Malnutrition, Infection, and Immune Function

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Although it has long been known that malnutrition and infectious diseases frequently occur together (1), it has only recently been established that the nutritional status of the host can alter the functional activity of the immune system and thereby affect the host's response to infections (2-12). In the traditional view of causality, dietary inadequacy has been considered the primary cause (13). It has been argued, however, that the reverse may be the more likely scenario, since frequent infections cause nutritional deterioration, progressive impairment of host defenses against infection, and ultimately result in fatal infections (14). It has been difficult to establish causality in humans, however, because marginal diet, frequent infections, and laboratory and/or clinical evidence of impaired immune function coexist in the populations at risk, largely in the third world. Regardless of which factor is primary, it is now clear that the relationship among the three is cyclical, with changes in one, in turn, influencing the other two (Fig. 1). This concept was first clearly presented just 20 years ago in the classic monograph by Scrimshaw, Taylor, and Gordon (1). Since then, application of contemporary clinical and laboratory studies has expanded the data base and established the validity of the basic concept. These data have been the subject of a number of excellent reviews in recent years. Rather than reiterate these data in detail, the interested reader is referred to the numerous excellent summaries already in print (2-12).

Up to this time, it has been difficult to determine the actual mechanisms involved, and considerable effort has been devoted to models of malnutrition in experimental animals, usually rodents, by providing a diet deficient in one or more nutrients or by restricting the amount of diet available. It must be remembered that such controlled animal experiments are not comparable to the human state and that direct comparison is precarious (8). People do not eat formulated diets that are lacking just one nutrient but are otherwise complete and balanced, and most people do not have access to the same amount of the same diet day after day. Rather, humans eat food according to its availability in the market and especially according to their own economic status and ability to pay for food. Human malnutrition is also rarely due to a defi-
Malnutrition may be initiated by primary or secondary dietary deficiency (e.g., malabsorptive states), or by the metabolic effects of infection. The consequence is impairment of host defenses, which in turn leads to an increased burden of infection and further malnutrition. (From ref. 12.)

 DOES INFECTION CAUSE MALNUTRITION?

The onset of infection initiates a number of physiologic and metabolic changes that alter, however briefly, host nutritional status. DuBois (15) established well over 50 years ago that regardless of cause, fever increased metabolic rate by a predictable amount. Using modern methods of calorimetry, an increase in resting metabolic expenditures of 15 to 40% can be demonstrated in hospitalized septic patients (16). When coupled with the usual anorexia and decreased food intake in the febrile infected patient (17), the consequence of infection is a significant shortfall in energy supplies, necessitating a switch to endogenous energy sources (3,6,9). As body
stores of carbohydrate are limited and other alterations in fat metabolism inhibit the use of lipid energy stores, the infected host must turn to gluconeogenesis as the major means to generate glucose, using amino acids, released from catabolism of muscle protein as the substrate (9,18–20). This leads to fasting hyperglycemia, an abnormal "diabetes-like" glucose disappearance curve, and a significant increase in both glucose pool size and glucose oxidation rates (16,18). Increased plasma insulin, glucagon, growth hormone, and corticosteroid levels also help to sustain this pseudodiabetic state.

Although muscle catabolism releases amino acids into plasma, circulating levels generally decrease, except for the nonreutilized amino acids (6,9,21). This is accounted for by increased uptake of amino acids in the liver and an increased rate of hepatic protein synthesis, which proceeds even in the presence of negative nitrogen balance and wasting of lean body mass (3,9,20,21). For example, there is a marked increase in synthesis of acute phase proteins, such as C-reactive protein, serum amyloid A, and the third component of complement. At the same time, amino acids are deaminated, converted to glucose, oxidized, and eliminated from the body as urinary nitrogen and CO$_2$ excreted via the lung (3,6,9,21–23). The normal priorities for protein synthesis are further distorted, as there is a concomitant decrease in production of export liver proteins such as transferrin and albumin. Turnover of immunoglobulins, phagocytic and lymphoid cells, and soluble protein mediators of host defense is also increased. In addition, tissues damaged by the inflammatory reaction may be in need of repair, further consuming available amino acid substrates.

To compound the energy problem still further, there is often a marked impairment in fat metabolism. Clearance of fatty acids from plasma is blocked due to depression of the key enzyme, lipoprotein lipase, resulting in profound hypertriglyceridemia (24). This is particularly prominent during gram-negative rod bacterial infections (25). Because lipid anabolic enzymes are also depressed, fat depletion from adipocytes occurs at the same time as utilization of ketones for energy is inhibited (26).

The net sum of these changes is pronounced wasting due to the combined effects of decreased food intake, increased energy consumption, marked catabolism of muscle protein, absolute losses of nitrogen stores, and depletion of glycogen and fat stores. In addition, when the gut is involved, other mechanisms can contribute to the nutritional deterioration attributable to changes in endogenous metabolism, including intestinal malabsorption, hypermotility, and mucosal damage due to enteric pathogens. Protein absorption can be reduced by 20 to 33% in the course of moderate diarrhea (as much as 400 mg/kg-day), and fecal fat losses can approach 40% of intake (27).

