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THE BIOLOGICAL IMMUNE RESPONSE - A REVIEW OF EFFECT OF DIETARY --ETC(U)
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increase the protein deficiency of the host and indirectly affect the immune response. These effects on the immune system are only observed during very severe PCM and would not account for the high incidence of infection that has been observed in the marginally malnourished child, which may be more related to the epidemic environment in which he lived. The consequence of altered immune response is not clearly defined and is largely circumstantial. During protein deprivation in experimental animals, most investigators have observed a decreased resistance to bacterial infections. An increase in susceptibility to infectious disease with accompanied elevated rates of morbidity and mortality have been observed in children and adults with PCM. In patients that have anergy (no DCH skin test response) before and after surgery, the incidence of sepsis and death is much higher than in those patients that convert back to their normal dermal responsiveness or have been normal both before and after surgery. Of interest is the observation that experimentally induced obesity in dogs resulted in reduced resistance and increased mortality to both bacterial and viral infections. These dogs were not protein-depleted or manifesting vitamin deficiency, suggesting that over-nutrition can also influence the immune response of the host defense against infectious diseases.

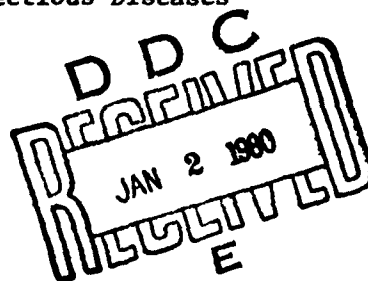
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The Biological Immune Response - A Review of Effect of Dietary
Amino Acids

By Robert W. Wannemacher, Jr.

United States Army Medical Research Institute of Infectious Diseases
Frederick, MD 21701



For Feedstuffs*

*Paper for the Annual Meeting of the Animal Nutrition Research Council

The views of the author do not purport to reflect the positions of
the Department of the Army or the Department of Defense.

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The immune system encompasses a wide variety of host defense activities and involves the cooperative interaction of leucocytes, a complement system, and other nonspecific factors.^{1,2} The cellular components in the immune system are made up of a number of specialized cells with specific functions: thymus-derived lymphocytes (T-cell), which can be further subdivided into killer, helper, or suppressor T-cells and are responsible for the cell-mediated immune response; antibody synthesizing lymphocytes (B-cells), which can be further subdivided into IgM, IgG, IgA, S-IgA, IgD, and IgE, that are responsible for the humoral immune response; and an extremely heterogeneous population of afferent and efferent accessory cells (A-cell), which includes polymorphonuclear leucocytes, monocytes, macrophages, eosinophils, basophils and mast cells. Thus, dietary manipulations could affect a variety of host defense activities including: phagocytosis, complement-mediated bacteriolysis, interferon production, cell-mediated cytotoxicity, antibody synthesis, and lymphokine elaboration. Because of this complexity and cooperative interaction of a number of the components of the system, often confusing and contradictory data have been obtained concerning the effects of diet and nutritional deficiencies on the various aspects of the immune response of the host.

Only a very limited number of studies have been done on the effects of individual amino acids on the immune response; therefore, most of this review will be directed toward the effect of dietary protein or protein-calorie deficiency effects on various aspects of the immune system.

Most of the information on the effects of dietary amino acids on immune response have been derived from either studies on experimental animals, malnutrition in children of underdeveloped societies, or

malnourished hospitalized patients. Studies in experimental animals have an advantage in that individual dietary components can be evaluated as to their effect on the immune system; however, because of the variation in the immune responses, it is often difficult to extrapolate the data to effects in the human population or domestic animals. The observations in malnourished children are complicated by multiple nutritional deficiencies, environmental variants,³ frequent presence of infections and parasite disease, and clinical limitations which involve studies on the children, at the time of hospitalization and after recovery. In addition, most of the investigations are from patients with severe forms of protein-calorie malnutrition, which is prevalent in only 1-7% of the population in certain pre-industrialized countries.⁴ Therefore, most of the data is obtained from a small, select group of severely malnourished children. Observations in the malnourished hospitalized patient are again complicated by multiple deficiencies, underlying diseases with accompanying therapies, and clinical limitations of obtaining data during illness and recovery stages of the malnutrition. Despite these difficulties, a definite pattern is emerging as to the effects of amino acids or protein deficiency on various components of the immune system.

