MALNUTRITION AND IMMUNOLOGICAL RESPONSE

Malnutrition and Immunocompetence: An Overview

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To a man with an empty stomach, food is God.

Mahatma Gandhi (1862–1948)

The association between famine and pestilence has been recorded in ancient history. Puranic literature and biblical references cite observations that suggest an increase in the prevalence and severity of infection among starved individuals and populations. Besides these historic observations, several types of evidence may be marshalled to support causal links between malnutrition, impaired immunity, and infectious illness.

EVIDENCE FOR CAUSAL INTERACTION BETWEEN MALNUTRITION, IMPAIRED IMMUNITY, AND INFECTION

Epidemiologic studies document increased mortality and morbidity in infants and young children with protein-energy malnutrition (PEM). In rural India, the probability of death among young children increases progressively with deterioration in nutritional status (Table 1). Most of the deaths are due to infectious illness and diarrheal illness. The attack rate as well as duration of diarrhea are increased in the malnourished (Table 2). The severity of infectious diseases is also increased in PEM. The most cited examples are measles and herpes simplex virus. There is, however, an unexplained difference in the natural history of measles in PEM in West Africa and Eastern India. There also is an impression among clinicians working in developing countries that common pyogenic infections are more common and severe in malnourished children. The prevalence of PEM among children with lymphoreticular malignancy complicated with Pneumocystis carinii infection is higher than in those without such infection (Table 3). Similar data are available for adults undergoing surgery in North American hospitals (Table 4). Finally, the pattern of organisms isolated from malnourished children resembles the findings in

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TABLE 1. Risk of death by nutritional status in infants in rural India*

<table>
<thead>
<tr>
<th>Weight-for-height (%) standard</th>
<th>Mortality rate (%)</th>
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</thead>
<tbody>
<tr>
<td>&gt; 80</td>
<td>0.45</td>
</tr>
<tr>
<td>71-80</td>
<td>2.16</td>
</tr>
<tr>
<td>61-70</td>
<td>9.87</td>
</tr>
<tr>
<td>&lt; 60</td>
<td>18.14</td>
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*Infants were aged 1 to 23 months.

patients with primary defects of immunity (Table 5), thereby pointing to the possible link between PEM, impaired immunocompetence, and infection.

Confounding Variables

Many studies conducted in the last 15 years have addressed the question of immunocompetence in PEM. Host defence is a composite protective umbrella consisting of antigen-specific mechanisms (Fig. 1) (immunoglobulin-antibody system, cell-mediated immunity) and nonspecific mechanisms (skin, mucous membranes, cilia, mucus, interferon, macrophages and microphages, complement system, etc.). Defect in one facet, e.g., phagocytes, as seen in inherited chronic granulomatous disease, may produce serious life-threatening disease, whereas in another instance, e.g., selective IgA deficiency, it may be asymptomatic. PEM produces many rents in the protective umbrella; even though a single defect may not be sufficient to increase susceptibility to disease, each deficit probably has an additive, even synergistic, effect.

At the same time, it must be pointed out that there are many confounding variables that impinge on the nutrition-immunity-infection nexis. Some of these are highlighted here.

1. Coexistent but unrecognized deficiencies of other nutrients are frequently present in PEM. Each of these nutrients may exert an important influence on immune response.

TABLE 2. Diarrhea morbidity

<table>
<thead>
<tr>
<th>Weight-for-height (%) standard</th>
<th>Episodes of diarrhea per 12 months</th>
<th>Duration of episode (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 80</td>
<td>3.0 ± 0.4*</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>&lt; 80</td>
<td>6.3 ± 0.8</td>
<td>8.9 ± 1.1</td>
</tr>
</tbody>
</table>

* Data are shown as mean ± S.E.
FIG. 1. Host protective factors and examples of primary and secondary immunodeficiency. A concert of nonspecific barriers and antigen-specific immune responses protects man from extraneous and internal injurious agents (left panel). Primary, often inherited, deficit of a protective mechanism, for example phagocyte defect, results in repeated severe infections (center panel). Malnutrition robs the host of many host defenses (right panel). Some bulwarks of immunity are impaired more often and to a greater extent than others. (From ref. 25.)
TABLE 3. Pneumocystis carinii infection in acute lymphoblastic leukemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum albumin (g/dl)</th>
<th>Serum transferrin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>2.7 ± 0.4*</td>
<td>121 ± 29</td>
</tr>
<tr>
<td>Noninfected</td>
<td>3.4 ± 0.3</td>
<td>198 ± 23</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.E.

2. Concurrent or recent infections may suppress immunocompetence and further increase nutritional deficiency. An example is measles which is associated with cutaneous anergy for several weeks, impaired lymphocyte response to mitogens such as phytohemagglutinin (Fig. 2), normal or slightly reduced proportion of rosette-forming T cells, and frequent reactivation of latent infections such as tuberculosis.

