EFFECT OF PROTEIN, SELECTED MINERALS AND VITAMINS ON IMMUNE SYSTEM

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INTRODUCTION

The association of malutrition and infection has been known for centuries. The high mortality rates caused by the effects of malnutrition upon human populations always have been an intriguing problem. At one end of the scale is the availability of food and at the other end is the overwhelming infection which invariably accompanies severe malnutrition. Both malnutrition and infections are common in the developing countries and the devastation of the combined effects of both of them prompted Schrimshaw et al. (1) to employ the term "synergism of malnutrition and infection" to describe their interactions.

An intimate relationship exists between nutritional status, immune response and infection. Nutritional deficiency impairs immunocompetence and increases the severity and frequency of infection. Infectious illness is frequently associated with negative nitrogen balance which may precipitate malnutrition and depresses the immune response. Primary immunodeficiency states are characterized by failure to thrive and a variable susceptibility to infection. Undernutrition and infection often coexist and augment each other, and impair immunocompetence to a variable extent. The mutually augmenting effects of malnutrition are seen not only in individuals with gross protein-calorie malnutrition, but also in those with deficiencies of individual nutrients such as iron and folic acid (2).

A basic knowledge of the immune system is essential to understand the interrelationship between nutritional status and immunocompetence. The purpose of this report is to a) review the immune system briefly

and b) describe the role of specific nutrients in the immune system.

IMMUNE SYSTEM

The maintenance of the body's integrity is the principal function of the immune system. The immune system has evolved mechanisms to repel or destroy invaders of extrinsic origin, ranging from virus through bacteria, fungi, protozoa, and even metazoa. Immunity entails three major responses: 1) cell-mediated immune response, the thymus-dependent system often referred to as delayed hypersensitivity, 2) humoral antibody response, the apparent bone marrow dependent system, and 3) the non specific immunity response, which includes phagocytosis and macrophage-mediated cytotoxicity (3).

Cell-mediated immunity

"Cell-mediated" reactions are commonly defined as those immunological reactions transferable by cells and not serum, and include such diverse manifestations as allograft rejection, allogenic disease and delayed hypersensitivity in addition to pathogenic organisms (4).

Cell-mediated immunity works through the T-cells, the thymus dependent lymphocytes, and its end results are affected by the lymphoid killer cells which develop from the T-cells and macrophages which are required and activated by T-cells (5).

T-lymphocytes

The yolk sac and later, the fetal liver of mammalian embroys contain stem cells, whose offspring can become any of several kinds of hemopoietic cell. In adults, stem cells are produced in bone marrow. A hemopoietic stem cell or its immediate descendents, after migrating into the thymus

will divide repeatedly and give rise to small lymphocytes of the T-type. Normally T-lymphocytes become antigen responsive after they leave the primary lymphoid organ (thymus) to seed into the secondary lymphoid tissues (spleen and lymph nodes). Subpopulations of T-lymphocytes include "killer" cells, "helper" cells, "suppressor" cells, and perhaps a fourth, "amplifier" cells. As the nomenclature of these cells suggest, the killer cells can destroy other cells and the helper cells can enhance B-cell responses (vide infra) in the humoral immune system. Suppressor cells may inhibit T-helper and B-cell responses. Amplifier cells may facilitate and/or enhance the response of killer, suppressor, or helper cells (4,5,6).

T-lymphocytes recognize antigens by means of specific membrane receptors on their surfaces. Strong evidences suggest that the antigenic receptors of some T-cells are least partially if not fully structurally related to immunoglobulins (4,5).

Lymphokines

T-lymphocytes bearing specific receptors on their surface are stimulated by contact with antigen to release protein factors, collectively known as lymphokines. Lymphokines may recruit and activate macrophages to deal with intracellular parasites and microorganisms. The lymphokines produced by T-lymphocytes also include the poorly understood transfer factor, interferon, chemotactic factors, and blastogenic factors that will include proliferation of both T- and B-lymphocytes and other cells (5,7). Each of these factors is capable of amplifying the influence of the T-lymphocytes several hundred fold on a cell to cell basis.

Humoral immunity

Humoral immunity is more broadly defined as those immunological responses transferable by serum. The responses are also transferable by antibody producing cells or their precursors. Manifestations of humoral immunity include immediate hypersensitivity or "allergic" responses (e.g. anaphylaxis) and Arthus reactions in addition to classical antibody mediated protective immunity (4).

B-lymphocytes

Another population of lymphoid stem cells arising from the mehopoietic stem cells in bone marrow undergoes a process of proliferation and differentiation at the site as yet unknown in mammals but known to be the Bursa of Fabricus (located in the hindgut) in birds. Current evidence favors the bone marrow or fetal liver and spleen as the most likely site of the bursa equivalent in mammals. B-lymphocytes and their progeny, plasma cells, are responsible for the functions of humoral immunity. The latter is expressed through the production of circulating plasma proteins termed antibodies or immunoglobulins (5,6).

B-lymphocytes recognize antigens by means of specific membrane receptors on their sufaces. The B-cell receptors are immunoglobulins which are easily visible by fairly sensitive methods such as immunofloresence (4,5).

Immunoglobulins

There are five classes of immunoglobulins - IgG, IgM, IgA, Igd, and IgE. IgM occurs naturally in blood plasma (agglutinins anti-A and anti-B). In certain transfusion reactions, these substances can cause the agglutination of red blood cells, which have antigens

(agglutinogens A and B) on their membranes. IgG occurs in plasma and tissue fluids and is particularly effective against bacterial cells virus and various toxins. IgG antibodies are highly effective opsonins (substances that promote phagocytosis) and can activate the complement system (vide infra) and so stimulate its different biological activities. IgA is the most predominant immunoglobulin class in body secretions. Two molecules of IgA and one molecule of secretory piece form secretory immunoglobulin (S-IgA). S-IgA provides the primary defense mechanism against some local infections owing to its abundance in saliva, tears, gastro-intestinal tract, pulmonary tree and urino-genital tract. IgE is found only in small amounts in serum. It binds with very high affinity to mast cells and is responsible for allergic manifestations and for anaphylaxis. IgD is present normally in serum in trace amounts. There are a few reports of IgD with antibody activity toward certain antigens (5,6).

Complement system

The complement system contains a group of at least eleven protein factors. This series of substances is "activated" by complexes of antigens with a certain kind of antibody. Combination of antigen and antibody leads to a structural change in the antibody molecule, a change which is recognized by one of the components of the complement system. A cascade reaction begins with activation of one member of the chain which leads to activation of others next in line. This eventually leads to the release of a number of active proteins or kinins and various chemotactic factors. The triggering of the chemotactic agent and influx of leucocytes, which migrate to the site of the antigen-antibody complex, enhances phagocytosis. This sequence may further

lead to cell death through membrane damage. The complement system is also concerned in certain hypersensitivity reactions involving the combination of an antibody with surfaces and immune complexes (6).

T and B cell cooperation

The requirement for cooperation between thymus-derived (T-cells) and bone marrow derived lymphocytes (B-cells) was established by studies showing that neither cell population alone could mount an immune response to antigens such as serum proteins, heterologous erythrocytes and hapten-protein conjugates, whereas an admixture of the cell populations resulted in the production of high levels of antibody (6). The B-cells are the precursors of antibody forming cells. The T-cells do not synthesize readily detectable amounts of immunoglobulins but are needed as "helper" cells which stimulate the B-cells, accessary cells such as macro cells which stimulate the B-cells to differentiate into antibody producers. In addition to T and B cells, accessory cells such as macrophages are necessary for an immune response to occur. The area of cell interactions in immune responses is a complex one and a variety of mechanisms have been proposed. At present it is probably reasonable to regard collaborative immune responses as a series of inter-related processes in which antigen-specific recognition is performed by immunoglobulins and in which non-specific mediators of various sorts function as modifiers to regulate the intensity and the quality of the responses (5,6).

Non-specific immunity

Non-specific immunity does not involve specific recognition of the foreign agent. The methods by which it operates include a) specific

or genetic insusceptibility to certain pathogens, b) physical barriers to infection--skin, mucous membranes, c) biochemical barriers--lysozymes, stomach acid, and complement system, d) celular mechanisms involving phagocytosis (3).

Phagocytosis is an important non-specific defense mechanism involving macrophages. The macrophages may be of the floating or fixed types. The floating types include the polymorphonuclear leucocytes and monocytes in the blood. Fixed macrophages include the kuffer cells and the alveolar macrophages.

The process by which they provide protection can be divided into ten stages: production, mobilization, opsonization, recognition of and attachment to the object of phagocytosis, ingestion, degranulation, metabolic activity with associated generation of microbicidal free radicals, killing and digestion of the inactivated particle. The ingestion of foreign particles by phagocytes is promoted by antibodies (opsonins), component C3 and other serum factors such as IgG. In addition T-cell lymphokines may make macrophages more metabolically active and therefore more effective in degrading the phagocytized bacteria (3,6).

Tests for immunocompetency

The standard means of quantifying the adequacy of T-cell functions include skin testing for delayed allergies, in vitro transformation of cells with appropriate mitogens, antigens, or cells, and quantification of production of T-cell lymphokines and killer T-cell activities (8). Skin testing for delayed hypersensitivity is done by the intradermal injection of an antigen, such as purified protein derivative (PPD) for tuberculin sensitivity into the volar surface of forearm. The reaction is read at 48 hours, at which time an area of induration 0.5 cm in

diameter is considered positive in an individual who has had a tuberculosis infection. For humoral immune response other antigens can be administered to elicit antibody response.