Effects on Cell-Mediated Immune Response

Lymphoid stem cells for the T-cell component are found in bone marrow, fetal liver and spleen.⁵ These stem cells migrate to the thymus and arrive at epithelial sites, where they pass through a series of steps in differentiation. Their surfaces display an extraordinary variety of alloantigens, which are related to the functional state of the T-lymphocytes.⁵ This differentiation of T-cells requires the presence of

a mediator protein which is produced by the epithelial cells within the thymus.⁵ Post-thymic T-cells appear to be essential components in defense against certain viruses, fungi, and facultative intracellular bacterial pathogens. Further, the T-cell immunity system probably has additional important functions, the significance of which have not been fully elicited. Killer T-cells can exercise direct or indirect cytotoxic activity through a secretion of a protein called "lymphotoxin." T-cells also release other lymphokines which affect phagocytic activity of macrophages, blood flow through changes in vascular reactivity, inflammation, and blood coagulation. Helper T-cells assist in the antibody responses to certain antigens, while suppressor T-cells can turn off both killer T-cells and antibody production.

Quantitation of the adequacy of T-cell function can be performed by a number of procedures including: (a) skin testing for delayed allergic response of so-called "delayed cutaneous hypersensitivity" (DCH) to a battery of antigens; (b) in vitro lymphocyte blastogenesis with appropriate mitogens or antigens; (c) quantitation of the production of T-cell lymphokines and killer T-cell activities; and/or (d) measurement of a number of circulating T-cells. As pointed out by Miller,⁶ quantitation of leukocyte function is a measurement of a variety of different cell types, activities and interactions. One of the most commonly reported procedures for measuring T-cell function, mitogenic blastogenesis, involves both A- and T-cell expression, as well as mediators present in serum.^{7,8} Another common measurement, DCH to injection of multiple antigens or recall testing with dinitrochlorobenzene requires interaction of A-, T-, and B-cells in the presence of mediator and complement in serum. Further, anergy can be produced by a multitude

of conditions (uremia, liver disease, infection, etc.), some of which will often be present simultaneously with nutritional deprivation. Therefore, the complexity and interactions of the immune system must be taken into account when interpreting data on the effects of dietary amino acids or protein on the immune response.

A number of reviews⁹⁻¹⁴ on immunity in malnutrition contain sections on cell-mediated immunity (CMI); more extensive review on CMI was published by Edelman.¹⁵ A generalized pattern of effects of protein-calorie malnutrition on CMI has emerged and will be summarized.

Most studies in experimental animals have suggested a decrease in CMI during protein deprivation in rodents, pigs and monkeys, but a few studies have reported opposite effect in certain protein-starved mice and rats. In general, however, decrease in CMI was only observed in severe malnutrition, perhaps explaining the contradictory results observed in mice and rat studies. Marginal deficiencies in methionine and choline or folate resulted in a decrease in CMI response of the rat.¹⁶⁻¹⁸ When rats were deprived only marginally of methionine and choline during gestation and lactation, CMI response was depressed when they were tested for immunological competence at 4-6 months of age.¹⁸ Williams *et al.*¹⁸ have suggested that these 1-carbon metabolites regulate the activity of the enzyme 5-methyltetrahydrofolate:homocysteine methyltransferase, which is the only pathway available for methionine salvage in immunocompetent cells. In other studies, a moderate leucine restriction markedly depressed cytotoxic CMI to allogenic mouse tumor cells, where only slight depressions were observed with dietary limitations of arginine, histidine, or lysine.¹⁶ Thus, in experimental

animals protein deprivation and marginal deficiency of certain amino acids can decrease T-cell-dependent induction and expression of immunity.

A large number of papers described defective DCH reactions to a variety of skin test antigens in protein-deficient humans.¹⁵ The degree of immunological impairment appeared to follow magnitude of the protein-calorie malnutrition (PCM) and in general and DCH reactions returned to normal with dietary repletion of the patients. The DCH reaction can be considered as being comprised of at least three separate components: sensitization (afferent), recognition or recall (efferent), and the inflammatory.¹⁹ All three components of the DCH reaction have been observed to be defective in severe PCM.^{15,19} Further, a small percentage of well-nourished patients have negative skin reactivity to nonspecific irritants, which could give a false interpretation of anergy to skin test antigens. This emphasizes the complexity of interpretation and the need for necessary control to define the defective T-cell response by DCH reactions.