3. Our knowledge of the threshold of severity of immunologic dysfunction which is clinically relevant is limited. How much reduction in T cells is biologically important? We do know that complement C3 concentration must fall to less than 40% of control values before it impairs opsonization. On the other hand, a coexistent deficit in antibody response may compound the immunologic problem; now, even a milder impairment of complement system may affect opsonization.

4. Different causes of nutrient deficiency may have different effects on immunity. For example, iron deficiency due to blood loss has relatively little effect on immunocompetence, whereas iron deficiency due to reduced dietary intake does.

5. The many variables of the study design should be similar before comparisons can be valid. This includes the nature and dose of antigen, duration of culture, etc.

6. Results obtained in experiments in vitro may have little relevance to findings in vivo.

7. The extrapolation of results of studies from one species to another is fraught with danger.

TABLE 4. Postoperative complications

<table>
<thead>
<tr>
<th>Preoperative nutritional status</th>
<th>Sepsis (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Malnourished</td>
<td>6.8</td>
<td>2.3</td>
</tr>
</tbody>
</table>
TABLE 5. Organisms commonly isolated from patients with PEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Klebsiella, Pseudomonas, Proteus, Staphylococcus, Mycobacterium</td>
</tr>
<tr>
<td>Viruses</td>
<td>Measles, herpes simplex</td>
</tr>
<tr>
<td>Fungi</td>
<td>Candida, Aspergillus</td>
</tr>
<tr>
<td>Parasites</td>
<td>Pneumocystis carinii, Giardia lamblia</td>
</tr>
</tbody>
</table>

LYMPHOID TISSUES

The association of starvation and profound involution of the thymus to the extent that it may be difficult to locate the organ was described well over a century ago. This led to the term "nutritional thymectomy," a finding dramatically observed in animal models of PEM (8). The thymus of undernourished children is usually small on X-ray examination. In patients with kwashiorkor or marasmus, the thymus, on average, weighs less than one-third of the normal organ. Histologic studies of PEM have shown a significant lymphocyte depletion of lymphoid organs (Figs. 3,4). In the thymus, the clear distinction between the densely packed cortex and sparse medulla is ill-defined and Hassal corpuscles appear degenerated, enlarged, and crowded together. This appearance is distinct from that of the thymus in primary defects of the immune system in which the epithelial components also may be absent or less prominent. The histomorphologic changes in the thymus are most marked in those patients who show lymphopenia before death. In the spleen, the periarteriolar cuff of lymphocytes is less prominent. In the lymph node, germinal centers are scanty and small. There is a reduction in the number of small lymphocytes as well as plasma cells.

IMMUNOCOMPETENCE

A number of immunity functions are deranged in PEM (22,30,39,60). The pattern of infections encountered in patients (52) and the marked histologic changes in the thymus in PEM (58) suggest that cell-mediated immunity (CMI) is impaired. This is a consistent observation and is reflected in changes in skin reactions, lymphocyte subpopulations, and in vitro responses. Other immune responses altered in PEM include antibody affinity, complement system, mucosal secretory antibody, phagocytes, and lysozyme (Fig. 1).

DELAYED HYPERSENSITIVITY

Cutaneous tests using ubiquitous recall antigens, such as candidin, trichophytin, streptokinase-streptodornase, mumps, and purified protein derivative of tuberculin, evaluate the memory response dependent on T lymphocytes and
inflammatory cells and are generally depressed in patients with protein-energy malnutrition (7,23). A direct correlation between the size of induration and concentrations of serum albumin and transferrin has been reported. Skin responses may be impaired in mild to moderate undernutrition. Many studies have also demonstrated marked impairment of the primary afferent or sensitization limb of delayed hypersensitivity, using agents such as keyhole limpet hemocyanin, BCG vaccine, and 2,4 dinitrochlorobenzene (9,45,50,57). Tuberculin conversion after BCG vaccination is impaired and the extent of defect correlates with nutritional status (Table 6).

LYMPHOCYTE SUBPOPULATIONS AND PROLIFERATION RESPONSES

Lymphopenia is seen in approximately 15% of malnourished children; it may be mild or profound. PEM is associated with a reduction in the proportion and absolute number of circulating thymus-dependent T lymphocytes identified by their ability to form rosettes with sheep red blood cells (Fig. 5; refs. 2,11,19,51). Severe malnutrition generally produces a more marked reduction in T-cell number. The mechanism(s) underlying this observation are not clear. Changes in surface membrane proteins including the putative receptor(s) for
FIG. 3. Thymus in malnutrition. There is a loss of corticomedullary differentiation, fewer lymphoid cells, and degeneration of Hassal bodies. (×25.) (From ref. 39.)