Determination of T-lymphocyte blast transformation is done by measuring DNA synthesis by the uptake of tritiated (H^3) thymidine. Two mitogens commonly used for assessing T-cells are Concanavalin A (CON A) and phytohemmagglutinin (PHA) where as lipopolysaccharides (LPS) are used to assess B-cell function. If the lymphocytes were sensitized previously to the antigen under test, significantly higher counts from incorporated H^3 thymidine will be found in the cultures to which the antigen was added.

Ability to develop contact allergy to 2-4 dinitrochlorobenzene (DNCB) is another good indicator of the integrity of T-cell immunity system. Fourteen to 21 days after DNCB has been applied to the skin, a state of delayed sensitization is induced and can be demonstrated by a second application of DNCB to a different skin area. If cellular immunity is intact, an erythmatous lesion and blistering on the second challenge is observed. Regarding humoral immunity, levels of immunoglobulins IgA, IgM, or IgG and C3 complement component may indicate adequacy of immune competence (8).

EFFECT OF NUTRIENTS ON THE IMMUNE SYSTEM

Protein

Protein and/or calorie deprivation may produce profound and sometimes paradoxical changes in the immune defense mechanisms against infection and malignancy. The animal's immune resistance could be either increased or depressed depending on the timing and the severity of the nutritional deprivation.

Aref et al. (9) observed that severe protein-calorie malnutrition from birth resulted in the appearance of kwashiorkor as early as 6 to 7 months of age. Kwashiorkor in these children was accompanied by hypogammaglobulinemia and deficiency of both cellular and humoral immune responses. Symthe et al. (10) found however that kwashiorkor in South African children is accompanied by severe cell-mediated immune deficiency while immunoglobulin levels are maintained or increased.

Schlesinger and Stekel (11) reported on certain parameters of cellular immune responses that have been evaluated in infants suffering from a severe marasmic type of malnutrition. Both healthy and infected well-nourished infants were used as controls. The purified protein derivative (PPD) reactions were negative in the marasmic infants. Seven of eight marasmic patients studied within the initial 30 days of rehabilitation failed to develop hypersensitivity to dinitrochlorobenzene (DNCB); three gave a positive reaction after 30 days. Subjects giving a negative reaction initially remained negative when retested several months after recovery. Blast transformation of lymphocytes stimulated with phytohemagglutinin (PHA) was normal in the three groups. In another study (12) same authors showed significant reduction in interferon production in the cultures of marasmic infants. Early observations by Jayalakshmi and Gopalan (13) and Hartland (14) showed that children with subnormal rate of growth due to dietary protein deficiency had an impairment of their delayed hypersensitivity response to tuberculin antigen. The reduction in the hypersensitivity response was related directly to the severity of weight deficit and was not absolute since injection of 50 tuberculin units (T.U.) or greater overcame the anergy. A short period of good nutrition with a high protein (4g/Kg) diet repaired the delayed hypersensitivity response.

Cooper et al. (15) examined the differential effect of protein insufficiency on the immunological response in successive progeny of normally fed and protein depleted mother mice. Isocaloric diets of 8 and 27% casein were fed; all other nutrients except protein were kept constant. Seven basic types of immunological experiments were done to demonstrate the differential effect of chronic protein insufficiency. Results showed that chronic protein insufficiency 1) had no effect on either the primary or secondary antibody response to Brucella abortus, 2) depressed the primary humoral antibody response to sheep erythrocytes, 3) enhanced the graft vs host reaction as measured by spleen indices, 4) accelerated rejection of skin allografts in non-neonatally thymectomized mice vs both normally fed, non-thymectomized mice and neonatally thymectomized protein deficient animals, 5) enhanced the PHA induced blastogenic effect on spleen cells, 6) enhanced phagocytic activity of peritoneal macrophages to Listeria monocytogens, and 7) enhanced resistance to viral infection with pseudorabies, while depressing resistance to bacterial infection with Streptococci.

Serum antibody response to Salmonella typhimurium (typhoid) vaccine has been reported to be impaired in protein-calorie malnutrition (PCM) (16). Within eight days of starting nutritional therapy, significant increases in serum typhoid antibody were noted by Suskind et al. (17). Kenney et al. (18) studied the effect of protein deficiency on the spleen and antibody formation in rats. Control animals received a protein free diet for either 4 or 5 weeks in two trials. They found that the spleen of protein deficient rats immunized with sheep red blood cells (SRBC) produced only one-third as many specific antibody forming cells as did controls. Serum hemagglutinin antibody was

depressed similarly to one-third of the control level. Despite absolute reduction in both antibody forming cells and serum antibody forming titers there was no decrease in antibody production per cell. Mathur et al. (19) reported that intrinsic B-cell function appeared to be intact in protein deficient animals. Although significantly depressed splenic IgM antibody response to SRBC was noted, a normal response could be restored in these animals by injection of normal, syngeneic thymocytes (genetically identical thymus cells). This implies that the functional impairment in SRBC response in protein deficiency involved helper T-cells and not B-cells.

Good et al. (20) used the guinea pig as a model to test the influence of protein insufficiency on both in vivo and in vitro cell-midiated and humoral immune responses. The control groups were given 27% casein, the others 9% (representing a moderate restriction of protein), 6% (marked restriction), or 3% (severe). On day 28 of the experiment, all animals were inoculated with bovine-globulin (BGG). Results shown in Table 1 indicated that chronic protein deficiency had an 1) adverse effect on the humoral limb of the immune system, as measured by the hemagglutinating antibody titers, which decreased as the percentage of protein in the diet was decreased, 2) adverse effect on the lymphocyte system, as measured by the total number of lymphoid cells (per kilogram of body weight) obtained from nodes draining the site of injection of the immunizing antigens, 3) adverse effect on the immune response, as measured by a skin test reaction to the sensitizing antigen BGG, that involved contributions from both humoral and cellular limbs of the immune system. Guinea pigs on the lowest protein (3%) showed no erythema or induration at the skin test sites; while those on 6% and 9%

protein intake showed decreased erythema but little decreased induration and the same frequency of delayed allergic reactions, and 4) at 3, 6, and 9% protein intake, lymphocytes from nodes draining the site of antigen injection showed normal or increased capacity on a cell for cell basis to produce migration inhibitory factor (MIF) after antigenic stimulation.

Bhuyan and Ramlingaswami.(21) stuided the early inductive phase of the cellular immune response by evaluating the development of tubercles in draining lymph nodes of protein deficient guinea pigs after intra-dermal immunization with Bacille Calmette Guerin (BCG). They found a marked delay and defective mobilization of macrophages resulting in a poorly formed primary BCG nodule. Draining lymph nodes were atrophic with almost no cellular proliferation and no histologic evidence of antigen stimulation. epitheloid cell transformation and transformation of mature granulomas were retarded, and bacilli persisted for long periods in the primary immunization site as well as the draining lymph nodes. This and other evidence indicate that both macrophage and T-cell function are markedly impaired in protein deficient guinea pigs.

Immunity to viral antigens seems to be less adversely affected by poor nutrition. Children with kwashorkor develop antimeasles immunity at a younger age than do healthy controls (22). Ifekwunigwe et al. (23) also showed that protein-calorie malnutrition did not effect the children's ability to develop adequate immune response to measles or smallpox vaccine. The ability to mount an adequate antibody response to polio viruses is especially resilient. A group of children with kwashiorkor who were immunized with yellow fever and polio vaccines produced high titers of anti-polio activity but responsed poorly to

Table 1

Effect of chronic protein deficiency on immune responses of guinea pigs to bovine γ-globulin (20)

Percent casein	Skin test reaction: Erythema	Skin test reaction: Induration (diameter)	Cellular immunity (percent PEC migration inhibition by MIF)	Humoral immunity (hemagglutinating AB titers/log^2)	Effect on lymphatic system (total mean numbers of lymph node cells[1])
27%-- Control	++++	++++	Normal M = 82 R = 58-93	Normal N = 15.5 R = 2.5-19.0	Normal (7.0×10^8)
9%-- Moderate CPD	+++	+++	Moderately increased M = 90 R = 87-96	Decreased M = 10.5 R = 10.0-13.5	Decreased (4.6×10^8)
6%-- Marked CPD	++	+++	Moderately increased M = 89 R = 75-94	Marked decrease M = 8.5 R = 6.5-11.0	Decreased (5.2×10^8)
3%-- Severe CPD	0	0	Normal M = 81 R = 53-87	Marked decrease M = 7.5 R = 6.5-9.0	Very marked decrease (2.8×10^8)

M = median, R = range, AB = antibody, MIF = migration inhibitory factor, PEC = peritoneal exudated cell

[1]From nodes draining site of injection per kilogram of body weight.

yellow fever (24). Yellow fever vaccine given to another group of children with kwashiorkor again failed to generate specific antibody production (25).

The effect of dietary protein restriction on immunity to xenogeneic, allogeneic and syngeneic tumor models has been studied. Jose and Good (26) in their study on rats with moderate chronic protein or calorie deficiency showed normal development of specific cell-mediated immunity to transplantation antigens. Complement dependent cytotoxic antibody and blocking antibody production to cellular antigens were markedly depressed in protein deficient animals. In further studies (20) they compared the response of Charles River male rats which had been normally nourished to those of rats on protein and calorie restricted diets, using xenogeneic tumor cells taken from the DBA/2 mouse as target cells (Table 2). Controls maintained on a 30% protein diet showed vigorous T-cell cytotoxicity and maintained both cytotoxic and blocking antibody responses. Animals suffering from a moderate protein restriction had an intact T-cell reaction, but an impaired humoral response to the xenogeneic cells, a phenomenon which actually served the animal well, for with an inhibition of the blocking antibody, T-killer cells were able to attack the foreign tumor cells in an unimpeded fashion. Calorie deprived animals in this experiment displayed a moderately decreased cytotoxic and blocking antibody production.