Severe PCM in children generally results in a decrease in circulating T-cells and atrophy of lymphoid organs.¹⁵ In vitro lymphocyte transformation to mitogen-induced proliferation tends to be reduced in children with PCM and appears to correlate with the defects in in vivo DCH reactions.¹⁵

In a typical study by Kulapongs et al.,²⁰ peripheral blood T-lymphocyte populations were significantly decreased in children on admission to the hospital with PCM, which returned to normal during recovery. Both blast-cell transformation and thymidine incorporation by stimulated lymphocytes was significantly reduced on admission compared to control or recovery values. Recently, it has been observed that a

number of infectious diseases are directly associated with a fairly pronounced, reversible depression of lymphocyte transformation, in the absence of malnutrition.²¹ This possibility will have to be evaluated in interpreting depression of in vitro lymphocyte function as a result of malnutrition. It has been demonstrated also that autologous serum in children with PCM is unable to support normal lymphocyte transformation.^{7,8} Thus, both in vivo and in vitro measurements of CMI appear to be depressed during PCM in children, but interpretation of the data are obscured by the complexity of the immune system and the difficulties of the in vivo and in vitro assays utilized to assess immune function.

Decreased or anergic DCH reactions were observed in a large percentage of hospitalized patients with PCM.^{6,22,23} A reduction was observed in the total number of lymphocyte and T-lymphocytes of patients with severe PCM.²³ In addition, the serum of patients with PCM contained an inhibitor of E-rosette assay for T-cells.²³ In contrast, lymphocyte blastogenesis was reduced only in patients with very severe PCM.^{6,23} In adult surgical patients with marasmus, Bistrian et al.²² observed a significant increase in the number of negative skin tests to Candida or streptokinase-streptodornase antigens, when compared to well-fed hospitalized controls or young adults. However, no significant change was observed in the number of T-cell lymphocytes or in the lymphocytic stimulation index.

Thus, in both children and adults with PCM, in vivo and in vitro CMI response was significantly depressed; these results were consistently observed only in the more severe types of PCM. Thus, it has been concluded that the basic immune interactions of A-, T-, B-cells for DCH,

blastogenesis, and cell-mediated cytotoxicity appear to have a high metabolic priority, in which the body preferentially utilizes its protein stores to maintain integrity of the immune system.⁶

Effect on B-Cell Function

B-lymphocytes are responsible for the humoral and secretory immune responses of the host. Stem cells for B-lymphocytes are present in yolk sac, fetal liver and bone marrow.²⁴ B-lymphocytes arise from the stem cells independent of the influence of antigens in the bursal-²⁴ equivalent (bone-marrow, spleen, and the far cortical regions of lymph nodes). They differentiate into B-cells with membrane immunoglobulins for all classes in decreasing order, IgM > IgG > IgA.²⁴ Upon contact with antigen, these B-lymphocytes may proliferate to form memory cells or antibody-secreting plasma cells. This proliferation requires interaction with the macrophage of the A-cell population and, for certain types of antigens, cooperation of T-cells. During clonal development, B-lymphocytes make specific surface membrane immunoglobulins at a relatively slow rate (2-4% of protein synthesis of the cell).²⁴ Following antigen-stimulated transformation into plasma cells, they synthesize and secrete a specific class of cytoplasmic Ig, which represents the major protein synthetic activity of the cell.²⁴

During clonal selection, B- and T-cells influence each other through direct cell-to-cell interactions.²⁵ Certain antigens do not require T-lymphocyte antibody response and probably can trigger B-cells directly because of their ability to bind to the surface of immunoglobulins. Other antigens lack the ability to bind directly to B-lymphocytes but may bind antigen recognition sites on T-cells. This recognition unit will be released to macrophages (A-cell population), which can then stimulate

B-cell antibody synthesis. T-cells can also influence antibody response to T-independent antigens by preventing antibody tolerance of large doses of antigen. Recent evidence has suggested that suppressor T-cells can interact with B-lymphocytes to shut off clonal differentiation and plasma cell immunoglobulin synthesis.²⁶

There are five classes of immunoglobulins (IgG, IgM, IgA, IgD, and IgE), which are made up of a class-specific pair of heavy chains and a pair of light chains.²⁴ The chains themselves are made up of variable and constant regions. Alterations in the variable regions are responsible for the unique ability of the Ig molecule to combine with specific antigens, whereas the constant regions are similar within each class. IgG is the major immunoglobulin involved in the host serologic defense and is divided equally between the intravascular and extracellular pools. Approximately 3% of IgG is turned over each day, with a biological half-life of approximately 25 days. This immunoglobulin is responsible for the majority of the antiviral, antitoxic, and antibacterial activity of serum. IgG antibodies are long-lasting and associated with the secondary and anamnestic response to antigen. They are good neutralizing, opsonizing, and agglutinating antibodies.