FIG. 4. Lymph node in malnutrition. The germinal follicles are inconspicuous and small. (×25.) (From ref. 39.)
TABLE 6. Tuberculin conversion 3 months after BCG vaccination

<table>
<thead>
<tr>
<th>Nutritional status at time of vaccination</th>
<th>Conversion rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>82</td>
</tr>
<tr>
<td>Mild PEM</td>
<td>68</td>
</tr>
<tr>
<td>Moderate PEM</td>
<td>54</td>
</tr>
<tr>
<td>Severe PEM</td>
<td>32</td>
</tr>
</tbody>
</table>

sheep red blood cells or the presence of serum inhibitors (3), such as C-reactive protein, IgE, microbial products, and alpha-fetoprotein, can prevent rosette formation with sheep red blood cells, lymphpothy due to elevated levels of free cortisol, impaired maturation and differentiation of T-cell precursors, reduction in pre-T cells due to impaired cellular multiplication, and a redistribution of cells to sequestered sites.

Various subsets of T lymphocytes can be distinguished on the basis of cell surface antigens using monoclonal antibodies. These are functionally heterogeneous as well. Our recent studies using monoclonal antibodies and cell sorting technique have demonstrated changes in T-cell subsets in PEM. There is a marked numerical and functional deficiency of T4+ helper cells, whereas T8+ cytotoxic/suppressor cells are affected to a lesser extent (37). These changes in functionally distinct T-lymphocyte subpopulations may explain the alterations in CMI in children with PEM.

The proportion and absolute number of B lymphocytes are similar in well nourished and PEM subjects (2,19), although the latter have a slightly higher
proportion of \( B_\alpha \) cells (21). Thus, the proportion of "null" cells without the conventional surface markers of T and B lymphocytes is markedly increased. The possibility that these cells may be undifferentiated T lymphocytes is suggested by a significant decrease in serum thymic hormone activity in infants and young children with PEM (Fig. 6; ref. 20) and an elevation in leukocyte terminal deoxynucleotidyl transferase (21). The addition of calf thymic extract in vitro to mononuclear cell preparations from undernourished individuals results in an increase in the number of rosetting cells.

Lymphocytes obtained from children with PEM respond poorly to mitogens, particularly when cell cultures are set up in autologous sera (3). Plasma of malnourished, often infected, patients contains inhibitory factors and/or may also lack essential supporting factors for cell proliferation. It is essential to use standard methods and plot dose-response curves since optimal concentrations of mitogen to produce maximum stimulation may vary for different cell preparations. The reduction in lymphocyte transformation response in malnutrition correlates with a decrease in the number of rosetting T lymphocytes.

**IMMUNOGLOBULIN-ANTIBODY SYSTEM**

Hypergammaglobulinemia is a common finding in malnutrition. This may be the result of frequent associated infections as well as reduction in T-suppressor-cell activity. The concentrations of serum immunoglobulins are generally elevated or within the normal range in PEM (22). Associated bacterial and viral infections and parasitic diseases are important determinants of changes in serum immunoglobulin levels, particularly elevated IgA. Plasma half-life of IgG is reduced in these patients (22). In the occasional malnourished child with concomitant or recent infection, serum IgG, and sometimes IgM
Malnutrition and Immunological Response

**FIG. 7.** IgG antibody-forming cells in the spleen of the F₁ generation progeny of starved (closed or hatched columns) and control (open columns) rats. Three-week-old rats were subjected to partial starvation for 6 weeks. One batch from each experimental and control group was immunized and the animals were killed after 4, 6, 10, or 14 days. Another batch of female rats from the starved and control groups was mated with healthy male animals. On weaning, the litter (F₁ generation) was given free access to food for 6 weeks. These animals were immunized with SRBC and studied for number of direct (IgM) (A) and indirect (IgG) (B) plaque-forming cells (PFC) in the spleen. Mean and SE are shown, based on data from ref. 14. Copyright 1975, American Association for Advancement of Science.

and IgA, may be low (9). In this situation, plasma half-life of IgG is prolonged (20). Serum IgE concentration is often elevated as a probable result of parasite infection and reduced T-suppressor-cell number and function.
Antibody response is regulated by a number of factors, including the number and function of B and T lymphocytes and macrophages, the dose and nature of the antigen, presence of adjuvant, previous exposure, presence of associated infection, and state of the local lymphoid tissue. Serum antibody response is generally adequate in PEM (9,30,50). Some T-cell-dependent antigens may not induce antibody response to the same extent in PEM as in the healthy state. For example, in nutritionally deprived rats and their offspring, spleen antibody-forming cell response to immunization with sheep red blood cells is reduced (Figs. 7A and 7B; ref. 14).