In another study (20) the effect of progressive reduction in dietary protein or protein and calories on humoral immunity was assessed by measuring serum hemagglutinin titer to allogeneic erythrocytes and tumor cytotoxic and blocking antibody titer to both allogenic and syngeneic tumors. T-cell cytotoxicity was measured by a ^{51}Cr-release assay. In both cases, cell-mediated cytotoxicity in deficient groups

Table 2

Immune response of rats to xenogeneic tumor cells (20)

Percent protein[1]	Calories/day[2]	CMC (percent lysis)	CMC (percent lysis) in presence of blocking factor	Percent cytotoxic antibody	
				No Complement	Complement
30	Normal	62	5	16	82
8	Normal	68	67	11	25
22	Half Normal	57	17	15	41
Non-immunized	--	0	66	0	19

CMC = cell-mediated cytotoxicity

[1]Normal protein = 22 to 30%

[2]Normal calories = 20 to 22

receiving either 11, 8 or 5% protein diets was comparable to or greater than that found for a control group receiving a 28% protein diet, but became extremely low when protein intake was reduced to 3%. Significant reduction in cytotoxic and hemagglutinin antibody titers was noted at dietary levels of 8% protein after allogeneic tumer cell immunization. Further slight reductions were noted if calories were also reduced to half-normal. Marked depression of these antibody titers was noted at a dietary protein level of 5%, with complete disappearance of cytotoxicity and hemagglutinating activity at a level of 3% protein. Serum blocking activity was more sensitive to changes in protein level, it was significantly reduced at 11% protein and disappeared entirely at protein levels of 5% and 3%. Similar results were obtained in the syngeneic tumor system. In the allogeneic tumor model repeated immunization resulted in increased serum blocking activity at all dietary levels, although it always remained less than that observed in animals on normal diets. From these studies it is postulated that the increase in cell-mediated immunity noted in animals which have been chronically deprived of protein may be due at least in part, to inhibition of the humoral blocking factor which under normal circumstances would inhibit T-cells from destroying neoplastic cells of syngeneic, allogeneic, or xenogeneic origin.

Autoimmunity represents a breakdown in normal immunoregulatory mechanisms. The pathogenesis is as yet unknown, although the loss of supressor cell activity may play a role. Fernandes et al. (27) demonstrated that nutritional manipulation can exert an important effect on the course of autoimmune disease. They studied the effect of dietary protein and fat content on the development of autoimmune phenomena in NZB mice. NZB mice provide a useful model for autoimmunity.

After normal development for 3-4 months NZB mice develop a Coombs-positive hemolytic anemia, autoantibodies, and progressive renal disease. These changes correlate with the appearance of antinuclear and anti-DNA antibodies and progressive loss of functions dependent on cell-mediated immunity. NZB mice maintained on high fat/low protein diets exhibited improved breeding performance but earlier development of Coombs-positive hemolytic anemia and shorter life spans (28). High protein/low fat diets resulted in increased longevity and later development of the hemolytic anemia, although eventual severity of the anemia was the same as in controls. In subsequent studies (27) moderate protein restriction resulted in significantly lower titers of autoantibodies against DNA. Cellular immune function, which normally undergoes progressive decline with age in NZB mice, was also better maintained in animals on chronic protein restricted diets. Protein restriction resulted in maintenance of more vigorous antibody response to thymus-dependent antigen SRBC, greater capacity to produce graft-versus-host reactions, more vigorous cytotoxicity response against DBA/2 mastocytoma cells after immunization, and some inhibition of the normally observed decline in PHA and concanavalin A reactivity with age. Life span, however was not prolonged by this diet, although the appearance of hemolytic anemia was delayed it was not prevented. These results demonstrate, however, that significant alteration in the course of the disease can be accomplished by dietary means.

Amino acids

Antibody production also can be affected in amino acid deficiency. The immune defects observed in PCM may result from deficiencies of essential amino acids. Bhargava et al. (29) studied the effects of

d or l methionine in chicks infected with New Castle disease virus. The "l" form of methionine resulted in higher antibody production than the "d" form. The optimum requirement for methionine for growth was 0.7% of the diet whereas for optimum antibody production, it was considerably lower. On the other hand, the requirement of the chick for valine was somewhat higher for optimum antibody production than for optimum growth. Gershoff et al. (30) found no effect of methionine deficiency or excess on antibody production against the synthetic antigren poly-Glu-Lys-Tyr or against SRBC.

Tryptophan is thought to play a crucial role in maintenance of normal antibody production (30). Kenney et al. (31) demonstrated that in rats supplementation of tryptophan deficient diets with tryptophan resulted in a fourfold increment in hemolysin titers to SRBC, attributed to both increases in the number of the antibody-forming cells and in the number of immunoglobulin produced per cell (Table 3).

Bounous and Patricia (32) investigated the humoral and cell-mediated responses in vivo of mice fed a series of diets in which either the concentration of a single amino acide varied over a wide range or there were multiple amino acid restrictions. In mice fed a diet moderately deficient in the amino acid phenylalanine (0.4 g/100 g), tyrosine (0.2 g/100 g), the plaque forming cell response and serum antibody titers to SRBC were enhanced. More severe limitation of phenylalanine-tyrosine or multiple essential amino acid restriction gave rise to a slight increase in the delayed hypersensitivity reaction to SRBC. These latter diets usually limit growth, nevertheless the humoral immune response to SRBC was not significantly depressed except in the mice fed a diet with severe restriction of seven essential amino acids.

Table 3

Effect of dietary tryptophan on antibody (Ab) production in rats (31)

Tryptophan added	Serum Ab	
	Hemolysin	Abblutinin
mg/g diet	log titer	log titer
0	1.72[1,2]	2.01[2]
0.38	—[3]	—[3]
0.75	2.09	2.80
1.50	2.20	2.90
2.25	2.46	2.80
	SE ± 0.13	± 0.20

[1]Significant linear regression of Ab on amount of added tryptophan ($p < 0.05$)

[2]Different from group supplemented with tryptophan ($p < 0.05$).

[3]No data.

Studies in mice and in melanoma patients suggested that some degree of tumor growth inhibition may occur with dietary restriction of phenylalanine and tyrosine or other essential amino acids. Jose and Good (33) measured the effect of different degrees of deficiency of essential and semi-essential amino acids upon specific immune responses to allogeneic tumor cells in mice. Synthetic amino acid diets were prepared from purified amino acids mixed in concentrations approximately equivalent to a casein diet containing 15% protein calories. The effect of deficiency of a single amino acid was tested feeding a series of diets in which the selected amino acid was reduced to 50, 25 or 10% of its concentration in the standard amino acid diet. Primary humoral and cellular immune responses to allogeneic tumor cells were assessed by in vitro assay in groups of mice fed the various diets. Moderate reduction of the amino acids phenylalanine-tyrosine, valine, threonine, methionine, cystine, isoleucine and tryptophan in the diet produced depression of hemagglutinating and blocking antibody responses, although cytotoxic cell-mediates immunity remained intact. Limitation of the amino acids arginine, histidine and lysine in the diets gave rise to only slight depression of the immune responses. Moderate leucine restriction resulted in a paradoxical depression of cytotoxic cell-mediated immunity with little effect on serum blocking activity. Similar tumor growth inhibition by phenylalanine-tyrosine restricted diets also has occurred in mice with sarcoma, hepatoma (34) and various adenocarcinomata (35). In studies (without controls) of the effect of phenylalanine-tyrosine restriction in the treatment of human patients with malignant melanoma (36) and other malignant tumors (33), regression of primary tumor growth, cessation of the metastatic spread and

improvement in hematological and subjective parameters have been reported. The relative roles of limitations of essential substrates for tumor growth and of increased immune resistance to the tumor through depression of serum blocking activity suggested in their studies are unknown.

Worthington et al. (37) evaluated in a syngeneic system the long term effect of dietary reduction of isoleucine, leucine or phenylalanine-tyrosine on carcinogenesis with methylcholanthrene (MCA), and immunity to a transplanted MCA tumor in inbred mice. The result suggested that 1) restriction of the selected amino acid does not inhibit chemical carcinogenesis with MCA, 2) phenylalanine-tyrosine deficiency may actually enhance chemical carcinogenesis with MCA and 3) selected essential amino acid deficiencies do not enhance immunity to a transplaned MCA tumor.

Although a few clinical and experimental observations pointed to enhanced host resistance to some viral infections in undernutrition, most data suggest an increased susceptibility of individuals with protein-energy malnutrition to infectious diseases. In recent years there has been increasing recognition of the frequent presence of protein-energy malnutrition in hospital patients. In a study of patients with lymphoreticular malignancies, the occurrence of complicating pneumocystis carinii infection was related to lower concentrations of serum proteins and albumin (38). This confirmed controlled observations in rats, whose susceptability to P. Carinii infection was enhanced by protein deficiency (39). Epidemiological studies and serological surveys have not shown a higher incidence of measles infection in populations that experience variable degrees of protein-

calorie malnutrition (40). Nevertheless measles stands out among the common viral diseases as the great scourage of the malnourished child (41). Furthermore severe complications of measles, such as giant cell pneumonia, bronchopneumonia and diarrhea are common events in the malnourished child and contribute to high fatality rates. Impairment of CMI may contribute to decreased resistance from infection with measles virus in malnourished patients. Altered response to BCG in protein malnutrition may be because of impairement of macrophage function in protein deficiency. Macrophages play a crucial role in mycobacterial infection. An important aspect of improved tumor immunity observed in animals on low-protein diets is the concomitant depression of B-cell derived blocking factors which can interfere with the lysis of the tumor cells by T-cell killer cells (42). In terms of function, T-cell immunity, including the T-cell mediated surveillance mechanism for elimination of malignant and virus infected cell, becomes vastly more effective due to the absence of inhibition by the serum blocking factors.