While these effects may be detected to a greater or lesser degree in all infected subjects, in the otherwise healthy individual, the losses are replaced during convalescence. Repletion of body stores begins at the point when the febrile disease ends, which signifies the onset of convalescence but is the nadir in nutritional status. Young children generally stop growing during the period of catabolic stress of infection and usually lose weight. However, a period of rapid catch-up growth will occur in convalescence, and if there is unlimited access to high-quality food, the rate of
catch-up growth can exceed seven times the normal daily growth rate (28). Reasonable estimates of the daily energy and protein requirements for growth in a 2-year-old child are 2% and 12% of the recommended intake levels, respectively. Thus, to provide for maximum catch-up growth of seven times the normal rate, the diet should supply 14% more energy and 84% more protein than ordinarily recommended. On this basis, it has been suggested that rehabilitation diets should provide 30% more energy and 100% more protein than usual (28). This amount of additional energy and protein is easily provided in affluent first-world populations but may be difficult, if not impossible, to attain in poor children in the Third World. This is partly because available dietary staples do not contain sufficient energy per gram of food to make it physically possible to ingest the amount of energy needed (29), since bioavailability of vegetable protein is much less than that of animal protein, and since the amount of food available is limiting as well. Thus, adequate catch-up may be significantly delayed. Since infections occur frequently in such individuals, the next episode often occurs when the host is still nutritionally subnormal, precluding full catch-up. When this happens repeatedly, a progressive downhill spiral in nutritional state is inevitable, until eventually overt protein-energy malnutrition develops (30).

DOES NUTRITIONAL DEFICIENCY RESULT IN IMMUNOLOGIC ABNORMALITIES?

Although its significance was not appreciated at the time as a marker of the impact of malnutrition on the immune system, reports of thymic atrophy in the presence of disease, stress, or impaired nutrition can be traced to as early as the 17th century (31-36). We now know that the term nutritional thymectomy, coined by Watts (37) almost 20 years ago, does describe a causal link between thymic involution and malnutrition. It is only the physiologic mechanisms involved in this process that remain to be determined.

T-Lymphocyte Abnormalities in Malnutrition

A seminal discovery in establishing the thymus gland as a critical organ of lymphocyte differentiation was published in 1944, when Saxton et al. (38) reported that underfeeding of the inbred AKR mouse strain reduced the incidence of spontaneous leukemia from 65% to 10% and resulted in thymic involution. The authors suspected that critical events regulating lymphocyte biology were occurring in the thymus, and indeed when young adult AKR mice were thymectomized, there was a significant suppression of tumor development (39).

In the past 20 years, it has been established that the effector arm of cell-mediated immunity, the T-lymphocyte system, is derived from stem cells that migrate to the thymus from the bone marrow or from fetal liver (40). Within the epithelial mesh-
work of the gland, these in-migrating T-lymphocyte precursors differentiate, proliferate into distinct immunocompetent cells with defined functions, and exit from the thymus (40) (Fig. 2). In-migration begins around week 8 to 9 of gestation, and the numbers of cells present increase until term. T-cells first leave the gland to populate the peripheral lymphoid organs after birth, continuing during childhood until the thymus involutes at puberty, with a sharp reduction in the output of new cells. Nonetheless, if mature T-cells are depleted by some insult, the adult thymus can still support their replacement (41). The processes of terminal differentiation of helper, suppressor, and cytotoxic T-cell populations and their functions and interactions in regulating the immune system have been reviewed recently (42).

On the basis of these data, we should expect that the "nutritional thymectomy" of protein-energy malnutrition will profoundly affect the cell-mediated limb of the immune system. This is what actually occurs (Table 1), but it may be a surprise that as recently as 20 years ago, this concept did not exist (1). The idea that thymic atrophy in kwashiorkor might be the cause of immunodeficiency first emerged in 1969 (37), and by 1971, this was clearly appreciated (43,44). As the understanding of the structural organization of the thymus gland increased, it was further appreciated that malnutrition caused lymphocyte depletion in cortical regions, with loss of corticomедullary differentiation, prominence of the epithelial and reticular tissue, and fibroblast infiltration, and that these changes were proportional to the duration and severity of the nutritional insult. Similar changes were present in regions of the spleen, tonsils, and lymph nodes now known to be populated by T-cells, including paracortical and periarteriolar regions, with preservation of B-cell-rich germinal centers and primary follicles (45–47). Such changes could also be detected clinically. For example, the tonsils and adenoids in kwashiorkor patients were smaller.

![Thymic Microenvironment Diagram](image)

**FIG. 2.** Major pathways in the immunologic network. Solid arrows, differentiation pathways; fine dashed arrows, inductive pathways; heavy dashed arrows, suppressive effects. (From ref. 8.)
by physical examination, and the thymic shadow observed on a chest X-ray was also diminished in size (45–48).

However, the most accessible population of lymphocytes in humans is the circulating pool of cells. Although the number of lymphocytes per mm³ is not usually diminished in protein-energy malnutrition, the number of mature T-cells identified by their ability to form rosettes with sheep erythrocytes (E-rosettes are defined as lymphocytes with three or more erythrocytes attached to the cell surface) is decreased (8). As there is no concomitant decrease in circulating mature B-lymphocytes (2,8,49–51), there must be a relative increase in already-committed, but immature, T-lymphocytes in the circulation. Several studies have provided evidence for this concept (52–54). As functional activity of T-cells correlates with cellular maturation, any decreases in mature cells would be expected to result in impaired function. The most frequently studied T-cell function in malnourished hosts is antigen- or mitogen-induced proliferation, largely because the major characteristic that distinguishes mature lymphocytes from other mature hematopoietic cells is their ability to proliferate when activated by exposure to antigens or mitogens. As expected, such proliferative responses are invariably reduced (2,4–8,10–12,44–47,55,56).