IgM forms a polymer of five units linked together to an additional J-chain.²⁴ This immunoglobulin has a much shorter half-life and is the first antibody formed in response to an antigenic challenge. IgM assists the reticuloendothelial system to clear the blood stream of pathogens; it is the major antibody to polysaccharide antigens (T-independent antigens) and gram-negative bacteria.

Serum IgA makes up about 10% of the body immunoglobulins and has no unique antibody humoral function.²⁴ The chief immunoglobulin of

secretion is the IgA secretory antibody (S-IgA), which is made up of a J-chain, and SC-chain, and two serum IgA molecules.²⁴ Secretory IgA is synthesized locally in plasma cells of submucosa and exocrine glands in the respiratory, gastrointestinal and genitourinary tracts and skin. Secretory antibody is crucial for the host defense mechanism in helping to prevent organisms from penetrating the submucosa. S-IgA antibodies neutralize some viruses, facilitate opsonization of antibody by monocytes, and promote complement fixation by activation of the alternate pathway.²⁴

IgD is in very low concentration in the body and has no unique antibody function. IgE is also in very low concentration in the body but may be important in protecting against intestinal parasitism and in augmenting local inflammatory responses, by activation of the alternate pathway of complement system.

Quantitation of the adequacy of the immunoglobulin in antibody response can involve the following: (a) measurement of proportion and number of B-lymphocytes; (b) determination of serum concentration and class distribution of immunoglobulins; (c) quantitation of antibody production in blood by measuring in vivo rates of immunoglobulin synthesis and catabolism or by in vitro production of antigen-specific antibody-forming cells; (d) use of sheep red blood cell immunization to measure "T-cell-dependent antibody synthesis" in experimental animals; and (e) measurement of secretory S-IgA concentration in nasal washings and respiratory fluids.

A number of earlier studies (before 1967) in experimental animals have suggested reduced antibody response to specific antigens during dietary protein deficiency, and most of this information has been

summarized in a monograph by Scrimshaw et al.¹⁰ Many of these studies, however, were performed before the understanding of the involvement of T-cells in antibody production. Therefore, it is difficult to discern whether this reduced antibody response represents a defect in T- or B-cell function. Studies by Jose and Good¹⁶ suggested that a moderate reduction of phenylalanine-tyrosine, valine, threonine, methionine, isoleucine and tryptophan in the diets of mice produce profound depressions in B-cell antibody response. T-cells are essential for the formation of antibodies against sheep red blood cells and are examples of the so-called "T-dependent antigens."²⁷ A number of investigators¹⁵ have described a deficient antibody response to sheep red blood cells when injected into protein-deficient rodents. Thus, in experimental animals, protein deficiency results in only minor alterations in B-lymphocyte function and in part reflects defects in the helper T-cell interaction.

During PCM in children and adults, serum IgE, IgG, and IgA concentrations are often elevated, while IgM and IgD are normal.²⁸ The elevation in the immunoglobulins in malnourished children probably represents effects associated with chronic infections. Further, the rate of immunoglobulin synthesis is 3-fold higher in adults living in hyperendemic areas of the world compared to that in developed countries.²⁸ Serum antibody responses following immunization with most antigens were normal in children with PCM, while the response to typhoid fever and some viral antigens is depressed.²⁸ It is difficult and almost impossible to interpret most of the data on the effect of PCM on antibody response because of lack of control of critical variables such as: concomitant infection, dose of antigen, severity of malnutrition, simultaneous treatment of PCM, liver function, or status of local lymphomatoid tissue.