The effect of malnutrition on host defense mechanisms are undoubtedly complex and no simple answer can be anticipated from above mentioned studies. However Good et al. (42) have summarized that: 1) Profound chronic protein, protein-calorie, or specific amino acid deprivation depresses both T- and B-cell functions. 2) An acute deprivation of protein, with or without calorie deprivation, increases antibody production, while it depresses proper functiong of the T-cell system. 3) Diets which induce chronic protein deprivation (CPD) enhance many aspects of the T-cell system. Humoral responses may be depressed or unaffected depending upon the nature of antigen employed.

Chronic protein insufficiency may lead to increased output of thymic hormone(s) (thymin). This may operate in several ways to enhance cell-mediated immunity, any of which may involve thymin overproduction. However the two most likely mechanisms for this enhancement are: 1) an absolute increase in T-cell population and 2) increased immunological competence of each effecter T-cell. In spite of all these findings, much more extensive studies on the interfaces between specific immunological functions and nutritional factors are needed and from such studies much useful information can be anticipated.

Zinc

Zinc is essential for the activity of many enzyme systems and a zinc deficiency can result in a variety of manifestations (43). Recent studies have begun to elucidate the important role that zinc can play in DNA synthesis and because of its role in DNA synthesis one might expect zinc to exert an important control over replicating cells involved in the immune response.

Four to eight week old A/Jax, C57BL/KS and CBA/H mice on zinc deficient diets showed a gradual loss of body weight, high mortality rate, skin changes, diarrhea, acrodermatitis and involution of the thymus (44). Following intraperitioneal immunization with sheep red blood cells (SRBC) the numbers of antibody forming cells in the spleen were significantly lower in animals fed a zinc deficient diet compared to pair-fed animals getting a zinc replete commercial stock diet (Table 4). Both direct predominantly IgM, plaque forming cells (PFC) and indirect predominantly IgG plaque forming cells were reduced. Secondary immune response following reimmunization with SRBC seven days after the first injection also was evaluated and was slightly improved over the primary

Table 4

Plaque forming cell (PFC) response in C57BL/Ks mice maintained on various diets (44)

Diet[1] Group	Duration weeks	Direct PFC per spleen	Indirect PFC per spleen
Zn (-)	2	29,127 ± 9,682[2]	23,149 ± 8,402[2]
Zn (+)PF[3]	2	25,428 ± 1,276	19,699 ± 920
Zn (-)	4	18,502 ± 5,606	14,913 ± 5,446
Zn (+)	4	38,392 ± 9,902	31,934 ± 8,549
Lab chow	6	64,281 ± 5,152	53,294 ± 3,144
Zn (-)	6	8,278 ± 2,651	5,128 ± 2,312
Zn (+)PF	6	80,983 ± 2,772	69,071 ± 6,749
Zn (+)ad lib	6	117,968 ± 15,858	95,446 ± 7,393
Lab chow	8	104,662 ± 18,937	74,647 ± 6,202

[1]At least four mice were immunized with SREC in each group.

[2]Data are shown as mean ± SEM

[3]PF, pair fed.

response but in contrast, the zinc replete animals showed a three to four fold increase (Table 5).

Haas et al. (45) have reported that zinc deficient mice were unable to mount a primary antibody response after immunization with keyhold limpet hemocyanin-P-azophenylarsonate, despite the fact that immunization was initiated on the first day of the deficient diet, when a normal population capable of recognizing and processing antigen was presumably present. In a subsequent study (46), however young adult A/J mice were immunized with the same antigen and then fed adequate, marginal, or severely zinc deficient diets for four weeks. There was no significant difference in antibody titers among the three groups, even though the thymuses of the deficient mice were markedly atrophic. When the mice were fed these zinc deficient diets for four weeks prior to immunization with SRBC, however the number of anti-SRBC plaque forming cells subsequently were markedly reduced to 10% of control levels in the severly deficient and 25% in the marginally deficient animals. These observations indicate that zinc deficiency produced thymic involution and impaired T-helper cell activity with little effect on B-cells.

Frost et al. (43) observed that in zinc deficient mice the splenic response to immunization with SRBC, was relatively depressed. There was a delay in developing the plaque forming response and it reached a lower absolute level.

Zinc deficiency in swine was shown to cause growth failure, atrophy of the thymus gland, reduced peripheral blood lymphocyte counts and depressed serum gamma-globulin level (47).

In rats, lymphocyte transformation in response to phytohemagglutinin was impaired by zinc deficiency (48). In a recent series of studies,

Table 5

Development of direct or indirect PFC response after immunization with SRBC in A/jax mice maintained on Zn(-) and Zn(+) diets for 8 weeks (44)

Diet	n[1]	Diet PFC per spleen	Indirect PFC per spleen
Zn (-)			
Primary	11	6,515 ± 261[3]	5,949 ± 2,158[3]
Secondary	7	13,938 ± 3,848	22,669 ± 7,996
Zn (+)PF[2]			
Primary	9	18,501 ± 2,321	17,406 ± 1,960
Secondary	7	8,928 ± 2,758	75,950 ± 17,933
Zn (+) adlib			
Primary	7	20,436 ± 3,438	18,870 ± 3,704
Secondary	6	8,737 ± 2,254	63,756 ± 19,679
Lab Chow			
Primary	5	21,355 ± 5,762	22,625 ± 7,897
Secondary	5	10,174 ± 3,374	82,070 ± 6,116

[1] n = Number of mice immunized;

[2] PF, pair fed;

[3] Data are shown as mean ± SEM.

Gross et. al. (49) used a battery of mitogens to define the blastogenic capacities of thymic, splenic and peripheral blood lymphocytes in zinc deficient and pairfed control rats. Weanling rats were placed on either a zinc deficient or a control diet for four weeks and then studied. The T-cell proliferative response to phytohemagglutinin (PHA) and concanavalin A was measured; pockweed mitogen (PWM) was also used to assess proliferative response requiring T- and B-cell cooperation. The response of splenic, thymic, and peripheral blood lymphocytes to PHA was depressed to approximately 50% of control levels (Table 6). Although lymphocytes from spleen and peripheral blodd of zinc deprived animals showed a significantly depressed response to Con A, the response of thymus cells were normal to Con A (Table 7).

Although Con A is a T-cell mitogen, it probably stimulates a different subset of T-cells than that responsive to PHA (50), which suggests that different subsets of T-cells may be differentially sensitive to zinc deficiency. Finally, the response of both spleen and thymus to PWM were significantly depressed in the deprived group. Although decreased, PWM response to peripheral blood lymphocytes of zinc deprived animals was not significantly different from that of the control (Table 8).

Zinc deficiency also may play a part in the thymic atrophy and infections associated with malnutrition. In a case study where eight children recently recovered from severe protein-energy malnutrition, were supplemented with zinc showed an increase in thymic size (51). A 17 year old decerebrate male with acquired zinc deficiency rendered a negative skin reaction to dinitrochlorobenzene (DNCB) (50). The patient's lymphocytes to incorporate tritiated thymidine into DNA and undergo mitosis in response to PHA stimulation was significantly depressed with a stimulation index of 47 $\pm$ 0.8 compared to 139.1 $\pm$ 77 for the controls

Table 6

Maximum response to PHA of spleen, thymus and peripheral blood lymphocytes from control and zinc deprived animals (49)

Organ	Mean maximum stimulation index			
	Controls	# of rats	Zinc deprived	# of rats
Spleen	146.2 ± 95.4	9	76.6 ± 78.2	11
Thymus	18.5 ± 17.0	10	6.6 ± 4.0	11
Peripheral blood	270.9 ± 20.7	17	140.0 + 125.1	21

Table 7

Maximum response to Con A of spleen, thymus and peripheral blood from zinc deprived and control animals (49)

Organ	Mean maximum stimulation index			
	Controls	# of rats	Zinc deprived	# of rats
Spleen	165.7 ± 104.2	8	91.8 ± 73.8	10
Thymus	31.0 ± 21.6	9	27.3 ± 24.1	10
Peripheral blood	221.4 ± 199.8	12	90.3 ± 112.9	19

($P < 0.02$).

McMohan et al. (53) studied the effect of zinc chloride on spontaneous SRBC rosette formation by human lymphocytes. In normal subjects, in vitro addition of zinc chloride caused a concentration dependent increase in the percentage of lymphocytes forming rosettes. A maximum increase of 36% (from 40 to 76%) was observed with the addition of 0.3mM zinc chloride. In addition, depressed rosette levels in 10 cancer patients showed markedly increase after addition of zinc, increasing from levels of 29% to maximal levels of 78%. Zinc also increased the number of SRBC bound to lymphocytes and increased both temperature and mechanical stability of the rosettes. The increased size and stability of the rosettes suggested that zinc acts either by increasing availability of functional receptors for SRBC on the lymphocyte surface or by neutralization of cell surface charge leading to increased net attractive force between lymphocyte and SRBC.