These findings provide an explanation for the old observation that prevalence of positive tuberculin skin test reactions and the size of the response are diminished in malnourished children with tuberculosis or following bacillus Calmette-Guérin (BCG) immunization (57–59). The tuberculin reaction is, in essence, an antigen-specific T-lymphocyte-mediated cellular immune reaction occurring in skin (60). Anergy to antigens that elicit delayed type skin hypersensitivity (DTH) is a common finding in malnourished hosts (1,2,4–8,44,46,47,49,61,62). Responses to sensitizing doses of new DTH antigens are also significantly impaired. These changes can usually be reversed after nutritional rehabilitation. Indeed, anergy to a battery of skin test antigens is currently used by some surgeons as a predictor of the risk of sepsis and death in surgical patients (61,62). Completely anergic patients are given a trial of nutritional rehabilitation in advance of elective surgical procedures.

Although some studies have shown an almost universal and severe depression in the number of E-rosetting cells in circulation in the malnourished patient (45–47,49,52), other studies reveal more heterogeneity. For example, the mean percentage of E-rosettes is significantly lower in Guatemalan children with kwashiorkor
compared with clinically healthy children of similar age. However, the ranges overlap, and some children who are malnourished by anthropometric and biochemical criteria have normal levels of T-lymphocytes (54). This may be due to a specific factor present in some, but not all, of the malnourished children, for example, concomitant zinc deficiency. Alterations in some nutrients can have widespread or localized effects on the lymphocyte network (Fig. 3). Similarly, when optimal nutritional therapy is provided for a 90-day period, mean values for E-rosettes increase. However, in individual patients, the changes do not necessarily parallel nutritional improvement as manifested by weight gain. In vitro addition to peripheral blood lymphocytes of either of the thymic peptides, thymosin fraction 5 (f-5) or thymopoietin, increased the percentage of E-rosettes (52–54,63). These effects of f-5 were dose-dependent and directly related to the initial percentage of nonrosetting cells (54). However, even after 1 month of optimal nutritional therapy in Guatemalan infants sufficient to result in improvement in weight-for-height, thymosin-responsive cells were still present in the circulation (63).

**B-Lymphocytes and Antibody Production in Malnutrition**

As already noted above, the number of B-lymphocytes in the central lymphoid organs and in the circulation is usually normal in the malnourished host. These findings are consistent with the normal to elevated immunoglobulin (Ig) levels found in the serum of malnourished patients (9). However, Ig levels are not the equivalent of antibody activity, which can only be assessed in functional assays. Because some antigens require the help of T-lymphocytes to activate B-cells (T-dependent anti-

**FIG. 3.** Localization of the specific effects of nutrients on the immunologic network. Dashed arrows, site or specific cell type affected by the various nutrients listed for effects elicited by nutrient deprivation or, in a few instances, by excess. (From ref. 8.)
gens), any defect in T-cell function can have an impact on the antibody response. Thus, the B-cell from a malnourished host can function totally normally in vitro, while in vivo function may be markedly abnormal (2,5,9,50).

One way to evaluate this is to examine the antibody response to vaccine administration, and a number of studies have done just that in malnourished humans (reviewed in refs. 9,50). The results are variable and not readily explainable on the basis of what we presently know about the control of antibody synthesis or about the effects of malnutrition on immune function. For example, typhoid O-antigen, one of the commonly tested vaccine antigens to which a subnormal response is often reported in malnourished hosts, is a polysaccharide that should be capable of directly stimulating B-cells without the involvement of T-lymphocytes (T-independent antigen). In contrast, tetanus toxoid, a T-dependent peptide antigen, invariably produces an adequate immune response. Variable results are also described after administration of live virus vaccines, with apparently normal responses to measles but impaired responses to oral polio vaccine (9,50). The failure of polio vaccine to induce adequate antibodies, however, could be independent of immune function. For example, it is certainly possible that it could be a consequence of viral interference in the gut due to the presence of other enteroviruses that inhibit colonization by the vaccine virus. Data on secretory immunoglobulins (s-Ig) in malnourished patients are much more uniform (5,9). The levels of s-IgA are consistently low in patients with protein-energy malnutrition, and when polio vaccine is used to probe the ability to make secretory antibodies, there is evidence of significant depression in response. If salivary secretions are a model of the rest of the mucosal immune system, then not only is the concentration of s-Ig and antibody diminished in the malnourished host, but the volume of secretions is also diminished. Such effects on the parotid gland result in an even more marked diminution of total secretory antibody content in saliva.

Complement Abnormalities in Malnutrition

Another host defense mechanism often affected in the malnourished host is the complement system (Table 2). The complement system consists of a cascade of proteins activated in sequence, resulting in the generation of products of major importance in mediating the inflammatory response or possessing direct or indirect effects on pathogen viability. Activation of hemolytic complement activity by the classic pathway (64-66) or the alternative pathway (66) is reduced in serums obtained from malnourished children. The levels of all classic pathway components except C4 are reported to be reduced (65). Factor B levels are very depressed (66), and there is often evidence of in vivo consumption of C3 as well (64). One functional consequence of this is a defect in the phagocytosis of bacteria that are opsonized by the complement system, in particular, gram-negative bacilli. In contrast, Staphylococcus aureus is opsonized primarily by immunoglobulin. An opsonic defect for Escherichia coli and not S. aureus in serums from Guatemalan children with kwashiorkor
TABLE 2. Effects of protein-energy malnutrition on the complement system

- Decrease in total hemolytic complement activity of both classic and alternative pathway.
- No apparent alteration in regulatory proteins.
- Probable decrease in synthesis of complement proteins, with blunted acute phase response.
- In vivo consumption of complement, with complement breakdown products in the circulation.
- Decrease in concentration of individual complement components in serum.
- Impairment in complement-mediated functions, such as opsonization.

has been reported. The defect was much greater for *E. coli* that are opsonized primarily by the alternative pathway rather than the classic pathway (67,68).

