Immune Function in the Malnourished Host

By GERALD T. KEUSCH, M.D.

The high rate of concurrence between malnutrition and infection in the developing countries has raised the question of the relationship between the two. Much research has been conducted to evaluate the influence of malnutrition on host defense and it is now generally accepted that malnutrition is the most prevalent cause of immunodeficiency.

Immune mechanisms and other host defenses are complex processes. They involve a multiplicity of cell types interacting with one another and regulated by diverse humoral mediators. The most common mediators are lymphokines produced by stimulated lymphocytes, and monokines produced by activated macrophages. Both may have more than a regulatory function. For example, lymphocyte activating factor (LAF), which is a monokine, appears to be identical with the leukocyte pyrogen that induces the fever response.

Macrophages also produce complement components. Activation of the complement cascade yields C3a anaphylatoxin and C5a chemotactic factor, which have direct effector roles in the inflammatory response and C3b opsonin, which is active in phagocytosis. The immune system is therefore appropriately described as a network in which both cellular and humoral factors function to regulate responses to various stimuli.

Diet inadequate in protein, calories, some vitamins and certain minerals do not depress immunity completely, but are selective in their effects. Excessive nutrients, resulting from overeating or megavitamin “therapy” also exert a profound influence upon the system. This article, however, will be limited to problems deriving from undernutrition.

METHODOLOGICAL CONSIDERATIONS

Definition of malnutrition is difficult because of the complexity of nutritional needs and heterogeneity of dietary sources. Malnutrition, therefore, has been considered a mosaic, varying over time as food intake and food quality change and various stress factors, such as infection, come and go.

Investigations of immune function in malnourished subjects have been conducted in man and experimental animals. Variability of deficiencies in human subjects suffering the common protein-energy malnutrition (PEM) has hampered immunological studies and led to conflicting results. On the other hand, manipulation of diets in experimental animals allows production of specific nutritional deficiencies susceptible to investigations giving reproducible results. Experimental nutritional science has been built on studies of the rat whose nutritional requirements are reasonably well defined. However, genetic variability, which may influence host defense more than nutrition and is impossible to control.

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in human studies, is quite easy in experiments with highly inbred strains of animals, particularly mice, whose immune responses have been thoroughly studied. Laboratory studies in man are also more limited than those in experimental animals. Studies of human lymphocyte function are restricted mainly to circulating peripheral cells, whereas in animals it is easy to examine thymic tissue, bone marrow, spleen or lymph nodes.

Circadian rhythms, hormonal responses, and continuous vs. intermittent feeding can also influence experimental results, and these considerations have often been neglected in experimental studies.

In spite of the many difficulties, an impressive body of knowledge has accumulated over the past decade, permitting some degree of precision in defining the role undernutrition plays in modulating host defense.

**EFFECTS OF MALNUTRITION ON LYMPHOCYTES**

**T. Lymphocytes**

Although the effect of PEM on involution of the thymus and lymphoid organs was observed well over a century ago, recognition of its importance for immune function dates back only a little more than a decade. The first link between nutrition and cell mediated immunity was the observation of depressed delayed type hypersensitivity (DTH) responses to tuberculin in children with kwashiorkor and marasmus. More recent studies have confirmed the early results (Table 1) and related the changes in PEM to a decrease in the number of circulating mature T cells (Table 2), without a corresponding decrease in the number of B cells. This abnormality was correctable by nutritional rehabilitation and correlated with increase in size of the thymus gland observed in roentgenograms and of tonsilar tissue seen directly.

The decrease in mature T cells is accompanied by an increase in lymphocytes free of identifying markers, the so-called null cells. Chandra has reported that null cells comprise about half the circulating lymphocytes in severe PEM compared to 5% to 10% in well nourished controls. A decrease in the number of functionally mature peripheral blood lymphocytes (PBL) has been demonstrated in vitro by assessing their response to mitogenic lectins such as phytohemagglutinin (PHA). In such tests, PBL function appears uniformly impaired in children with kwashiorkor, and somewhat decreased in children with marasmus (Table 3).