The growth retardation in zinc deficient animals is related to both a loss of appetite and a defect in several enzyme systems. The extensive depression of lymphoid organ weight could be related to two facts. First, new lymphoid cells are being produced constantly in the bone marrow and are being modified by the thymus into T-cells and by the bone marrow and spleen into B-cells. Second, the response to antigen requires an active proliferation of resting antigen sensitive cells. Either of these steps would be impaired by metabolic deficiencies which hamper DNA synthesis (43). Thymic atrophy seen in protein-energy malnutrition could be due to a combination of high levels of circulating steroids, zinc deficiency or pyridoxine deficiency (49). Acute and chronic illness and a high level of circulating steroids in themselves

Table 8

Maximum response to PWM of spleen, thymus and peripheral blood from zinc deprived and control animals (49)

Organ	Mean maximum stimulation index[1]			
	Control	# of rats	Zinc deprived	# of rats
Spleen	28.3 ± 14.3	9	14.1 ± 10.9	11
Thymus	8.7 ± 8.2	10	4.3 ± 4.1	11
Peripheral blood	30.8 ± 39.9	12	15.5 ± 13.6	16

[1]Stimulation index is expressed as mean ± SD; statistical analyses are by one way analysis of variance.

lead to a fall in plasma zinc values. Thus insufficient zinc may be a common mediator for the thymic atrophy seen in various conditions. Zinc deprivation also might result in membrane labilization with subsequent changes in membrane receptor stie availability or function (49). Since the humoral response to most antigens is dependent on fruitful interactions between helper T-cells and B-cells, an inadequate response is generated in zinc deficiency due to the reduction in the generation of plasmacytes caused by an insufficient number of functional helper T-cells. However, further studies are needed to determine the extent of impairment to the immune system caused by zinc deficiency.

Iron

On the basis of clinical impression and anecdotal experience, iron deficiency has been associated with increased susceptibility to infection (54). Several authors purport increased rate of infections in iron deficient patients when compared with controls. On the other hand, other workers have failed to confirm these findings.

Iron deficiency has biochemical and morphological effects on various tissues. There are a number of enzymatic and metabolic abnormalities in iron deficiency which would theoretically affect the various components of the immune system.

Macdougall et al. (55) evaluated the cellular defense mechanism in 20 children with iron deficiency anemia and in seven with latent iron deficiency. Delayed hypersensitivity reactions were performed with diptheria toxoid, candida and streptococcal antigens. Lymphocyte transformation was measured by using phytohemagglutinin (PHA) and candida antigen. Whereas 12 of 14 control children had a positive reaction to diptheria toxoid, only two of eleven anemic children and one of the

latent iron deficient children had a positive reaction. With candida antigen, 11 of 14 children had a positive reaction, as compared with 7 of 11 anemic and 3 of 7 latent iron-deficient children. The response to streptococcal antigen was poor in all the three groups. Stimulation of DNA synthesis in lymphocytes by PHA or candida antigen was impaired (Table 9). Treatment with iron restored most of teh cellular immune function to normal.

Chandra and Saraya (56) in a study of 20 iron-deficient children in whom PCM had been carefully ruled out, found normal peripheral blood lymphocyte counts in the deficient children with the exception of two with slight lymphopenia. Circulating T-cell levels were significantly depressed (49% compared with the control level of 63%). There was impaired cutaneous hypersensitivity to candida antigen and a less than normal stimulation of DNA synthesis in lymphocytes by PHA in deficient children.

Joynson et al. (57) deseribed in vivo and in vitro deficits in the cell-mediated immune response in patients with iron deficiency anemia. A significant skin reaction to candida antigen was found in all 12 controls, but in only 3 out of 12 iron-deficient patients. A positive skin reaction to purified protein derivative (PPC) was found in ten of the controls but in only five of the iron-deficient subjects. Lymphocyte transformation (as measured by the uptake of labeled thymidine) was significantly decreased in preparations from 12 iron deficient patients when PPD was used as the antigen, but did not achieve statistical significance when candida was used as the antigen (Table 10). Further production of macrophage migration inhibitory factor (MIF) by lymphocytes stimulated with candida antigen and by PPD was less than that

Table 9

Mean lynphocyte index after PHA and candida stimulation (55)

	Control	Iron-deficient Pre-treatment Anemic	Iron-deficient Pre-treatment Latent	Iron-deficient Post-treatment Anemic	Iron-deficient Post-treatment Latent
No. of patients	11	11	4	6	4
PHA	13.37 ± 3.98	9.74[1] ± 4.62	6.60[2] ± 1.47	16.22 ± 3.49	17.23 ± 2.98
Candida	2.28 ± 0.70	1.50 ± 0.69	1.40 ± 0.54	2.1 ± 0.50	1.93 ± 0.69

[1] $p < 0.02$ vs controls

[2] $p < 0.05$ vs controls

Table 10

Mean mitotic index after PHA
and candida stimulation (57)

Mean mitotic index	Controls	Iron deficent subjects	Significance
Candida	3.32 ± 2.03	1.16 ± 0.08	$p < 0.25$
PPD	22.26 ± 4.29	7.73 ± 2.07	$p > 0.005$

produced by lymphocytes from iron repleted control subjects. Unfortunately, Joynson et al. (57) were unable to demonstrate a significant improvement in the in vivo and in vitro proliferative responses to PPD and Candida with iron therapy, except for a significant improvement in the MIF response after treatment. Fletcher et al. (58) reported oral candiasis in 12 out of 23 patients with iron deficiency. The iron deficient patients had significantly lower peripheral lymphocyte counts than did controls. There was no significant difference in PHA response with regard to the presence or absence of oral candiasis, both groups were significantly depressed compared to the controls. Levels of folate and B_{12} in the serum were normal. In 6 patients treated with iron only, both lymphocyte count and PHA response returned to normal. Bhaskaram and Reddy (59) reported a reversible reduction in the blastogenic ability of the lymphocytes of the iron deficient children.

Kulapongs et al. (60) studied eight severely iron deficient Thai children (mean hemoglobin level 3.5 $\pm$ 0.4 mg/ 100 ml) in whom other nutrient deficiencies had been carefully ruled out. The in vitro cell mediated immune response was evaluated by blast cell transformation and in vitro incorporation of tritiated thymidine into PHA stimulated lymphocytes. They found that both the percentage of lymphocytes undergoing blast transformation in response to PHA and the proliferation index, measured by ^{3}H thymidine uptake were normal. The proliferation index for the iron-deficient group was higher (139.7 compared with 84.4 for controls), but not significantly so. After iron therapy had restored hemoglobin level to normal the patients were retested and neither percentage blast transformation nor stimulation index was changed from admission values.

Although most of the patients in the above mentioned studies had a well defined iron deficiency state, other mild nutritional deficiencies were not definitively excluded. Serum folate levels were reported only by Kulapongs et al. (60). Yet folic acid deficiency might be associated with iron deficiency particularly in poorly nourished populations. Gross et al. (61) reported that deficiency of folic acid alone or in combination with iron deficiency results in depressed cell-mediated immunity as measured by skin responsiveness to dinitrochlorobenzene (DNCB) and PHA stimulated lymphocyte transformation. They found that iron deficiency alone did not affect skin reactivity and that lymphocyte transformation was only slightly depressed. Treatment of those patients with folic acid, restored cell-mediated immunity to normal.

As far as humoral immunity is concerned iron-deficiency does not alter antibody response to a variety of antigens. Macdougall et al. (55) could not demonstrate immunoglobulin deficiency in iron-deficient children. The anemic children had mean IgG and IgA concentrations significantly higher (IgG $p < 0.02$, IgA $p < 0.005$) than the latent iron deficient group, but not significantly different from the control subjects. The IgG and IgA concentrations in the latent group were slightly lower than the control group, but the differences were not significant ($p < 0.02$). The mean salivary IgA concentration was normal in all three groups. The mean C3 component of complement, however, was significantly higher in both anemic group and latent iron deficient group when compared with that of the control group. In the study of Chandra and Saraya (56), quantities of IgG, IgA and IgM were normal in blood and saliva in iron-deficient patients. Concentration of serum complement C3 were slightly lower. Antibody production during primary and secondary response was normal to both T-cells dependent and independent antigens.

Naldar et al. (62) determined the specific antibody titer in rats immunized with tetanus toxoids. Unlike the normal response in man, antibody production in response to tetanus toxoid immunization was significantly reduced in rats that received inadequate dietary iron.

The mechanism by which iron-deficiency impairs antibody production is not known. Since synthesis of antibody production requires energy at the rate of two pyrophosphate bonds of ATP per amide bond constructed (12). The possibility exists that energy available for protein synthesis may be decreased in iron-deficiency.

Regarding iron status and phagocytic functions, conflicting results have been reported. Chandra and Saraya (56) found phagocytosis by polymorphonuclear leucocytes (PMNS) and opsonic activity of plasma comparable in patients with iron deficiency anemia and healthy controls. However intracellular bacterial killing by PMNS and oxidative reduction of nitroblue tetrazolium (NBT) were both significantly less in iron deficient children. Macdougall et al. (55) found impaired bactericidal capacity of phagocytes from 20 children with iron deficiency. However in their patients NBT reduction was normal. Patients with latent iron deficiency also demonstrated impaired bactericidal capacity. Contrarily, Kulapongs et al. (60) found normal phagocytic killing function in 7 out of 8 iron-deficient children. The deficit was in serum and not in the leucoytes, since it was corrected by the addition of control serum to the patient's leucocytes. Similarly Masawe et al. (63) could demonstrate no defects in phagocytic killing in a group of patients with iron-deficiency. The mechanism through which iron deficiency causes dysfunction of PMNS is not certain. Lack of iron diminishes the activity of several enzymes that either contain iron or are dependent on it. These

include myeloperoxidase, which contribute significantly to the metabolic processes activated inside PMNS for bacterial dynsfunction.