IS THERE A CONNECTION BETWEEN THE IMMUNE SYSTEM AND NUTRITIONAL STATE?

In the past decade, this question has been clearly answered in the affirmative, and the mechanisms have begun to be unraveled. The first insights resulted from studies on the molecular mechanism of fever, in particular, the role of a monocyte-derived pyrogenic factor, initially called endogenous pyrogen (EP) but since renamed interleukin-1 (IL-1) (69). It is now known that IL-1 acts on the thermoregulatory centers of the hypothalamus to reset the thermostatic set point by increasing the production of prostaglandin E2 (69). Years later, leukocytes activated in the inflammatory response were found to produce factors inducing abrupt changes in plasma levels of divalent cations and alterations in hepatic protein synthesis. The responsible factor was termed leukocyte endogenous mediator (LEM) (70,71). When EP was finally purified, it co-purified with LEM activity. It has since been proved that EP and LEM are two functional activities of the same molecule, IL-1 (Fig. 4) (72). When IL-1 also turned out to be the lymphocyte activating factor (LAF), the link between metabolic events in infection and the immune system was firmly established (72).

The gene for human IL-1 beta has now been cloned and expressed in *E. coli* (73), allowing preparation of large quantities of recombinant IL-1 sufficient for thorough investigations of its properties (74,75). These studies have shown how diverse the metabolic effects of IL-1 are (Fig. 5) and have demonstrated its key role in activation of host defenses.

During the past 5 years, another cytokine affecting metabolism and host response in infection has been identified by assaying for its bioactivity. As with IL, the assay for biological activity was useful in studies to clone and express the gene, which resulted in production of enough of the recombinant molecule to study its properties (76,77). This mediator was first discovered by Rouzer and Cerami (78), who noted that chronic *Trypanosoma brucei* infection caused severe cachexia and marked
FIG. 4. Initiation of the acute-phase response and the diverse effects of interleukin-1 on host nutrition and immune responses. (From ref. 74.)

FIG. 5. Schematic diagram of the tissue targets for interleukin-1 (IL-1) and/or cachectin-tumor necrosis factor (C-TNF) in the metabolic response to infection, including pathways and manifestations.
hypertriglyceridemia in cattle or rabbits. Since gram-negative rod infections were known to cause similar changes in serum lipid patterns (25), administration of endotoxin was studied and was found to produce an identical response in C3H/HeN mice. No effect was observed in the endotoxin-resistant C3H/HeJ mouse strain, but serum harvested from HeN mice a few hours after giving the endotoxin elicited typical lipid changes in the HeJ mouse, suggesting the involvement of a mediator (79). This hypothesis was later proved, and the monokine was named cachectin (80). Subsequent analysis of structure showed that cachectin was identical to the previously described monokine, tumor necrosis factor (76), and the mediator is generally referred to as TNF. At the present time, however, the name cachectin/tumor necrosis factor (C/TNF) is the preferred, albeit awkward, name, for this is most consistent with both historical precedent and physiologic role.

The mechanism of the lipid abnormalities caused by C/TNF was a defect in lipid clearance due to inhibition of lipoprotein lipase (LPL) activity (79). Elicited peritoneal macrophages or a macrophage cell line, RAW 264.7, also responded to endotoxin in vitro by releasing a mediator that suppressed LPL activity in cultured cells (79,80). Recent studies have defined the remarkable way in which C/TNF causes these effects, using TA1 cells, a fibroblast line that undergoes differentiation in cell culture into an adipocyte (81). During this process, certain genes for enzymes involved in lipid metabolism are activated (including the anabolic enzymes and LPL), transcribed and translated, and fat is synthesized and stored. In the presence of cachectin, however, these genes are not activated, and neither messenger RNA nor the enzymes are synthesized. This inhibition is specific and does not involve genes unrelated to lipid synthesis, for example, actin. Regulation of this process by C/TNF is at the transcriptional level. This was shown by isolating nuclei from control and C/TNF-exposed cells, followed by incubation with labeled precursors of RNA, and identification of the newly synthesized messenger RNAs (mRNAs) by hybridizing products with known complementary DNAs (cDNAs). Only the lipid genes were affected.

As already noted, when cachectin was purified and the initial amino acid sequence data were obtained, it became obvious that it was closely related to TNF, a monokine capable of lysing certain tumor cells in vitro. Subsequent sequencing of human, mouse, and rabbit cachectin at the gene and peptide level has proved that cachectin and TNF are the same, and the conservation of its sequence across species is striking (82). Like IL-1, human C/TNF is a 17,000-kd peptide, and it consists of 157 amino acids. The mouse and rabbit molecules are identical, except for 1 or 3 amino acid deletions, respectively. All derive from a larger precursor, which is about 86% conserved. Production of C/TNF is also regulated by steroids, and in the presence of dexamethasone, activation of the C/TNF gene by lipopolysaccharide (LPS) is inhibited, and translation is completely shut off (83). These data are consistent with there being a large pool of untranslated specific C/TNF mRNA in normal cells and with activation by LPS involving increases in both transcription and translation. Since C/TNF appears to reproduce the symptoms and signs of endotoxic shock and has been proposed as one of the key mediators in the response (84,85),
down-regulation by steroids of C/TNF is consistent with the therapeutic effects of these drugs. In addition, similar transcriptional and post-transcriptional regulation of IL-1 production has also been shown (86), and this mediator also has the capacity to induce the septic shock syndrome and may be the major mechanism underlying the toxic shock syndrome (87).