A modest but significant decrease in the concentration of secretory IgA has been observed in nasal washings of children with protein calorie malnutrition.²⁹ This deficiency improved slowly with dietary treatment. The concentrations of IgG and albumin in the nasal washings were only slightly below normal, suggesting that the reduced concentration of secretory-IgA was probably selective and unrelated to generalized reduction of proteins in nasal fluids. Since Sirisinha *et al.*²⁹ were unable to correlate secretory-IgA defect with the presence of infection, the relative deficiency of S-IgA by itself probably could not account for the predisposition of these children to infections.

Effects on the A-Cell Component

The A-cell component is made up of three specialized cell types, polymorphonuclear leukocytes (PMN), monocytes, and macrophages, which are responsible for the phagocytic activity of the immune system. The first type arise from undifferentiated stem cells in bone marrow, where they undergo a series of cellular differentiations before being released into the circulation.^{30,31} From its primal beginnings, the PMN requires a week to mature, at which time it leaves the bone marrow.³ The PMN circulates in blood for 8-12 hrs, after which it enters tissue, where it dies, probably as a result of a phagocytic encounter.³¹ An adult human has approximately 3×10^{12} PMN, of which approximately 1% are found in circulating blood, 60% are associated with the differentiating cells and bone marrow, and about 39% are with other organs of the body.³⁰ Approximately 10% of the PMN are turned over each day; extremely little is known as to what governs the rate of stem cell differentiation, PMN release, or survival in peripheral blood.

The PMN is a wandering phagocyte and is generally the first cell type of the immune response to arrive at a site invaded by microorganisms. This chemotactic response of the PMN apparently involves attraction to agents which are humoral factors elaborated from the interaction between microbes and the host tissue. The major chemotactic factors are low molecular-weight fragments of C_3 and C_5 components of the complement system, which can be activated by antibody-antigen complex, microbial surface, or opsonizing antibodies.³¹ Proteases from the complement system, bacteria, blood cells, or damaged tissue can cleave C_3 or C_5 to C_{3a} and C_{5a} , which are the chemotactic fragments. Inhibitors of this system block in vitro chemotaxis of PMN. A number of other factors released from lymphocytes, granulocytes, bacteria, and other cells also can stimulate in vitro chemotaxis. Once the PMN comes in contact with a particle, it is moved along the plasma membrane by locomotion to an actin-binding protein, which results in ingestion of the particles. The mechanisms involved in locomotion and ingestion are currently an area of intense research. After enclosure of the microbe within the inverted membrane vesicle (phagosome), the pH becomes acid and is moderately microbicidal for certain microorganisms. A number of enzymes are released from the PMN granules to the phagocytic vesicle, which are capable of degrading microbial macromolecules. Some of these enzymes are capable of reducing oxygen to H_2O_2 , superoxide anions, hydroxyl radicals and singlet molecular oxygen. The best-characterized aspect of the system involves synergy between degranulation and oxygen metabolism, in which myeloperoxidase potentiates the reduction of oxygen in the presence of halide ions, which can generate H_2O_2 , a potent

antimicrobial agent.³¹ Energy for this system is derived from the hexose monophosphate shunt activity. The PMN is an essential element of the immune system but its precise role is difficult to determine. It appears to be most important in maintaining peripheral defense, mucosal integrity, and in promoting healing of infected wounds.

The monocyte originates from stem cells in bone marrow and has a turnover rate which is slightly slower than that of the PMN.³² A monocyte differentiates into either a fixed or wandering macrophage upon being stimulated by antigen or after entering tissue. The macrophage can pinocytose small molecules and phagocytize larger particles.³² This process is stimulated by opsonization of the particle with certain specific and nonspecific immunoglobulins or by activation C_3 . A product released from lymphocytes, migration inhibitory factor, is also capable of stimulating the phagocytic activity of macrophage. The stimulated macrophage is capable of synthesis and secretion of a number of products including: lysozyme, a protein which is bactericidal to some gram-positive organisms and can enhance complement-mediated bacteriolysis of gram-negative organisms; C_2 and C_4 components of the complement system; collagenase, a product which probably plays a role in inflammatory response; plasminogen activator, a product involved in transformation of fibroblasts, clotting and complement activity; endogenous pyrogen, a mediator of fever; interferon, an inhibitor of viral replication; and products, such as "LEM" which stimulates a number of nonspecific responses of the host.^{32,33} Mechanisms of intracellular antimicrobial action are not as well defined in the macrophage as they are in the PMN. The halide-myeloperoxidase peroxide system is present in the monocyte but disappears in the differentiated macrophage.³²

Lysosomal enzymes in combination with complement components may play some role in bactericidal activity; interferon may be involved in an intracellular virucidal activity, but the method by which the macrophage contents often kill protozoa, many viruses, bacteria and fungi have not been delineated. As described earlier, the macrophage apparently plays a role in T-dependent antigen processing.