An increased susceptibility to infection has been documented in several clinical and experimental conditions characterized by iron overload. Idiopathic hemochromatosis severs as an example of plasma iron overload in human beings although the iron content of the different tissues varies. In particular, the concentration of iron is relatively low in the reticuloendothelial system. Thus the immune response may be spared the effects of iron overload since the infection is not a problem in uncomplicated hemochromatosis (54). Parenteral iron therapy results in marked but transient hyperferemia, but there have been only a few suggestions in the literature that this might effect host resistance (64).

McFarlane et al. (65) observed increased mortality in children with kwashiorkor and speculate that this might have been due to iron, some of it apparently given as a part of nutritional therapy. In children with kwashiorkor and low serum transferrin levels, any increase in free circulating iron may result in overwhelming infection and death.

Masawe et al. (63) noted that clinical attacks of malaria frequently occurred following parenteral or oral iron therapy in patients with latent malaria. Fletcher and Goldstein (65) investigated the effect of iron injections on pyelonephritis in rats and mice primed by an intravenous inoculation of various bacteria. Injection of iron sorbitol citrate allowed growth of bacteria with the development of frank renal absesses.

Infections are enhanced in a number of animal models when the concentration of iron is increased with amounts calculated to saturate greater than 60% of the transferrin. In man, in a number of conditions characterized by a high concentration of plasma iron and increased

transferrin saturation, increased susceptibility to bacterial, fungal and viral infections has been noted (54). The major factor in most of these is the saturation of host iron binding proteins which renders iron more available for use by microorganisms.

The frequent association of reversible immunologic abnormalities in iron-deficient subjects has been demonstrated in several studies and denied in others. The discrepencies between the results of these various studies could be due either to subject variability to the laboratory methods used. It is extremely difficult to rule out concomitant deficiencies of other essential dietary components in iron deficient infants and children, except in cases where deficiency is clearly due to blood loss. Therefore scome elements of general malnutrition may have been present in many subjects tudied. The association of marked iron overload and infection is probably related to the interaction between the iron binding protein of host and microorganisms that determines the availability of free iron than to an immunological defect. However, delineation of correlation between iron and infection needs vigorous investigation instead of anecdotes.

Vitamin A

Numerous clinical studies have demonstrated that vitamin A deficiency results in increased frequency and severity of infections because of an impaired immune system.

Vitamin A deficient rats receiving a commercial vitamin A free diet showed marked leukopenia and a significant depression in the ability of rat splenic lymphocytes to respond to mitogenic stimulation (67). When an equal number of viable spleen cells were incubated with the mitogens concanavalin A (Con A), phytohemagglutinin (PHA) and E. coli

Lipopolysaccharide S (LPS), lymphocytes from deficient rats showed one-third the transformation response of the ad libitum and pairfed groups (Table 11). When supplemented with vitamin A, the splenic response of the deficient group returned to control values in 3 days. Panda and Combs (68) found that the sear of chicks which had been fed a diet partially deficient in vitamin A (975 I.U.) exhibited a lower ($P < 0.01$) average agglutinin response to Salmonella pullorum antigen than that of controls. Weanling swine made vitamin A deficient had a marked reduction in agglutinin titer against S. pullorum, the reciprocal titer in controls being 12 fold higher.

Moderate depression of agglutinating antibody production to diptheria toxoid has been reported (69). In C3Hf/Bu mice the injection of 250 I.U./g/day of vitamin A aquasol for 5 days preceding or following sensitization with SRBC elevated the production of hemagglutinin antibodies (70).

Acute hypervitaminosis A in guinea pigs significantly suppressed the expression of delayed hypersensitivity, without exerting any effect on reticuloendothelial function or antibody production. If given prior to skin testing, vitamin A suppressed the delayed cutaneous hypersensitivity (DCH) response to diptheria toxioid and bacteriophage X 174 (71).

An adjuvant like effect also has been shown in mice for both antibody (72) and cellular immune (70) responses. Cohen and Cohen (72) found that anti-SRBC plaque forming cell response was significantly increased ($P \leq 0.05$) in mice pretreated with 1000 to 3000 I.U. vitamin A for 4 days. Plaque forming cells/spleen increased from 12,000 in untreated controls to 28,000 in animals pretreated with 1,000 I.U. of vitamin A and to 65,000 in those pretreated with 3,000 I.U. of vitamin A. At 9,000 I.U./day, which caused toxic symptoms, the vitamin A induced

Table 11

Effect of vitamin A deficiency on the transformation of rat splenic lymphocytes[1] (67)

Diet	Control	Con A	Stimulant PHA	LPS
	(cpm)	(cpm)	(cpm)	(cpm)
Ad-lib fed	4,399	56,535	24,517	6,565
Supplemented (AL)	1,008	11,250	5,788	1,082
Pairfed	3,991	96,438	37,827	7,598
Supplemented (PF)	789	12,484	7,570	1,300
Vitamin A	2,437	24,767	7,239	1,792
Supplemented (DEF)	4,96[2]	4,209[3]	6,20[3]	355

[1]Results are expressed as ± SEM.

[2]Significantly different ($p < 0.05$) from the Al group.

[3]Significantly different ($p < 0.01$) from both groups.

enhancement of the immune response was no longer seen. Vitamin A pre-treatment also increased the antibody response to immunization with dinitrophenylated ovalbumin (DNP-OVA), a hapten protein conjugate (Table 12). Concomitant vitamin A treatment (3000 I.U. for 4 days) resulted in a completely normal anti-SRBC response in animals given 0.025 or 0.25 mg of hydrocortisone/day. The same dose of vitamin A did not reverse the supressive effects on the immune response of the relatively massive dose of 2.5 mg of hydrocortisone/day.

Cohen anf Elin (73) showed that supplementation of 3,000 I.U. of vitamin A palmitate in mice resulted in significantly decreased mortality after mice were challenged with either gram-negative bacteria Pseudomonas aeruginosa ($P < 0.01$), grampositive bacteria L. monocytogens ($P < 0.0003$) or fungus C, albicans ($P < 0.01$). Vitamin A deficient chicks infected with Newcastle disease virus had a rapid loss of lymphocyte from the thymus and bursa. Bang et al. (74) observed that chicks deprived of vitamin A from the time of hatching to 1 month of age showed depletion of lymphocyte and plasma cell populations in the upper-respiratory tract; this coincided with injury to epithelium and failure to replace damaged cells. There also was depletion of lymphocyte in bursal lymphoepithelial tissues. Infection of these birds with Newcastle disease whowed further depletion in plasma cells, increased oculonasal epithelial metaplasia or atrophy; inflammatory responses were depressed and plasmacyte populations were either further altered morphologically, replaced by other types of cells or eliminated.

The effect of concurrent administration of vitamin A on allograft rejection also has been studied (70). In C57BL/6 female mice grafted with isologus male skin, injection of 150 I.U./g of vitamin A from 5 days before to 10 days after grafting significantly reduced the mean

Table 12

Effect of vitamin A on the immune response to DNP-OVA (72)

Vitamin A	Mice/group	Serum DNP binding capacity (RSD_{33})[1]
I.U.		
0	5	21.2 ± 5.7
2000	6	90.5 ± 10.2[2]
5000	6	122.3 ± 20.4[2]

[1]Data reported as the mean resiprocal of the serum dilution binding 33% of 5 x 10^{-9}M ^{3}H-DNP-lysine (RSD_{33}) ± standard error of the mean.

[2]Probability < 0.01 for value compared to control.

rejection time of grafts. Graft survival time was reduced from 35.5 days for controls to 23.7 days for animals treated with vitamin A.

Finally, in another measure of cell-mediated immune reactivity, Levis and Emden (75) found that vitamin A added to human peripheral blood lymphocytes in vitro resulted in 50-100% increase in tritiated thymidine incorporation by lymphocytes after stimulation with Tricophyton and Candida antigens. Enhancement of lymphocyte proliferative response was achieved with nanogram and microgram amounts of vitamin A and was does dependent.

Vitamin A also may provide potential protection from carcinogens. Various carcinogens bind much more tightly to DNA in cultured tracheas isolated from vitamin A deficient hamsters than to DNA in tracheas from healthy animals (76). Roger et al. (77) found that vitamin A deficient rats were more susceptible to induction of colon tumors by aflatoxin B_1 than normal rats. The activity of vitamin A might be due to its labilizing action against lysosomes. Depletion of lysosomes has been reported to stimulate proliferation of lymphoid cells. This may be the reason of protection offered by vitamin A against the immunosuppressive effects of cortisone. The deleterious effects of vitamin deficiency upon antibody production may be related to degeneration or lack of proper development of lymphoid tissues. Lymphoid tissues become hypoplastic in vitamin A deficient germfree rats (68). Since vitamin A is known to play a role in the differentiation of epithelial cells into specialized tissues, it also might play a role in the development of epithelial tumors, tumorigenesis involves a loss of differentiation.

Folic acid

The effect of folic acid deficiency is of particular interest because

of its central role in DNA synthesis. The biochemical lesion in folic acid deficiency involves the inability to methylate deoxyuridylate to thymidylate. This is an essential step in DNA synthesis and requires 5, 10-methylene tetrahydrofolic acid, which is absent in folic acid deficiency. The defect in DNA synthesis found in megaloblastic anemia suggests that the DNA synthesis in lymphocytes associated with cell-mediated immunity might be similarly affected (78,81).