Histological studies of tissues obtained post mortem from children with PEM reveal that the thymus is depleted of lymphocytes. This is particularly evident in the cortex where normally the more mature thymocytes are found. There is a relative prominence of epithelial and reticular tissue, loss of cortico-medullary demarcation, and a striking decrease in Hassall's corpuscles. These observations have a parallel in the continued on page 1008
TABLE 2
E-ROSETTING PERIPHERAL BLOOD LYMPHOCYTES IN MALNOURISHED HOSTS

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>% E-Rosetting Lymphocytes</th>
<th>No. of Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Range)</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>34.0 (24-43)</td>
<td>7</td>
</tr>
<tr>
<td>Marasmus</td>
<td>34.6 (21-42)</td>
<td>5</td>
</tr>
<tr>
<td>Unclassified PEM</td>
<td>32.7 (17-47)</td>
<td>6</td>
</tr>
<tr>
<td>Controls</td>
<td>62.9 (50-74)</td>
<td>18</td>
</tr>
</tbody>
</table>

*Average of the mean values recorded in 18 studies.7

TABLE 3
PHYTOHEMAGGLUTININ RESPONSES IN MALNOURISHED HOSTS

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>PHA Stimulated 1H-Thymidine Uptake</th>
<th>Stimulation Index —% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Range)</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>33.5 (20.9-52.4)</td>
<td></td>
</tr>
<tr>
<td>Marasmus</td>
<td>51.2 (16.5-70.6)</td>
<td></td>
</tr>
<tr>
<td>Unclassified PEM</td>
<td>48.9 (29.2-81.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Summary of 14 studies utilizing different methodologies with or without autologous plasma present.1

continued from page 1005

peripheral lymphoid organs in which the T dependent areas (paracortical and periarteriolar collections) are also severely depleted, whereas the germinal centers and primary follicles, containing B lymphocytes, are relatively spared. This selective depletion indicates that the decrease in circulating T lymphocytes is not due to failure to release cells to the periphery, but to factors within the thymus affecting maturation of the cells. However, the possibility of a defect in the early lymphoid precursors in patients with PEM has also been suggested.16,17

Thymic influences on the development of T cells seem to depend on both cell-to-cell interactions with thymic epithelium and on soluble thymic factors, or hormones. Recent in vitro studies have demonstrated that addition of thymic factors to PBLs obtained from children with kwashiorkor resulted in a significant increase in cells with markers identifying them as T lymphocytes.18,19 This is consistent with the report of decreased circulating thymic hormone in patients with PEM.20

Further evidence of an increase in the proportion of circulating immature T cells in children with PEM comes from measurements of the T lymphocyte enzyme, terminal deoxynucleotidyl-transferase (Tdt). The enzyme is restricted to immature thymic lymphocytes in the bone marrow and to some circulating ones; it is not present in the mature cells. Tdt activity in the PBLs of patients with PEM is increased tenfold over that found in normally nourished individuals.21

Suppressor cells or soluble factors may also be responsible for the functional deficits in patients with PEM. Null cells obtained from a few such patients and treated with mitomycin C suppressed the response to PHA of PBLs from a normal donor.22 Serum of Gambian patients with kwashiorkor and, to a lesser extent, serum of marasmic patients also inhibits the PHA response of normal PBLs, but the suppression lasts only as long as the serum is present.22,23 The response is restored when the cells are incubated in serum from a normal donor. No inhibitor has been demonstrated and supplementation of serum from a patient with kwashiorkor with a low molecular weight filtrate of normal serum restores the response to normal. Moreover, the suppressive characteristics of the serum diminish progressively during nutritional rehabilitation. Thus kwashiorkor serum apparently lacks a factor essential for a normal proliferative response. The nature of this factor and its prevalence are unknown.

The abnormalities of function of the lymphocytes are more consistent and more profound in kwashiorkor than in marasmus.7 However, skin reactions testing DTH responses are similar in patients with kwashiorkor and marasmus. Apparently it is the efferent, or inflammatory, loop of the response that fails. When PEM patients are exposed to sensitizing antigens, they fail to respond. They do, however, show a positive response to antigen challenge after nutritional rehabilitation, indicating that the afferent loop was intact during sensitization.24

There is other evidence that the inflammatory response fails in severe malnutrition. The phagocytic cells, usually the first to appear when an inflammatory stimulus is applied, may be defective in PEM and fail to respond to humoral signals.25-27

B Lymphocytes

Most studies of human B cell function in PEM have employed vaccine probes to induce antibody.14,28 The
ignition of particles are defective in PMNLs of patients with PEM. More extensive observations clearly show that internalization of opsonized bacteria or latex particles is normal.  

In PEM patients, if the PMNLs can reach the site of infection, and opsonization is normal, phagocytosis is expected to proceed efficiently. On the other hand, there is no apparent defect in fusion and lysis of lysosomes within the phagocytic vacuole in the phagocytes of patients with PEM. In vitro examination of these cells reveals a significant decrease in iodination of bacteria by heme-peroxidase-halide bactericidal system.  

In vitro: investigation of normal microbicidal activity of these cells is consistent with the metabolic assays and indicates a decrease in the killing of bacteria.  

Increase that is usually moderate and unlikely to be significant by itself.