The release of these mediators appears to explain the mechanism of altered protein metabolism in infection. IL-1 or IL-1 breakdown products are capable of inducing muscle proteolysis (88,89). A small peptide has also been found in serum of septic patients, which initiates proteolysis in an in vitro muscle preparation. The relationship of this peptide to IL-1 was suggested by the correlation between the serum level of this proteolysis-inducing factor (PIF) with the fever response in the patient (90). If PIF or IL-1 is injected into the peritoneal cavity of rats, it results in increased uptake of amino acids by liver and synthesis of acute phase proteins (91). Both IL-1 and C/TNF have been shown to be capable of regulating the hepatic acute phase response, including the increase in synthesis of certain proteins and the decrease in synthesis of export proteins such as albumin or transferrin (Fig. 5). Gene expression in primary mouse hepatocytes or cultured human hepatoma cells is regulated by the two cytokines, and the level of specific mRNA parallels production of the particular protein (92–94). IL-1 and C/TNF activate the genes for several acute phase reactants, including factor B, C3, alpha-1 acid glycoprotein, alpha-1 antichymotrypsin, and inter-alpha-trypsin inhibitor, while at the same time the mediators turn off transcription of the albumin and transferrin genes (95–97). Since these two cytokines have distinct receptors on adipocytes, and therefore probably on hepatocytes as well, it is likely that the very similar regulation of multiple genes must be carried out by distinct signal recognition systems. This suggests either that a common second messenger is involved or that common mechanisms of gene regulation are activated by different transduction signals.

IL-1 is also involved in some of the alterations in carbohydrate metabolism in infection. For example, the hyperinsulinemia that is characteristic of infection can be induced in rats by the injection of endotoxin. There is a 4-hr lag, reminiscent of the kinetics of LPS-induced fever (94). This is consistent with the reduction in the lag period to 1 hr after administration of IL-1 itself (94). In vitro studies have shown that IL-1 has direct effects on the pancreas as well. For example, isolated islets of Langerhans respond to low levels of IL-1 both by increasing insulin content and by insulin release (96). The mechanism appears to be transcriptional regulation of insulin biosynthesis, since IL-1 induces alterations in the level of specific mRNA for preproinsulin as well as insulin content (97), not unlike the regulation of hepatic acute phase proteins by IL-1 and C/TNF.

As additional studies have been carried out, the similarity in biological activity of IL-1 and C/TNF has become even more striking (77,98). C/TNF is also an inducer of IL-1 (99), and in some experimental situations, for example, attempts to recreate the alterations in energy metabolism in normal rabbits by infusion of cytokines, a combination of the two peptides is required (100). Thus, the effects of either IL-1 or C/TNF, or both, can account for many of the metabolic events that occur in the sep-
tic host, and both appear to play important roles in the host defense response. Their broad and critical activities, their ability to act synergistically together, and the possibility that excessive quantities may cause lethal shock in severe sepsis indicate the need of the host to regulate production of IL-1 and C/TNF closely. Since the outcome of infectious diseases may be greatly conditioned by IL-1 and C/TNF, individually or together, it is of great interest to ask what happens in the already malnourished host. Some evidence suggests that these mediators are produced in infected malnourished hosts: for example, the typical anabolic events, changes in energy metabolism, the acute phase response, and change in pattern of hepatic protein synthesis, all known to be responses to IL-1 and/or C/TNF, certainly do occur. Nonetheless, it is generally believed that malnourished hosts fail to develop fevers commensurate with the clinical illness. Several recent studies have suggested that in vitro production of IL-1, measured by bioactivity, is diminished in such patients. In response to activation with opsonized zymosan, S. epidermidis, or LPS in vitro, human peripheral blood monocytes from malnourished patients do not produce as much endogenous pyrogen, leukocyte endogenous mediator, or LAF bioactivity as cells isolated from healthy controls (101,102). While these data suggest that undernourished hosts may be at a disadvantage because they are unable to produce necessary mediators in optimal amounts, it is also possible that the cytokines are being produced in vivo to a normal or even greater extent, and as a result, the isolated mononuclear cells are simply less able to respond to stimuli in vitro. This remains to be determined.

In addition, there is evidence that cells from malnourished patients also produce inhibitors of the LAF response (103). It is also possible that other functional inhibitors may be found in vivo with specificity for one or another of the many activities of IL-1 or C/TNF (104). While it seems clear that IL-1 and C/TNF are key mediators in activation of the immune response and the metabolic responses in infection, their release and role in the clinical events in malnourished patients remain to be determined. It seems clear that the next frontier in studies of nutrition–infection interactions is the cytokines and their positive or deleterious effects on outcome of infection (105,106).

COMPREHENSIVE SUMMARY

Malnutrition and infection are commonly found together in children in the developing world. Although it has been believed that dietary inadequacies lead to nutritional deficiencies and predispose to infection, newer evidence suggests that infection may be the initial problem, resulting in nutritional deterioration of the host. The link between malnutrition and infection is impaired host defense mechanisms (Fig. 1). To evaluate these theses, there are three major questions to ask: (a) Does infection cause malnutrition? (b) Does malnutrition result in impaired host defense? (c) Is there a connection between the immune system and nutritional state?
Does Infection Cause Malnutrition?