Das and Hoffbrand (78) in their study of cell-mediated immunity in human megaloblastic anemia, observed a decrease in the capacity of phytohemagglutinin (PHA) stimulated periopheral lymphocytes to undergo blast transformation and to synthesize DNA in the one folate deficient patient they observed. Axelrod (79) showed a severe defect in antibody response of white rats made folate deficient. The process of antigen recognition may be defective, since methotrexate, a folate antagonist, renders immunoblasts unable to transform into small lymphocytes in regional lymph nodes after skin sensitization with antigen (80). The animals used to demonstrate this did not become sensitized to the antigen, presumably because of the lack of small lymphocyte effector cells.

Gross et al. (61) studied cell-mediated immunity in 5 groups of pateints. Group I consisted of 11 nonobstetric patients, having megaloblastic anemia arising from nutritional folate deficiency. Group II was comprised of 7 obstetric patients with megaloblastic anemia of pregnancy. Group III had 5 patients with megaloblastic anemia and associated iron deficiency. Group IV had 5 patients with iron deficiency alone. Group V consisted of 8 normal age matched control volunteers, and 4 age and race matched, hematologically normal obstetric patients. Cellular immunity was studied in these patients, using primary delayed

cutaneous hypersensitivity (DCH) to dinitrochlorobenzene (DNCB), in vitro lymphocyte transformation in response to PHA, and inhibition of rosette formation with sheep red blood cell (SRBC) by antilymphocyte globulin (ALG). Only one of the 23 patients from the three folate deficient groups responded to DNCB skin testing. Positive response occurred 6 to 23 days (mean 15 days) after folate supplementation (Table 13). PHA stimulated lymphocyte transformation was significantly depressed in all the three folate deficient groups (Table 14). With folate therapy PHA response returned to normal in as early as 2 days; values increased steadily to levels well above normal, with maximum response occurring between days 7 and 14. The level and response then fell gradually, in most cases reaching normal by the 24th day of treatment. Rosette inhibition by ALG showed that the minimum inhibitor titer of ALG was the same for both folate deficient patients and controls. This suggests that the function of the T-cells responsible for spontaneous rosette formation, presumably receptor synthesis and/or density, is not affected by folate deficiency.

Williams and Gross (81) showed deranged immune response in folic acid deficient rats. Wistar-Lewis rats were fed a control or a folate free diet from weaning. Folate deficient animals received Neomycin (4 g/ml) in their water supply to eliminate folate synthesis by intestinal bacteria. After 4 weeks they were sensitized with full thickness skin-grafts from Brown Norway (BN) rats. Thereafter sensitization was continued by weekly intraperitoneal injections of 3×10^7 BN thyocytes. At age three months, when compared with controls, the folate deficient rats had mild megaloblastic changes in the bone marrow and the blunting of villi in the small intestine, a mild decrease in cellularity in both the thymus and the thymus-dependent areas of the spleen, decreased serum

TABLE 13

Dinitrochlorobenzene skin test before treatment in folic acid deficient, iron-deficient and control patients (61)

	Degree of dinitrochlorobenze skin test reaction No. of patients		
Group*	0	+	++
I	11	0	0
II	6	6	1
III	5	0	0
IV	0	2	3
V	1	4	8

*Group I consisted of 11 nonobstetric patients having megaloblastic anemia arising from nutritional folate deficiency. Group II was comprised of 7 obstetric patients with megaloblastic anemia of pregnancy. Group III had 5 patients with megaloblastic anemia and associated iron deficiency. Group IV had 5 patients with iron deficiency alone. Group V consisted of 813 normal central patients.

Table 14

PHA response in folic acid-deficient, iron-deficent, and control patients before treatments (61)

Group*	PHA Stimulation of Lymphocytes, Dpm	Unstimulated lymphocytes dpm
I	8,800 ± 3,600(3,091-13,994)	510(219-1202)
II	6,800 ± 2,900(3,691-12,323)	480(219-1202)
III	6,000 ± 3,280(3,527-10,049)	330(130-501)
IV	23,300 ± 5,470(15,005-28,896)	390(162-672)
V	26,340 ± 5,680(18,740-33,725)	370(130-719)

*Group I consisted of 11 nonobstetric patients, having megaloblastic anemia arising from nutritional folate deficiency. Group II was comprised of 7 obstetric pateints with megaloblastic anemia of pregnancy. Group III had 5 patients with megaloblastic anemai and associated iron deficiency. Group IV had 5 patients with iron deficiency alone. Group V consisted of 13 normal control patients.

folate levels, and decreased overall body weight. Hematocrit levels and weight of spleen and thymus did not differ significantly from those of controls. However the cytotoxicity activity in vitro of splenic lymphocytes from folate deficient animals exposed to BN thymocytes decreased significantly (Table 15). Uptake of tritiated thymidine by PHA stimulated spleen cells was decreased in the deficient group (Table 16). The number of T-cells in the spleen and peripheral blood of folate deficient rats was significantly lower than in controls; the number of T-cells in the thymus of deficient rats also was lower than in the control group, but not significantly so (Table 17).

The results of these studies show that indeed both in vivo and in vitro measures of cell-mediated immunity are depressed in megaloblastic anemia due to folate deficiency. Folate deficiency has been found to be one of the most common deficiencies in the industrialized world as well as in developing countries, and pregnancy seems to be the leading cause (82). Congenital defects in folate metabolism, inadequate dietary intake, intestinal disease, surgical and mechanical disorders are some other causes of folate deficiency. If as indicated by these studies, folate deficiency impairs the cell-mediated immune response, large segments of the population may have a lowered resistance to certain viral, fungal, parasitic, bacterial, and mycobacterial infections that the cell-mediated portion of the immune system aid in suppressing.

Vitamin C

There is little information available on the effect of ascorbic acid deficiency on cell-mediated immunity and conflicting reports on its effect upon humoral immunity.

Table 15

Cytotoxicity assay of splenic lymphocytes from folate-deficient rats (81)

	Control	Folate-deficient	Significance
Cytotoxicity	29.1 ± 3.7	5.2 ± 1.5	$p < 0.005$
	(16.3 - 40.0)	(0 = 13.9)	
	n = 7	n - 8	

Table 16

^{3}H-thymidine uptake by PHA stimulated spleen cells (81)

	Control	Folate-deficient	Significance
PHA stimulation			
No PHA	1,315 ± 312	1,405 ± 102	
	(887 - 1701)	(1789 - 8248)	
+PHA	19,398 ± 1,014	4,263 ± 579	
	(14,501 - 23,412)	(1789 - 8248)	
Stimulation			
Index	14.8	3.0	$p < 0.005$
	n = 8	n = 10	

Table 17

T-cell labelling by ^{3}H-uridine (81)

	Control	Folate-deficient	Signifiance
Number of T-cells			
Spleen	70.3% ± 2.4	4.6% ± 1.0	$p < 0.01$
	(61 - 78)	(35 - 52)	
Thymus	81.8% ± 1.5	73.0% ± 2.0	N.S.
	(76 - 88)	(68 - 83)	
Blood	67.0% ± 1.7)	43.6% ± 2.8	$p < 0.01$
	(63 - 71)	(35 - 51)	
	n - 6	n = 11	

Long (83) observed no significant differences on the primary response to diptheria toxoid by guinea pigs fed an ascorbic acid deficient diet, but the secondary antitoxin response of the deficient animals was greatly reduced. On the other hand Kumar and Axelrod (84) found no change in primary or secondary antibody response with the same antigen in guinea pigs in which severe scurvy was induced by feeding a highly purified ascorbic-acid deficient diet. Prinz et al. (85) investigated the effect of vitamin C supplementation on the human immune defense system. Twenty-five healthy human volunteers given 1 g of ascorbic acid daily for 75 days showed significantly increased serum IgA ($p < 0.005$), IgM ($p < 0.05$) and C3 complements levels compared with controls receiving no ascorbic acid supplementation.

Immunization of BALB/c mice receiving L ascorbate (250 mg %) in their drinking water, with sheep red blood cell (SRBC) and Lipopoly-saccharides (LPS) showed no significant effect on humoral antibody response to any of the antigens. However, in vitro stimulation of the spleen lymphoid cells with concanavalin A (Con A) showed significantly greater ($p < 0.025$, $p < 0.05$) thymidine incorporation in animals on a vitamin C regimen, suggesting a significantly increased cell-mediated immune response (86).

Anthony et al. (87) examined the effect of ascorbic acid deficiency on the cell-mediated immunity and the primary humoral response in ascrobic acid deficient, pairfed and ad libitum-fed control guinea pigs. Using chicken erythrocytes as target cells, the cytotoxicity of spleen lymphoid cells was assessed by a ^{51}Cr release technique. The percentage of spleen lymphoid cells binding rabbit erythrocytes (T-cell marker), the number of peripheral white blood cells, the percentage of T-cells

and the hemagglutinating serum antibody titer to chicken erythrocytes in blood also were measured. Results showed that the spleen was significantly larger in the deficient animals. Ascorbic acid deficiency had no effect on the ability of the guinea pig to produce agglutinating antibodies to chicken erythrocytes. The mean value of ^{51}Cr release for the ascorbic acid deficient guinea pigs was significantly less than either pairfed controls or ad libitum fed controls (Table 18). The percentage of splenic lymphoid cells that formed rosettes with rabbit erythrocytes, a T-cell marker, was the same in all three dietary groups (Table 18). Since ^{51}Cr release of labeled chicken erythrocyte target cells is considered to be a cell-mediated immune response involving primarily thymus dependent or T-cells, a reduction in cytotoxicity may reflect a decrease in the number of such cells to effect cytotoxicity. Since the same number of splenic lymphoid cells was used in each cytotoxicity assay and the percentage of T-cells was about 25% in all dietary groups (Table 18) it appears unlikely that a change in T-cell number can account for the differences. Therefore the defect in ascorbic acid deficiency could represent a selective dysfunction in a cell type (T lymphocyte) to mediate cytotoxicity.

