, Hämmerling U. Intracellular signaling by 14-hydroxy-4,14-*retro*-retinol. Science **254**:1654–1656, 1991.
40. Roitt IM, Brostoff J, Male DK. Immunology. London: Gower Medical Publishing, 1985.
41. Faherty DA, Bendich A. Characterization of immunomodulatory activity by retinoids. Methods Enzymol **190**:252–259, 1990.
42. Ahmed F, Jones DB, Jackson AA. Effect of vitamin A deficiency on the immune response to epizootic diarrhea of infant mice (EDIM) rotovirus infection in mice. Br J Nutr **65**:475–485, 1991.
43. Brown KH, Rajan MM, Chakraborty J, Aziz KMA, Phil M. Failure of a large dose of vitamin A to enhance the antibody response to tetanus toxoid in children. Am J Clin Nutr **33**:212–217, 1980.
44. Sijtsma SR, Rombout JH, West CE, van der Zijpp AJ. Vitamin A deficiency impairs cytotoxic T lymphocyte activity in Newcastle disease virus-infected chickens. Vet Immunol Immunopathol **26**:191–201, 1990.
45. Thurnham DI. Vitamin A deficiency and its role in infection. Trans R Soc Trop Med Hyg **83**:721–723, 1989.
46. Mark DA, Nauss KM, Baliga BS, Suskind RM. Depressed transformation response by splenic lymphoyctes from vitamin A-deficient rats. Nutr Res **1**:489–497, 1981.
47. Mark DA, Baliga BS, Suskind RM. All-trans retinoic acid reverses immune-related hematological changes in the vitamin A deficient rat. Nutr Rep Int **28**:1245–1252, 1983.
48. Butera ST, Krakowka S. Assessment of lymphocyte function during vitamin A deficiency. Am J Vet Res **47**:850–855, 1986.
49. Friedman A, Meidovsky A, Leitner G, Sklan D. Decreased resistance and immune response to *Escherichia coli* in chicks with low or high intakes of vitamin A. J Nutr **121**:395–400, 1991.
50. Trinchieri G. Biology of natural killer cells. Adv Immunol **47**:187–376, 1989.
51. Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MP, Park LS, Schooley KA, Coffman RL, Mosmann TR, Paul WE. Lymphokine control of in vivo immunoglobulin isotype selection. Annu Rev Immunol **8**:303–332, 1990.
52. Bowman TB, Goonewardene IM, Pasatiempo AMG, Ross AC, Taylor CE. Vitamin A deficiency decreases natural killer cell activity and interferon production in rats. J Nutr **120**:1264–1273, 1990.
53. Griffin DE, Ward BJ, Lauregui E, Johnson RT, Vaisberg A. Natural killer cell activity during measles. Clin Exp Immunol **81**:218–224, 1990.
54. Henderson RH, Keja J, Hayden G, Galazka A, Clements J, Chan C. Immunizing the children of the world: Progress and prospects. Bull WHO **66**:535–543, 1988.
55. Galazka A. Immunization of pregnant women against tetanus. WHO 1–13, 1983.
56. Dresser DW. Adjuvanticity of vitamin A. Nature **217**:527–529, 1968.
57. Semba RD, Muhilal, Scott AL, Natadisastra G, Wirasasmita S, Mele L, West KP Jr, Sommer A. Depressed immune response to tetanus in children with vitamin A deficiency. J Nutr **122**:101–107, 1992.
58. Lavasa S, Kumar L, Chakravarti RN, Kumar M. Early humoral immune response in vitamin A deficiency—An experimental study. Indian J Exp Biol **26**:431–435, 1988.
59. Kinoshita M, Pasatiempo AMG, Taylor CE, Ross AC. Immunological memory to tetanus toxoid is established and maintained in the vitamin A-depleted rat. FASEB J **5**:2473–2481, 1991.