A number of complex changes in host metabolism occur with the onset of infection and fever. These changes involve all aspects of host metabolism, including energy, protein, and mineral metabolism. It is readily apparent that fever itself imposes demands on energy in order to fuel the generation of body heat. The metabolic demands are even greater, for simply increasing body temperature accelerates all enzymic reactions, with a concomitant drain on energy sources. Because loss of appetite (anorexia) so commonly accompanies the fever, food intake is sharply curtailed and the patient must rely on endogenous energy stores in the form of carbohydrate, lipid, and protein. The carbohydrate stores are inadequate for more than a day at most, and alterations in metabolism during infection often impair utilization of lipid. The patient is therefore forced to break down proteins and convert amino acids into glucose (gluconeogenesis) in the liver. Hormonal changes, including elevations of insulin, growth hormone, glucagon, and corticosteroids in plasma, act to support the new energy pathways. The result is that the patient behaves as a diabetic with respect to glucose, showing fasting hyperglycemia, abnormal glucose tolerance, and an apparent peripheral insulin resistance. The increased energy flow and elevated glucose oxidation rates also support the increased demands for protein synthesis, to produce cells and proteins involved in host defenses, and to repair tissues damaged in the inflammatory response.

The major available protein reserve in the body is contractile proteins in muscle. Infection initiates a marked proteolysis of muscle, with release of amino acids into plasma. These are taken up by the liver and used for gluconeogenesis and new protein synthesis. During infection, there is a marked increase in synthesis of a group of proteins, collectively referred to as acute phase proteins, which are not normally produced or are present in very low levels in the uninfected host. These proteins include C-reactive protein, serum amyloid A, and the third component of complement, together with a number of others. A likely beneficial role can be assigned to these molecules in combatting infection or its consequences. At the same time, the synthesis of normal liver export proteins, such as albumin and transferrin, is inhibited, leading to marked decreases in their concentration in serum.

The net sum of these alterations is pronounced wasting due to the combined effects of decreased food intake, increased energy consumption, catabolism of muscle protein, negative nitrogen balance, and depletion of glycogen and lipid stores. It is necessary to replete these nutrients during convalescence, a process that can take as much as four times as long to accomplish.

Does Malnutrition Result in Impaired Host Defense Abnormalities?

It is now clear that protein-energy malnutrition causes abnormalities in the thymus gland, impairing the differentiation of T-lymphocytes. Mature T-lymphocytes are depleted from thymus, spleen, and lymph nodes, and there is a marked decrease
in their number in circulation. The major consequence is that all cell-mediated immunologic processes dependent on mature T-cells are impaired. Thus, immune responses to viruses, certain protozoa, fungi, and facultative intracellular bacteria are inhibited. But because of the critical role of T-lymphocytes in coordinating the network of immunocompetent cells, there is an impact on other immune mechanisms as well, e.g., production of antibodies to certain antigens dependent on T-cell help to initiate immunoglobulin synthesis. Many different nutrient deficiencies affect the function of immunocompetent cells (Fig. 2), and these may act together in causing clinically significant defects. Correction of these deficits should improve host responses to present and subsequent infections.

Complement activation is also reduced in protein-energy malnutrition, probably due to a combination of diminished synthesis and blunted acute phase responses of the complement components, and in vivo consumption because of concurrent infection. Since complement-derived products are essential for normal phagocytic cell responses to invading microorganisms (including chemotaxis, diapedesis, and opsonization), intracellular microbicidal activity is also affected. Hence, the defenses against pyogenic and other nonintracellular bacteria and against certain fungi and protozoa are inhibited. For example, the frequency of gram-negative bacterial sepsis is probably largely due to complement defects in the malnourished child.

Is There a Connection Between the Immune System and Nutritional State?

In the past 5 years, it has clearly been shown that peptide mediators made by stimulated immunocompetent cells not only regulate the immune response, but also initiate and sustain the metabolic response to infection. This close coordination between immunologic activation and metabolic alteration suggests that the latter is in direct support of the former. Two endogenous mediators in particular serve this function, IL-1 and cachectin-tumor necrosis factor. These peptides affect the brain, liver, pancreas, fat cells, and muscle to cause dramatic changes in energy, protein, and mineral metabolism (Fig. 3).

Conclusions

Infection results in the release of potent mediator peptides, primarily from immunocompetent macrophage/monocytes, which exert beneficial effects in activating immune responses. These same mediators alter host metabolism in a fashion that must be useful to the host but that is debilitating and leads to malnutrition if continued for too long. In turn, nutritional deficiencies adversely affect immune function and reduce the effectiveness of host defenses if not repaired before the next infection occurs. The frequency of infection in children in the Third World means that deficits are rarely corrected, that the next infection therefore occurs in a less immunocompetent host, and that a progressive worsening in clinical state occurs in the cyclical
fashion depicted in Fig. 1. The end result is usually growth faltering and constant morbidity, but all too often, the consequence is death. Any public health program designed to deal with malnutrition must simultaneously act to reduce the incidence and impact of infection.

REFERENCES


**DISCUSSION**

*Dr. Suroto:* I have three questions. Does immune response impairment correlate with the degree of malnutrition? Do children with marasmus and kwashiorkor demonstrate the same impairment? Finally, is it contraindicated to vaccinate a child who has marasmus or kwashiorkor?

*Dr. Keusch:* It is hard to document a correlation, because of the many instances in which the immune system may be relatively intact while anthropometry is significantly abnormal. The problem with malnutrition is that it is not a simple dietary deprivation but involves diverse, complex factors. What is necessary is a more complete assessment of the functional state of the individual. This is not easy because of the difficulty in measuring accurately the functional integrity of the immune system, especially *in vivo*. Sampling peripheral blood is not central enough to where the action is in order to give the necessary clues to predict immune function in individuals. It is probably better than anthropometry, but it is still not enough.
Comparing marasmus and kwashiorkor, the most severe infections and laboratory-detected abnormalities are generally in children with kwashiorkor.