Fraser et al. (88) reported the effects of supplemental doses of Na-ascorbate on in vitro mitotic activity of peripheral blood lymphocytes from guinea pigs immunized with bovine serum albumin (BSA). Groups of guinea pigs were pairfed an ascorbate free diet but supplemented with 0, 25 or 250 mg vitamin C, given in daily intraperitoneal doses for 28 days. Lymphocytes from guinea pigs receiving 250 mg Na-ascorbate per day incorporated in vitro the highest amounts of tritiated thymidine both in the absence of nonspecific mitogen and in the presence of Concanavalin A (Con A) or Phytohemagglutinin (PHA). Responses to

Table 18

Cytotoxicity assay of splenic lymphoid cells and percentage of T-cells in splenic lymphoid cells[1] (87)

Group	Cytotoxicity (% release) 50:1	Cytotoxicity (% release) 50:1	T-cell (% rosette-forming cells)
Control	2 ± 1	2 ± 2	27 ± 2
Control immunized	41 ± 3	27 ± 3	25 ± 2
Pairfed immunized	31 ± 3[2]	32 ± 4	26 ± 3
AA[3] deficient	2 ± 1	1 ± 1	19 ± 5
AA deficient immunized	20 ± 2[4,5]	13 ± 3[5,6]	26 ± 2

[1]All values expressed as mean ± SEM.

[2]Different from control immunized $p < 0.01$.

[3]AA = Ascorbic acid.

[4]Different from control immunized, $p < 0.001$.

[5]Different from pairfed immunized, $p < 0.01$.

[6]Different from control immunized, $p < 0.05$.

lipopolysaccharide S were not conclusive (Table 19). Anti-BSA titers were comparable from unsupplemented and supplemented animals. The percentage of B-lymphocytes increased from 43 to 63 and T-lymphocytes decreased from 50 to 35 in 28 days in scorbutic animals. Opposite changes were observed in vitamin C supplemented animals.

Effect of vitamin C status on phagocytic cell function was studied by Shilotri (89). Shilotri observed significantly impaired bactericidal activity of leukocytes from ascorbic acid deficient guinea pigs against E. coli. In addition, resting levels and phagocytosis-induced activation of both glycolytic and hexose monophosphate shunt pathways were markedly impaired. Since glycolysis provides energy for particle uptake and hexose monophosphate shunt activity provides energy for particle destruction by phagocytes, these metabolic defects were felt to be responsible for the observed impairment in bactericidal activity. In another experiment Shilotri and Bhat (90) supplemented five human subjects with 200 mg of ascorbic acid for 15 days. During the next 2 weeks the subjects were given 2 g of vitamin C per day. They found that supplementation of 200 mg of vitamin C significantly increased resting hexose monophosphate shunt activity as well as shunt activity during phagocytosis. Intake of 200 mg of ascorbic acid per day did not affect bacterial killing by leukocytes. On the other hand, intake of 2 g of ascorbic acid per day reduced bactericidal activity. The impaired bactericidal activity was restored to initial levels after withdrawal of ascorbic acid.

Ascorbic acid does not appear to effect either primary or secondary responses to SRBC or to diptheria toxoid in scourbutic guinea pigs. The differences in the study of Long (83) and others (84,86) may be because of the differences in methodology, in the composition of the diet, in

Table 19

Effect of Na-ascorbate on transformation of plasma lymphocytes stimulated by concanavalin A (Con A) phytohemagglutinin (PHA), or lipopolysaccharide (LPS) in immunized guinea pigs (86)

Na-ascorbate/ day	Day	n	Uptake of H-thymidine (mean cpm/group ± SEM			
			No mitogen	Con A (1. g/ tube)	PHA (1.5 g/ tube)	LPS (25 g/tube)
mg						
0	0	5	2,271 ± 385	117,370 ± 45,896	123,484 ± 51,066	2,782 ± 371
(Scrobutic)	14	5	29,916 ± 11,237	132,537 ± 58,691	172,598 ± 77,069	26,785 ± 15,078
	28	3	16,380 ± 15,464	20,502 ± 19,612	24,758 ± 23,301	39,702 ± 31,045
25	0	5	2,803 ± 1,139	105,023 ± 36,044	134,455 ± 41,249	3,399 ± 1,437
(Maintenance)	14	5	36,782 ± 16,278	122,455 ± 47,927	221,175 ±102,460	29,515 ± 10,480
	28	4	18,200 ± 9,204	77,357 ± 23,985	112,515 ± 46,071	10,275 ± 4,417
250	0	5	3,073 ± 683	53,360 ± 12,061	110,859 ± 33,107	3,014 ± 1,146
(Megadose)	14	5	53,906 ± 20,291	147,182 ± 21,813	184,575 ± 42,878	34,606 ± 10,747
	28	4	61,134 ± 49,257	140,138 ± 24,695	231,780 ± 41,583	47,334 ± 39,253

of the animals, in the dosage, and route of administration of antigens. The data on cell-mediated immunity provide in vitro evidence that cell-mediated immune response is impaired in ascorbic acid deficiency. As far as phagocyte function is concerned, too little or too much ascorbic acid can adversely affect overall phagocyte function.

SUMMARY

Experimental studies indicate dietary manipulations can profoundly influence immunity reactions in the body. How much the immune system is affected depends upon type of nutritional deficiency, period of nutritional deprivation and many other factors.

Immunity includes three major responses:

1. Cell-mediated immunity that works through T-cells. T-lymphocytes are transported away from the thymus by the blood and tend to take up residence in various organs of the lymphatic system. In order to respond to a particular antigen, T-lymphocytes attach themselves to antigen-bearing particles directly. This may involve the secretion of a specific toxic substance (lymphotoxin) from the T-lymphocyte, which is lethal to the cells being attacked.

2. Humoral immunity that works through B-lymphocytes. These cells also are distributed by the blood and tend to settle in various lymphatic organs. B-lymphocytes act indirectly against the antigen they recognize by producing and secreting globular proteins called antibodies.

3. The non-specific immunity which includes phagocytosis and macrophage mediated cytotoxicity. The ingestion of foreign particles by

phatocytes is promoted by antibodies (opsonins), complement component C3 and other serum factors.

The adequacy of T-cell function can be tested by skin testing for delayed hypersensitivity and T-lymphocyte blast transformation by measuring DNA synthesis. The levels of immunoglobulins and C3 complement component may indicate adequacy of humoral immunity.

In case of protein-calorie malnutrition, immune response either increases or decreases depending upon the age of onset of malnutrition and type of antigen or mitogen applied. Also, the quality and quantity of protein profoundly affect immune function, with specific amino acids being more important. Increased incidence of infection in malnourished children may be due at least in part to defective phagocytic function.

Both clinical and experimental evidence suggests that zinc deficiency causes suppression of lymphoid organ weight and depresses both cell-mediated immunity and humoral immunity. The cause of this may be the impairment of different enzyme system in Zn-deficiency. However, further studies are needed to determine the extent of impairment to the immune system caused by zinc deficiency.

The relationship of iron deficiency to increased suceptibility to infection, as well as its role in lymphocyte and/or phagocyte function is not yet clear. The discrepencies between the results of various studies on iron deficiency could be either due to subject variability, in the laboratory methods used or due to other associated nutritional deficiencies. For a clear understanding of the role of iron in immune function, studies in the future should carefully define the deficiency state, rule out coexisting nutrient deficiencies and infection, and evaluate specific response to iron therapy.

Evidence suggests that adequate vitamin A nutrition is required for optimal humoral and cellular immune response. Although its exact role in immune response is not clear, suggestions have been made that because of its labilizing action against lysosome and its role in the differentiation of epithelial cells into specialized tissues, it may be important as an adjuvant or to provide protection against carcinogen. However, further work is required in this area.

Folic acid as well as ascorbic acid depresses cell-mediated immunity, but there is need for studies on the humoral immune system. As far as phagocyte function is concerned, too little or too much ascorbic acid can adversely affect overall phagocyte function.

In conclusion, the effect of malnutrition on the host defense mechanism is undoubtedly complex. There is much need for future research, particularly regarding individual nutritional deficiencies.

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EFFECT OF PROTEIN, SELECTED MINERALS AND VITAMINS ON IMMUNE SYSTEM

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Various studies have suggested a possible relationship between malnutrition and immune response. This report has reviewed the impact of dificiencies of protein/or calorie, zinc, iron, vitamin A, folic acid and vitamin C on immunocompetence. Malnutrition seems to affect the different functions of T- and B-cells involved in the immune system. Results of different studies have shown that protein/or calorie malnutrition increases or decreases the immune response depending upon the age of onset of malnutrition, quality and quantity of protein and type of antigen used. Zinc deficiency depresses both cell-mediated immunity as well as humoral immunity. The relationship of iron-deficiency to immune response is not clear. The frequent association of reversible immunologic abnormalities in iron deficient subjects has been demonstrated in several studies and denied in others. Adequate vitamin A nutrition is required for optimal humoral and cellular immune response. Folic acid as well as ascorbic acid depresses cell-mediated immunity but there is need for further research regarding these nutrients on the humoral immune system.