60. Gershwin ME, Lentz DR, Beach RS, Hurley LS. Nutritional factors and autoimmunity. IV: Dietary vitamin A deprivation induces a selective increase in IgM autoantibodies and hypergammaglobulinemia in New Zealand black mice. J Immunol **133**:222–226, 1984.
61. McMurray DN, Barotw RA, Mintzer CE, Hernandez-Frontera E. Micronutrient status and immune function in tuberculosis. Ann NY Acad Sci **587**:59–69, 1990.
62. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med **323**:160–164, 1990.
63. EPI Global Advisory Group Meeting (EPI-GAG). Programmes for the control of vitamin A deficiency: The role of the EPI in new initiatives for the 1990's. WHO 1–23, 1988.
64. WHO/UNICEF. Vitamin A for measles [Statement]. Lancet **9**:1067–1068, 1987.
65. Chan M. Vitamin A and measles in third world children. Br J Med **301**:1230–1231, 1990.
66. Arroyave G, Calcaño M. Decrease in serum levels of retinol and its binding protein (RBP) in infection. Arch Latinoam Nutr **29**:233–260, 1979.
67. Baker PJ, Amsbaugh DF, Stashak PW, Caldes G, Prescott B. Regulation of the antibody response to pneumococcal polysaccharide by thymus-derived cells. Rev Infect Dis **3**:332–341, 1981.
68. Keusch GT. Vitamin A supplements—too good not to be true. N Engl J Med **323**:985–986, 1990.
69. Rahmathullah L, Underwood BA, Thulasiraj RD, Milton RC, Ramaswamy K, Rahmathullah R, Babu G. Reduced mortality among children in southern India receiving a small weekly dose of Vitamin A. N Engl J Med **323**:929–935, 1990.
70. Kimura S, Eldridge JH, Michalek SM, Morisaki I, Hamada S, McGhee JR. Immunoregulation in the rat: Ontogeny of B cell

responses to types 1, 2 and T-dependent antigens. J Immunol **134**:2839–2846, 1985.
71. Smith SM, Hayes CE. Contrasting impairments in IgM and IgG responses of vitamin A-deficient mice. Proc Natl Acad Sci USA **84**:5878–5882, 1987.
72. Carman JA, Smith SM, Hayes CE. Characterization of a helper T-lymphocyte defect in vitamin A deficient mice. J Immunol **142**:388–393, 1989.
73. Carman JA, Hayes CE. Abnormal regulation of IFN-g secretion in vitamin A deficiency. J Immunol **147**:1247–1252, 1991.
74. Forni G, Cerruti Sola S, Giovarelli M, Santoni A, Martinetto P, Vietti D. Effect of prolonged administration of low doses of dietary retinoids on cell-mediated immunity and the growth of transplantable tumors in mice. JNCI **76**:527–533, 1986.
75. Panda B, Combs GF. Impaired antibody production in chicks fed diets low in vitamin A, pantothenic acid or riboflavin. Proc Soc Exp Biol Med **113**:530–534, 1963.
76. Friedman A, Sklan D. Impaired T lymphocyte immune response in vitamin A depleted rats and chicks. Br J Nutr **62**:439–449, 1989.
77. Friedman A, Sklan D. Antigen-specific immune response impairment in the chick as influenced by dietary vitamin A. J Nutr **119**:790–795, 1989.
78. Ahmed F, Jones DB, Jackson AA. The interaction of vitamin A deficiency and rotavirus infection in the mouse. Br J Nutr **63**:363–373, 1990.
79. DeCarlo JD, VanHorn DL, Hyndiuk RA, Davis SD. Increased susceptibility to infection in experimental xerophthalmia. Arch Ophthalmol **99**:1614–1617, 1981.
80. Beisel WR. Synergism and antagonism of parasitic diseases and malnutrition. Rev Infect Dis **4**:746–750, 1982.
81. Parent G, Rousseaux-Prevost R, Carlier Y, Capron A. Influence of vitamin A on the immune response of Schistosoma mansoni-infected rats. Trans R Soc Trop Med Hyg **78**:380–384, 1984.