The data regarding vaccinations are somewhat reassuring. There is little evidence that you can do any harm by immunizing a malnourished child, even with a live virus vaccine. On the other hand, the malnourished child may not have an optimal immune response. That is one source of problems for the Expanded Programme for Immunization programs. Adequate distribution of vaccines can never be interpreted as adequate immunization. EPI programs need surveillance of the response of the target population to the antigens.

Dr. Guesry: You state that the first step in the depression of the immune system during protein-energy malnutrition (PEM) is a depression of lymphokines, and for some nutrients, such as vitamins or trace minerals, you have been able to indicate whether the activity was a direct one on the cell or was through hormone secretion. With regard to a theoretically pure protein or energy deficiency, would there be a direct effect on the cell or would the effect be through the secretion of hormones such as cortisol?

Dr. Keusch: When we get down to the primary mechanism of the failure of T-cell maturation, we really do not understand it in either molecular or physiologic terms. It probably involves the decreased production of thymic hormones and may represent an alteration in the thymic epithelial cell surface, possibly related to vitamin A or some other nutrient factor. Whatever the underlying cause, infection is contributing to nutrient deficiencies, at least because it contributes the catabolic processes. We should be able to account for such metabolic losses in balance studies. If we can analyze the mechanisms for the catabolic response, we may be able to devise interventions to mitigate the problem.

Dr. M. Mehta: In India, because of the prevalence of tuberculosis, we treat each moderately to severely malnourished child with antitubercular drugs. Regarding immunization, one must also immunize all children who are vulnerable to infectious diseases such as polio and measles. The only negative aspect is that they may not respond.

Dr. Keusch: I agree. Concerning tuberculosis, while a positive skin test does not mean that one has clinical tuberculosis, if confirmation is not possible, then one is limited to the tuberculin skin test. The problem is that in PEM the skin test will often be suppressed. Under such circumstances, drug therapy is quite safe and relatively cheap. The concerns are compliance and drug resistance.

Dr. Soriano: In children with secondary malnutrition, such as nephrotic syndrome, one sees severe pneumococcal infections that have been activated as a result of compromised splenic function. The question is whether the defective splenic function might be secondary to the protein deficiency.

Dr. Keusch: There are relatively few studies of macrophage function in PEM. Animal studies essentially conclude that there is very little wrong with peritoneal macrophages in their antimicrobial functions. A recent animal study, in which alveolar macrophages were examined using very sophisticated techniques to evaluate the lipoxygenase and the cyclooxygenase pathways, described a significant alteration in the lipoxygenase pathway and depression of the leukotriene metabolites that could have significant effects on tissue responses, with the same alveolar macrophages having essentially normal antibacterial function.

The macrophage is an interesting cell, because it is not only an antigen-processing cell and an antibacterial cell, but is also very important in tissue injury and tissue repair. These functions are separable, and one function may be affected while the other is not. It is time to initiate studies of macrophage function in children with PEM.

Dr. Tanner: In the human, infection seems to be the factor that starts the process. If that is true, then in animal models without inflammation or infection, immune studies should show
an intact immune mechanism even though there is protein-energy malnutrition. Is that what has been found?

**Dr. Keusch:** Animals put on protein or food-limiting diets tend toward increased longevity and better activation of the cell-mediated immune system. This is contrary to what is seen in the human. However, it is difficult to compare the results, because animal studies look at single, consistent deficiencies, with a constant diet from day to day. In addition, the animals are protected from infection and injury. Human experience is much more variable. The constant influences in the animal studies probably have a strong influence on the changes in the subpopulations of the lymphoid cells.

**Dr. Warrier:** Can you comment on the effect of iron and protein deficiencies on antibody synthesis?

**Dr. Keusch:** Iron deficiency is probably the most common nutritional deficiency in the world. There are studies suggesting that iron deficiency is protective against infection and the impact of infection by depriving pathogens of iron, while others suggest iron deficiency is immunosuppressive by altering the membrane structure of the mature peripheral T-cells. There are receptors on the surfaces of these cells for iron and iron-binding proteins that regulate their function. However, it is very difficult to show a relationship between a test of immune function in vitro and a host response to a real infection.

**Dr. Suskind:** In animal studies, we have found that specific nutrients, such as iron, zinc, or vitamin A, are probably more important in affecting the cellular immune system than are protein and calories. When cellular immunity and nutritional status were studied in sickle cell disease, we found children who both were zinc-deficient and had a depressed cellular immunity. The immune deficiency was reversed after zinc supplementation. That indicated, to us, that single trace elements and vitamins are probably more significant factors in modulating the cellular immune system than are protein and calories.

**Dr. Aggett:** Could you comment further on the effect of iron deficiency as a potential protection against infection?

**Dr. Keusch:** As in humans, microorganisms require iron for their growth. They have adapted a mechanism for responding to their iron requirements through production of iron-binding proteins with an extraordinary affinity for iron. They are thus able to compete with the host for available iron. When the concentration of free iron is limited in vitro, the microorganism will not grow very well, limiting its potential as an important pathogen. The studies suggesting that oral iron supplementation increases the incidence of severe infection are generally not convincing, nor do they support the idea that iron deficiency is protective. My feeling is that iron therapy, in the form of oral iron, is safe and advisable in treating iron deficiency states.

**Dr. Truswell:** Please comment on the frequency and severity of diarrheal disease and their effects on the malnourished child.