Experimental protein deficiency in the rat had no effect on in vitro chemotactic migration or bactericidal activity of cells from peritoneal exudates, but chemotactic activity of peritoneal wash or serum opsonic activity was significantly reduced compared to well-fed controls.³⁴ Thus, while A-cell function was normal, major abnormalities were observed in complement-dependent function in fluids from protein-deprived rats. Further, metabolic activity of phagocytic cells as measured by oxygen consumption and decarboxylation of 1-[¹⁴C]glucose was diminished during PCM in rats.³⁴ In vervet monkeys fed a protein-free diet, the mobility of the PMN and their phagocytic and killing indices, with and without leukokinin stimulation, were decreased compared to monkeys fed regular chow.³⁵ Thus, A-cell function and stimulative factors in body fluids can be decreased during PCM of experimental animals.

In protein-calorie malnourished children, in vitro studies on PMN functions suggest impaired chemotaxis, normal phagocytosis and defective candidal and bactericidal activity.³⁶⁻³⁸ Alterations in leukocyte metabolism from children with PCM include: increase in the resting activity of the hexomonophosphate shunt; elevated resting reduction of nitro-blue tetrazolium; and a slight decrease in iodination of the halide-myeloperoxidase-peroxide system. Serum opsonic activity was also

depressed in serum from children with PCM.³⁷ Some of these effects on A-cell function and opsonic activity of serum may be related to underlying infections, which were present in many of the children with PCM.³⁹

In surgical patients who were anergic, a decrease was observed in the stimulated and random chemotactic migration of blood PMN.²³ Serum from these patients had a reduced chemotactic activity when incubated with PMN from normal control patients. Mean phagocytic and bactericidal function of the PMN from malnourished patients was not markedly altered but a significant correlation was observed between phagocytosis, bactericidal function, and distance migrated PMN of individual patients. While A-cell function in the immune system is a complex series of relationships with other components, the evidence in experimental animals, children and adults with PCM suggests that a decrease in the chemotactic mobility of PMN and monocytes, as well as a decrease in chemotactic activity of serum. The chemotactic response of A-cells is critical, not only in the bactericidal activity, but also in the maintenance of delayed hypersensitivity skin response.¹⁵

Effects on Complement System

The complement system represents the series of nine protein components, which interact to form compounds essential for the host's defense against infectious disease.⁴⁰ The complement system is made up of two pathways depending on the sequence of activation. The classic pathway is stimulated by antigen-antibody complex by fixation of C₁ component in the constant region of the antibody molecule.⁴⁰ This pathway was originally thought to be activated only by specific antibody-antigen complex, but recent studies have indicated that C-reactive protein, one of the "acute-phase reactants" of serum, can initiate the

reaction of the entire sequence of the classic pathway.^{41,42} The alternate, or properdin, pathway interacts with the classic pathway via C_3 and is activated by polysaccharide, lipopolysaccharide (LPS), antigens or aggregated immunoglobulins, of which IgM has been best studied in this regard.³⁸ Various components or fragments of the complement system assist in a number of aspects of host defense against infection including: enhancement of neutralization of antibody-coated viruses; stimulation of cells to trigger release of histamine leading to vasal dilation and to the tumor and rubor of inflammation; stimulation of chemotaxis migration of A-cells; requirement for opsonization of some microbes including "T-dependent antigens"; endotoxin inactivation; and lysis of virus-infected cells and most bacteria.⁴⁰ Thus, the complement system is one of the major biological amplification systems through which the immune mechanisms produce an effective host defense against bacteria, fungi, and viruses.