82. Darip MD, Sirisinha S, Lamb AJ. Effect of vitamin A deficiency on susceptibility of rats to *Angiostrongylus cantonensis*. Proc Soc Exp Biol Med **161**:600–604, 1979.
83. Chandra RK. Reduced secretory antibody response to live attenuated measles and poliovirus vaccines in malnourished children. Br J Med **2**:583–585, 1975.
84. Sirisinha S, Darip MD, Moongkarndi P, Ongsakul M, Lamb AJ. Impaired local immune response in vitamin A-deficient rats. Clin Exp Immunol **40**:127–135, 1980.
85. Puengtomwatanakul S, Sirisinha S. Impaired biliary secretion of immunoglobulin A in vitamin A-deficient rats. Proc Soc Exp Biol Med **182**:437–442, 1986.
86. Majumder MSI, Abdus Satter AKM, Mohiduzzaman M. Effect of vitamin A deficiency on guinea pig Peyer's patches. Nutr Res **7**:539–545, 1987.
87. Majumder MSI, Abdus Sattar AKM. Peyer's patch immune function of vitamin A deficient guinea pigs. Nutr Rep Int **36**:143–150, 1987.
88. Dennert G. Retinoids and the immune system: Immunostimulation by vitamin A. In: Sporn MB, Roberts AB, Goodman DS, Eds. The Retinoids. Orlando, FL: Academic Press, Vol **2**, pp373–390, 1984.
89. Dennert G, Crowley C, Kouba J, Lotan R. Retinoic acid stimulation of mouse killer T-cells in allogenic and syngeneic systems. JNCI **62**:89–94, 1979.
90. Friedman A. Induction of immune response to protein antigens by subcutaneous coinjection with water-miscible vitamin A derivatives. Vaccine **9**:122–128, 1991.
91. Nuwayri-Salti N, Murad T. Immunologic and anti-immunosuppressive effects of vitamin A. Pharmacology **30**:181–187, 1985.
92. Cohen BE, Cohen IK, Vitamin A: Adjuvant and steroid antagonist in the immune response. J Immunol **111**:1376–1380, 1973.
93. Bangham AD, Dingle JT, Lucy JA. Studies on the mode of action of excess of vitamin A. Biochem J **90**:133–140, 1964.
94. Sidell N, Ramsdell F. Retinoic acid upregulates interleukin-2 receptors on activated human thymocytes. Cell Immunol **115**:299–309, 1988.
95. Prabhala RH, Maxey V, Hicks MJ, Watson RR. Enhancement of the expression of activation markers on human peripheral blood mononuclear cells by in vitro culture with retinoids and carotenoids. J Leukocyte Biol **45**:249–254, 1989.
96. Sidell N, Conner MJ, Chang B, Lowe NJ, Borok M. Effects of 13-cis retinoic acid therapy on human antibody responses to defined protein antigens. J Invest Dermatol **955**:597–602, 1990.
97. Demetriou AA, Franco I, Bark S, Rettura G, Seifter E, Levenson SM. Effects of vitamin A and beta carotene on intra-abdominal sepsis. Arch Surg **119**:161–165, 1984.
98. Medawar PB, Hunt R. Anti-cancer action of retinoids. Immunology **42**:349–353, 1981.
99. Malkovsky M, Edwards AJ, Hunt R, Palmer L, Medawar PB T-cell-mediated enhancement of host-versus-graft reactivity in mice fed a diet enriched in vitamin A acetate. Nature **302**:338–340, 1983.
100. Jurin M, Tannoch IF. Influence of vitamin A on immunological response. Immunology **23**:283–287, 1972.
101. Patek JQ, Collins JL, Yogeeswaran G, Dennert G. Anti-tumor potential of retinoic acid: Stimulation of immune mediated effectors. Int J Cancer **24**:624–628, 1979.