**Nuclear Phagocytes**

There is a defect in the mobilization of monocytes in PEM patients. This defect may be due to abnormalities of T lymphocytes, either in production or function of lymphokines with adhesion or immobilizing (MIF) properties directed at monocytes. Studies in animals and humans suggest that deficiency in humoral mediators of inflammatory response may be more important than depression of mononuclear cells to any intrinsic abnormalities of the cellsanova.

Experimental data on in vivo phagocytosis in protein-deficient monkeys and rodents have indicated a defect in uptake of colloidal carbon and of *Escherichia coli* by the fixed (tissue) mononuclear cells.  

However, more recent studies in mice, which confirmed the depression of phagocytosis by the mononuclear cells in protein-deficient animals, have demonstrated that this decrease was proportional to the size of liver and spleen. These data indicate that there is a decrease in the total of phagocytic cells in the animal, but that each phagocytic unit is functioning normally.


deficiencies of various single nutrients affect function of macrophages in experimental animals. Guinea pigs deficient in vitamin C show a decrease in function of macrophages into the peritoneal cavity or as of experimental pulmonary silicosis. Mice with liver deficiency experience an inhibition of growth of Salmonella. Other experiments indicate continued on page 1012...

**EFFECTS OF MALNUTRITION ON HUMORAL IMMUNITY**

Serum immunoglobulin levels are normal or increased and the number of circulating B cells is normal in patients with PEM. In vitro: In addition, circulating plasma cells are normal and the plasma cells — rare findings in normal humans — are found in peripheral circulation.  

It appears that there is activation of the B lymphocyte system, presumably due to constant antigenic stimulation related to the high frequency of infection.  

In spite of this state of B cell activation and adequate serum immunoglobulin concentration, the antibody response to bacterial vaccines is often subnormal especially in the malnourished patients who are also infected at the time of immunization. The antibody response can be increased by the addition of protein in the diet at the time of immunization, although the mechanism governing this effect is unclear.  

Response to viral vaccines is usually intact, even in severely malnourished children.  

Experimental studies in protein-malnourished mice give clues to the mechanisms underlying variability in antibody responses to malnourished children. The normal response by the B cells requiring triggering by T cells is absent, because the latter cells are deficient.  

Antigens not requiring triggering of B cells by T cells evoke normal responses.

**EFFECTS OF MALNUTRITION ON PHAGOCYTIC CELLS**

**Polymorphonuclear Neutrophilic Leukocytes (PMNL)**

The reported observations are quite variable (Table 4). Rebuffa window studies in patients with PEM have demonstrated a delay in mobilization of monocytes, and a concomitant increase in migration of the PMNLs several hours after abrasion of the skin.  

When the early PMNL response has been studied in a Boyd chamber there was depression during the first hour of incubation, but subsequently the responses approximated those of control cells. The release of PMNLs from bone marrow also may be impaired in PEM.
that minerals may exert some regulatory control at the cell surface, but it is uncertain what importance these effects may have in vivo.²⁻⁵³

EFFECTS OF MALNUTRITION ON COMPLEMENT

Complement proteins appear to be rapidly turned over under normal circumstances.²⁵ In the course of inflammation, concentration of C3 rises in plasma as complement is being consumed.⁵⁷ This activity requires an anabolic response to the stress.

There is a decrease of concentration of all the components except C4, of the classical pathway of complement activation in PEM and a significant impairment of hemolytic classical pathway complement activity (CH₅₀) as measured with sensitized sheep erythrocytes.¹⁰⁻¹⁴⁻¹⁷⁻⁶⁰ We have recently observed that levels of functional C₄ hemolytic activity are unaltered in acute childhood PEM as the CH₅₀ is reduced to 37% of control values.⁶¹ We have also noted that factor P and hemolytic complement activity(APH₅₀) of the alternative pathway are reduced. In fact, the defect in APH₅₀ activity (16% of control) was both more profound and more frequently observed in our patients than was the depression in CH₅₀. We found no increase in the regulatory proteins of the complement system, C1 inhibitor or beta-1H, to account for the depressed hemolytic activity. The defect is therefore likely to be a consequence of the decreased levels of the individual C components, as we recently reported.⁶²

Depression of complement components and activity in acute PEM appears to be a result of consumption of complement,²⁵⁶⁰ which can be initiated by endotoxin.⁶¹⁶⁴ Recovery of complement levels and function in acute PEM requires more than two weeks of optimal nutritional rehabilitation.⁵⁹ During this period these patients are vulnerable to gram-negative septicemia, which is often lethal. There is no available information about the effects of deficiencies of single nutrients on the complement system.