When compared to control values from children of the same age and environment, the mean serum concentration of all components except C_4 of the complement system in children with PCM was significantly lower than that of normal children.⁴³ All the serum components were increased following dietary treatment. Functional integrity of the complement system was evaluated by studying total hemolytic complement activity before, during and after nutritional repair. Hemolytic activity was significantly reduced in malnourished children and returned to control values following dietary treatment.⁴³ Since most of the malnourished children had underlying infectious disease, decrease in the serum concentration components of the complement system and reduced hemolytic complement activity could be related to the accompanying

infections. Chandra⁴⁴ found that serum C₃ and hemolytic complement activity were increased with infections in well-nourished children but were significantly reduced in malnourished children with or without infections. Thus, the decrease in the concentration and activity of the complement system in children with PCM could be related to an increased consumption of complement proteins during acute sepsis. To test this possibility, Sirisinha et al.⁴³ measured the anti-complement activity before, during and after nutritional repletion. Twenty-eight percent of the children with PCM had detectable anti-complement activity on admission to the hospital compared to 60% with decreased serum complement hemolytic activity.⁴³ This increase in anti-complement activity of the serum was correlated with their endotoxemia and gram-negative sepsis.⁴⁵ Therefore, some of the children could have decreased complement concentrations because of elevated consumption associated with infectious disease, but less than half of the children with depressed hemolytic activity had detectable anti-complement activity in the serum, suggesting an accompanying decreased synthesis of complement proteins in severely malnourished individuals. These observations suggest that dietary protein can influence both the concentration and activity of serum complement system, which is an important biological amplification system in the immune response of the host.

Effects on Nonspecific Factors

In addition to the specific immunological mechanisms, the body possesses a variety of nonspecific factors that contribute to host resistance against all types of microorganisms.^{46,47} Body surface structures constitute an anatomical barrier that prevents invasion of

body tissues by pathogenic organisms; these include intact dermal mucosal tissues, as well as epidermal surface and secretion. Severe protein deficiency states can be accompanied by atrophic changes of the skin with extensive desquamation, atrophy of the gastrointestinal mucosa, tissue necrosis, ulcer formation, and poorly walled-off infection.⁴⁸ These anatomical changes could provide a portal of entry for infectious microorganisms.

Elevations in body temperature or febrile response is characteristic of most infectious disease. Fever may be helpful in combating diseases, such as syphilis and gonorrhea, but it has no effect on virus and amplifies the loss of body nutrients.⁴⁹ Body temperature is reduced in children with PCM, and may be related to decreased synthesis and release of the mediator of fever, endogenous pyrogen, that is produced by the A-cell components of the immune system.^{32,33,47} This could reduce the resistance to certain infectious diseases but might also decrease protein wasting associated with the febrile response.

Changes in the normal intestinal flora can render the host more susceptible to pathogenic organisms. Most children with PCM have known bacterial pathogens isolated from their stool and marked alterations in metabolism and structure of the intestinal mucosa.^{10,45,47} Passage of bacterial pathogens across the intestinal mucosa could explain the high incidence of endotoxemia observed in children with PCM.⁴³

Secretory and plasma antimicrobial factors are an important nonspecific component of the host's resistance to infectious disease. As discussed earlier, secretory IgA is decreased in the nasal washings of children with PCM.²⁹ Another well-studied factor, interferon, is synthesized and released when body cells are activated; it is a protein

secretion that assists other cells in resisting the invasion and replication of viral pathogens.⁵⁰ Since interferon secretion requires RNA and protein synthesis, protein deprivation could influence this normally very effective host factor in resistance to viral infection. Lysozyme, a protein enzyme, is found in the respiratory tract, intestinal mucosa, saliva, tears, breast milk, sweat, serum, and cytoplasm and granules of the A-cell population.⁵¹ This enzyme is capable of hydrolytic effects on the cell wall of certain bacteria with a particular polysaccharide component.⁵¹ Lysosomal enzyme activities are increased in plasma and decreased in PMN during infection in both well-nourished and malnourished children, but the concentration in plasma is lower in both infected and noninfected children with PCM.⁵² Properdin is a distinct protein in the alternate complement pathway which can be affected by the nutritional state of the host.^{47,51} Kallikrein-kinin system and the vasoactive amines from mast cells and basophils are an important amplification system for immune response and require active RNA and protein synthesis, which could be influenced by dietary protein intake. Plasma acute-phase reactants are synthesized by the liver and play a role in biological amplification of the immune response.⁵³ Severely malnourished children have elevated plasma concentrations of acute-phase proteins despite marked depressions in albumin.⁵⁴ Thus, it has been suggested that the synthesis of these acute-phase proteins has a high priority even in the protein-depleted host.