**Dr. Keusch:** The host defense mechanisms against most of the agents of diarrheal disease are unknown. In our Guatemalan studies with the Institute of Nutrition of Central America and Panama (INCAP), the only immunologic indicator of increased risk was a decrease in T-cells. Other immunologic parameters had no predictive value. It is possible, however, that changes in the gut mucosal immune system will impact on the susceptibility to diarrheal disease, and these are more difficult to assess in humans.

**Dr. Monckeberg:** Recent reports affirm that stress can suppress the immune response. The alterations appear very similar to the ones described in malnutrition. In children with marasmus, who are chronically stressed, perhaps it is the stress rather than nutrient deficiency that causes the alteration in the immune response. In our studies, marasmic children treated with
psychomotor stimulation and affection have shown an immediate, significant decrease in their infection rate. When the stimulation program is stopped, the rate of infection increases.

**Dr. Keusch:** It is possible that the central nervous system has a major impact on regulating immune function. However, we must define stress very specifically in terms of mediators that act at the level of the brain or are produced by the brain and act peripherally.

**Dr. Barclay:** Regarding the connection between zinc and immune status, a report from Senegal showed that in malnourished children, the activity of thymulin in the thymus gland is reduced (1). In zinc deficiency, low circulating thymulin activity can be restored to normal levels by incubating the serum with zinc (2). Could you comment on this?

**Dr. Keusch:** I am not sure what is meant by serum thymic hormone level, since these peptides probably act within the gland and not in the circulation. I do, however, agree that some of the manifestations of the immune deficiency state of PEM may be attributable to zinc. Thymic hormones are peptides that bind zinc, which is needed for activity at the thymic level within the gland. You can readily measure the circulating hormone, but it is hard to biopsy the thymus in a living child.

**Dr. Tanner:** A recent study examined IgG subclasses in malnourished children with cystic fibrosis and concluded that high levels of IgG antibody to Pseudomonas lipopolysaccharide may be inhibitory to pulmonary macrophage function (3). It would be interesting to have more information on IgG subclasses in malnutrition.

**Dr. Jackson:** With regard to iron therapy, evidence from studies (4) demonstrated that in severely malnourished children, plasma ferritin may be very high. In addition, there is a close correlation between a high plasma ferritin and the risk of a child’s dying. The implication is that free iron in the liver is increased (5). This fits with the suggestion made by Viteri (6) that during the process of becoming malnourished, red cell production falls and an anemia develops. Thus, increased amounts of iron are made available, which must go into storage because they cannot be excreted. As a result, although there may be a decrease in total body iron, there is an increase in storage iron. During rehabilitation, as the red cell mass expands, the storage iron is taken back into newly synthesized red cells, and, at some point, iron deficiency develops. This evidence strongly argues against the use of iron therapy until rehabilitation is initiated in the malnourished child.

**Dr. Suskind:** Patients with anorexia nervosa, who are chemically protein- and energy-depleted, are not immunosuppressed, as are children with marasmus. The decisive factor may be the supplemental vitamins and minerals that the majority of those children take.

**Dr. Keusch:** When someone with anorexia nervosa develops an inflammatory process, production of catabolic mediators occurs. This catabolic response also alters vitamin and mineral metabolism. For example, the anemia of chronic infection can be related, at least partially, to the effects of interleukin-1 (IL-1) on the uptake and sequestration of iron from the usable iron pool. The difference between kwashiorkor and marasmus may be that the marasmic patient can regulate the mediators appropriately, and they are neither over- nor underproduced, as may happen in children with kwashiorkor. This adaptation may be at another level of response, and some of it may relate to the adequacy of nutrients like zinc. At least some of what we see in PEM may be related to zinc abnormalities as well as other factors.

**Dr. Suskind:** We have looked at nutrient modulation of the immune response in vitro by producing media deficient in specific nutrients, such as zinc, iron, or manganese. When the individual nutrient was added back to the media with specific mitogens, there was a dose response curve of the isolated lymphocytes to increasing quantities of the specific nutrient. This in vitro system may be another way of defining the role of specific nutrients in lymphocyte metabolism.
Dr. Keusch: Anything that produces a suboptimal nutrient environment in cell culture will alter growth and metabolism. How that relates to what happens in vivo, however, is hard to determine.

Dr. Karyadi: Would you comment on studies showing the relationship between vitamin A megadose supplementation and reduced mortality?

Dr. Keusch: The major impact is probably at the level of epithelial maturation and epithelial surface functions, whether it is respiratory or gastrointestinal, since vitamin A has an important role to play in membrane maturation and function.

Dr. Brunser: We have just finished a study on the effect of chronic iron supplementation in infants. Children who were subjected to a higher risk of enteric infection, as a result of the microbiological contamination of their environment, received an iron-enriched milk. Infants who were less than 8 months old were found to be at increased risk of developing episodes of diarrheal disease. This suggests that, in the presence of chronic iron intake, when enteropathogens gain access to the lumen of the small intestine, children will tend to develop episodes of diarrhea in circumstances in which they might only have become carriers.

Dr. Ballabriga: We have observed a special form of malnutrition in infants presenting with acquired immunodeficiency syndrome (AIDS). Many of the infants with positive antibodies after the age of 5 to 6 months become marasmic before they present with candidiasis or interstitial pneumonia. The marasmus is progressive and is practically the first manifestation of AIDS. Could you comment on this form of the disease?

Dr. Keusch: This is likely to be a result of hyperproduction of catabolic mediators, a form of endogenously induced cachexia. There is evidence of macrophage malfunction in AIDS, and the production of IL-1 and cachectin (or tumor necrosis factor) may account for this endogenous wasting. This endogenous factor may be more important in the wasting of malnutrition than the specific infection or the presence of intestinal malabsorption.

REFERENCES