EFFECTS OF DEFICIENCY OF SPECIFIC NUTRIENTS

Animal experimentation has revealed that deficiencies of minerals and vitamins can affect function of lymphocytes.⁴

Zinc Patients with acrodermatitis enteropathica, which is characterized by zinc malabsorption, and those who were inadvertently made deficient in zinc during parenteral alimentation with zinc-free formulas have thymic atrophy, a decrease in circulating thymic hormone levels and in certain T cell populations, resembling the observed deficiencies in PEM.¹⁴ Supplementation with oral zinc of PEM patients results in a rapid increase in the size of thymus and an increased delayed type hypersensitivity response of the skin.¹³

Patients with Down's syndrome, which is often accompanied by zinc deficiency, also have a reduced number of circulating T cells, lower levels of thymic hormone, decreased DTH skin test responses and corresponding decrease in PHA induced proliferation of lymphocytes in vitro. These abnormalities can be reversed by supplementation with zinc.³⁴

In Danish cattle, a genetic lethal trait, A 46, is analogous to acrodermatitis enteropathica. The affected animals show depletion of cortical thymic lymphocytes, hypoplasia of the spleen, lymph nodes and Peyer's patches, depressed DTH reactivity in the skin tests and decreased humoral responses to tetanus toxoid. These changes are reversed by dietary zinc supplementation.⁶⁵ Experimentally induced zinc deficiency in rodents leads to identical abnormalities.⁶⁶ Other studies in experimental animals also support these observations.

Iron In addition to its critical role in oxygen transport by hemoglobin, iron is an essential component of many enzymes and metabolic cofactors, among which are cytochrome c, cytochrome c reductase, succinic dehydrogenase, peroxidase and xanthine oxidase. Although the data are conflicting¹⁰⁻¹ⁱ and not as clear-cut as they are with respect to zinc deficiency, there is sufficient evidence to warrant a strong suspicion that deficiency of iron is also immunosuppressive.¹²⁻¹⁴ Iron supplementation apparently can reverse the abnormalities.¹⁵

Vitamin A There are relatively few reports relating to the effects of vitamin A deficiency on the immune system of man. An early study of tuberculin skin reactions in malnourished children reported a significant difference between negative skin tests and biochemical evidence of vitamin A deprivation.⁷⁶ In a later study, Indian children with normal anthropometric indices, but deficient in vitamin A exhibited a significant decrease in the percentage of peripheral T lymphocytes and a poor mitogenic response to PHA.⁷³ Because of methodological flaws this study can be questioned, but the results are consistent with observations in experimentally deprived Holtzman rats, which show atrophy of thymus and spleen compared to pair-fed, vitamin A supplemented controls.⁷⁷ In Lewis rats, vitamin A deficiency did not result in deficiency of lymphocytes, but did cause functional abnormalities in splenic cells, which was reversible by administration of vitamin A.⁷⁸ Dietary supplementation with vitamin A or retinoids appears to enhance lymphocyte functions in various experimental animals.⁷⁹⁻⁸⁷ In a recent study of children in Bangladesh, however, no effect of vitamin A palmitate was noted on antibody response to unadjuvanted tetanus toxoid and
upon the DTH reaction to PPD and *Candida* antigen. The primary or secondary response to tetanus was not altered with 200,000 IU, nor was DTH affected. The same toxoid preparation given to mice along with 3,000 to 15,000 IU of vitamin A significantly increased antibody response.

The discrepancy between the results in the human and animal studies may be attributable to the high dose of vitamin A relative to body weight in the mice. Such a high dose would be toxic in man. There may be other differences related to the species as well.

**Pyridoxine** Experimentally induced deficiency of pyridoxine in animals alters lymphocyte function and decreases circulating levels of thymic hormone.

In man, diet deficient in pyridoxine decreases antibody response to tetanus toxoid and typhoid vaccine. Only a few patients have been studied, however, and the data are insufficient to draw general conclusions.

**SUMMARY**

Deficiencies of protein, energy, and specific vitamins and minerals impair function of various components of the immunological network and therefore weaken host defense. These various deficiencies may affect specific loci within the network, but because of the interrelations between the different systems, the ultimate effect may be quite broad. Patients with PEM often suffer infections, which may also impair the immune function. It is difficult to separate effects of malnutrition from those of infection, a fact recognized in the recent coinage of the term malnutrition-infection complex.

On the basis of the available information it appears that PEM and associated deficiencies of nutrients cause a major impairment in the T lymphocytes and the complement system. They have relatively less effect on the B lymphocytes and the phagocytes directly; however, normal B cell activity is dependent on regulatory cytokines generated from the activation of complement. Therefore these cells also are functionally defective in PEM. The consequence of these deficiencies of function of the immune system in the malnourished host is heightened susceptibility to and a less vigorous response to infections. These defects can be generally reversed by nutritional rehabilitation.

**REFERENCES**


27. Schopler K, Douglas SD: Neutrophil function in children with kwashiorkor.