Infectious disease causes a number of metabolic alterations in the host, which can be influenced by nutritional status.^{33,35,56} one of the major effects is the marked wasting of body protein. Thus, during experimentally induced tularemia in volunteers, the average loss of body nitrogen was almost 60 g despite prompt treatment.⁵⁵ If normal volunteers were pair-fed the same dietary protein-calorie intake as that consumed during tularemia infection, they lost only 15 g of body nitrogen. Therefore, the anorexia is not the stimulus for increased wasting of body protein associated with febrile infection. During prolonged and repeated fevers, such as malaria, the continuing rate of loss of body nitrogen is diminished as the patient becomes protein-depleted.

A number of changes in the protein metabolism of the host during infectious disease are responsible for this catabolic response. Total body protein synthesis and degradation were both increased with a more marked elevation in the degradative process. There is a flux of amino acids from skeletal muscle and connective tissue to liver and other visceral cells. In skeletal muscle amino nitrogen is utilized to synthesize alanine at an increased rate and this substrate is utilized for glucose production to meet the elevated metabolic requirements of the febrile host. Infected individuals do not develop ketonemic adaptation to reduced food intake, which means they must continue to utilize glucose, from gluconeogenesis of amino acids, as a major energy substrate for tissues such as brain and leukocytes. Amino acids are utilized at an increased rate for the synthesis of serum in acute-phase proteins even in the protein-depleted host. Thus, during sepsis and anorexia, proteins of skeletal muscle, skin and intestinal mucosa all contribute amino acids to the body's extracellular pool. In skeletal muscle and skin

the rate of protein synthesis is decreased and degradation is elevated while intestinal mucosa rate of synthesis is reduced with little change in degradation. Amino acids are utilized for the synthesis of obligatory proteins which are necessary for homeostasis in various tissues, but the rate of anabolism equals catabolism with no change in protein content. The liver utilizes about 67% of the amino acids that are taken up for synthesis of glucose. This gluconeogenesis accounts for a large portion of the increased excretion of urea nitrogen and the catabolic effects of infectious disease. In liver, the amino acids are also utilized in increased amount of synthesis of acute-phase proteins and certain hepatic enzymes. Amino acids are utilized, also, at an elevated rate for the immune response. If there is a requirement for tissue repair and growth, amino acids utilized in these processes contributed very little to the extracellular pool and this compartment is considered a "nitrogen trap." The alterations in protein metabolism can lead to a very rapid depletion of the protein stores of the body. Protein degradation follows first-order kinetics and is a function of both the rate constant of degradation and protein content. Thus, as the protein content of the skeletal muscle is decreased, the flux of amino acids from this tissue compartment is reduced, with less being available for the anabolic processes, including gluconeogenesis or synthesis of acute-phase proteins and proteins associated with the immune system. Therefore, the depleting effects of an infectious illness can in themselves have a marked effect on the immune response of the host.

Conclusion

Despite the complexity and interacting components of the immune system, a definite pattern is emerging as the effects of dietary amino acids and

proteins on the immune response, which include: (a) T-lymphocyte function can be altered by dietary proteins and amino acids; (b) only minor alterations of B-cell function have been observed in the protein-calorie malnutrition in humans; (c) A-cell chemotaxis is decreased during PCM in humans; (d) concentration and hemolytic activity of serum complement, as well as opsonic activity decrease during PCM; and (e) the metabolic response to infectious disease can increase the protein deficiency of the host and indirectly affect the immune response. These effects on the immune system are only observed during very severe PCM and would not account for the high incidence of infection that has been observed in the marginally malnourished child, which may be more related to the epidemic environment in which he lived.³

The consequence of altered immune response is not clearly defined and is largely circumstantial. During protein deprivation in experimental animals, most investigators have observed a decreased resistance to bacterial infections.⁵⁷ An increase in susceptibility to infectious disease with accompanied elevated rates of morbidity and mortality have been observed in children and adults with PCM.^{9,15} In patients that have anergy (no DCH skin test response) before and after surgery, the incidence of sepsis and death is much higher than in those patients that convert back to their normal dermal responsiveness or have been normal both before and after surgery.²³

Of interest is the observation that experimentally induced obesity in dogs resulted in reduced resistance and increased mortality to both bacterial and viral infections.^{58,59} These dogs were not protein-depleted or manifesting vitamin deficiency, suggesting

that over-nutrition can also influence the immune response of the host defense against infectious diseases.

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