102. Cohen BE, Gill G, Cullen PR, Morris PJ. Reversal of postoperative immunosuppression in man by vitamin A Surgery **149**:658–662, 1979.
103. Penn ND, Purkins L, Kelleher J, Heatley RV, Mascie-Taylor BH Belfield PW. The effect of dietary supplementation with vitamins A, C and E on cell-mediated immune function in elderly long-stay patients: A randomized controlled trial. Age Ageing **20**:169–174, 1991.
104. Cohen BE, Elin RJ. Enhanced resistance to certain infections in vitamin A-treated mice. Plast Reconstr Surg **54**:192–194, 1974.
105. Cohen BE, Elin RJ. Vitamin A-induced nonspecific resistance to infection. J Infect Dis **129**:597–600, 1974.
106. Hof H. Effect of hypervitaminosis A on infection of mice with *Salmonella typhimurium*. Afr J Clin Exp Immunol **2**:319–324, 1981.
107. Hof H, Emmerling P. Stimulation of cell-mediated resistence in mice to infection with listeria monocytogenes by vitamin A. Ann Immunol (Inst Pasteur) **130c**:587–594, 1979.
108. Crowle AJ, Ross EJ. Inhibition by retinoic acid of multiplication of virulent tubercle bacilli in cultured human macrophages. Infect Immun **57**:840–844, 1989.
109. Hatchigian EA, Santos JI, Broitman SA, Vitale JJ. Vitamin A supplementation improves macrophage function and bacterial clearance during experimental Salmonella infection. Proc Soc Exp Biol Med **191**:47–54, 1989.
110. Moriguchi S, Werner L, Watson RR. High dietary vitamin A (retinyl palmitate) and cellular immune functions in mice. Immunology **56**:169–177, 1985.
111. Gergely P, González-Cabello R, Jakab I, Vien CV, Bodó I. Effect of vitamin A treatment on cellular immune reactivity in patients with CLL. Acta Med Hung **45**:307–311, 1988.
112. Vien CV, González-Cabello R, Bodó I, Gergely P. Effect of vitamin A treatment on the immune reactivity of patients with systemic lupus erythematosus. J Clin Lab Immunol **26**:33–35, 1988.
113. Johnson AG, Tomai MA. A study of the cellular and molecular mediators of the adjuvant action of a nontoxic monophosphoryl lipid A. Adv Exp Med Biol **256**:567–579, 1990.
114. van de Wijgert JHHM, Verheul AFM, Snippe H, Check IJ, Hunter RL. Immunogenicity of *Streptococcus pneumoniae* type

14 capsular polysaccharide: Influence of carriers and adjuvants on isotype distribution. Infect Immun **59**:2750–2757, 1991.
115. Pasatiempo AMG, Kinoshita M, Ross AC. Coimmunization with lipopolysaccharide and pneumococcal polysaccharide (SSS-III) restores the antibody response to SSS-III in the vitamin A-deficient rat. FASEB J **6**:A1659, 1992.
116. Barua AB, Olson JA. Chemical synthesis of all-*trans* retinoyl β-glucuronide. J Lipid Res **26**:1277–1282, 1985.
117. Pinnock CB, Douglas RM, Badcock NR. Vitamin A status in children who are prone to respiratory tract infections. Aust Paediatr J **22**:95–99, 1986.
118. Committee on the 10th Edition of the RDA. In: Food and Nutrition Board, National Research Council, National Academy of Sciences, Ed. Fat-Soluble Vitamins. chap **7**, pp78–92, 1989.
119. American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc Committee on standards for nutritional studies. J Nutr **107**:1340–1348, 1977.
120. Ongsakul M, Sirisinha S, Lamb AJ. Impaired blood clearance of bacteria and phagocytic activity in vitamin A-deficient rats. Proc Soc Exp Biol Med **178**:204–208, 1985.