**Secretory IgA**

The concentration of secretory IgA and mucosal antibody responses are decreased in moderate to severe PEM (12,53,59). Nasopharyngeal and salivary secretions and tears of malnourished individuals show a reduction in secretory IgA relative to total protein and albumin content. Secretory IgA-antibody response to live attenuated measles and poliovirus vaccines is reduced in malnourished children (12). Antibody is detected less frequently, the time of its first appearance is delayed, the maximum level is significantly lower (Fig. 8). The impairment of the secretory immune system may have several possible consequences. It may contribute to more prolonged and severe illness. It may also permit systemic spread, explaining, in part, the frequent occurrence of gram-negative septicemia in malnutrition. The absorption of macromolecules is increased (13), the clinical significance of which is not clear.

![FIG. 8. Secretory IgA-antibody response to live attenuated poliovirus vaccine (arrow) in malnourished (closed circle) and control (open circle) children. (From ref. 12.)](image-url)
Antibody Affinity

The effect of diet on antibody affinity has been investigated in genetically inbred high- and low-affinity mice (54). Protein deficiency decreased the relative affinity of antibody to human serum albumin in animals normally producing high-affinity antibody. Affinity was not further reduced by protein deprivation of the low-affinity mice.

Complement System

Serum concentrations of almost all complement components are reduced in PEM (18,44). The levels of C4, C5, and C1 inactivator are often within the normal range. Acute starvation and anorexia nervosa also depress C3 levels. In moderate degrees of energy-protein undernutrition, levels of complement component C3 correlate with the extent of weight deficit and biochemical indices of malnutrition as well as with days of febrile illness. Samples from some patients show anticomplementary activity which may be the result of the action of endotoxin, other microbial products, or antigen-antibody complexes. Infection superimposed on malnutrition further depresses the complement system, in contrast to the findings in well nourished subjects in whom complement levels rise in the presence of infection. Degradation products of C3 indicative of consumption are often detected. This, together with reduced hepatic synthesis, may be responsible for the marked reduction in serum C3 concentration seen in nutritional deficiency. The alternate pathway is also altered in PEM. The total activity as well as levels of factor B are decreased (20,44).

Polymorphonuclear Leukocytes

Malnourished children show a slight delay and reduction in the mobilization of monocytes in traumatic Re buck skin windows and in response to BCG (6). Chemotaxis of polymorphonuclear leucocytes (PMN) initially may be slightly sluggish in vitro but, given enough time, the number of cells traversing the barrier are comparable in well nourished and PEM groups, unless infection is present (29). The cellular component of ingestion of opsonized material is adequate, but PMN show several deficits in postphagocytic metabolic burst of oxidative and glycolytic activity. The intracellular killing of ingested bacteria is variably reduced (55,56).

Other Nonspecific Mechanisms

Lysozyme is decreased in the serum and secretions of children with PEM (20,59). Endogenous pyrogen production is decreased. Data on interferon production are inconsistent; some studies have shown a reduction in interferon
release from virus-infected leukocytes of PEM patients, others have found a normal response. It is probable that differences in the subjects studied and assay techniques may be important determinants of the different results obtained. Tissue changes may contribute to inadequate physical barriers to the entry of pathogens and may allow easier spread of infection in patients with PEM. These and other nonspecific mechanisms of host defense have been reviewed (20,30,39).

FETAL MALNUTRITION

In developing countries, small-for-gestational-age (SGA) low-birth-weight infants constitute 9 to 30% of all neonates. Infection among these infants is a common cause of morbidity and mortality. Malnutrition occurring during fetal life may be expected to have a more profound and longer-lasting effect on the immune system than acquired postnatal malnutrition. Many studies have indicated that fetal growth retardation is associated with involution of the thymus and impaired CMI (15,33,49). The number of circulating T cells is reduced (Fig. 9) and lymphocyte transformation response to mitogens is decreased. Serum levels of IgG, especially IgG_1_ and IgG_3_, and antibody titers are lower as a result of decreased placental transfer of immunoglobulins (Fig. 10; ref. 16). Serum concentrations of C3 are decreased and opsonization is suboptimal. The levels of factor B are reduced and correlate with opsonic activity (33). The depression of cell-mediated immunocompetence in SGA low-birth-weight infants has been shown to persist for several months to years (24,31,41,42). In contrast, appropriate-for-gestational-age (AGA) infants of comparable weight recovered their CMI function by the age of 3 months (24). The profound effect of fetal growth retardation on thymic hormone activity

![FIG. 9. Rosette-forming T lymphocytes in healthy, preterm appropriate-for-gestational-age, and small-for-gestational-age (SGA) infants. Values shown are means ± SD. (From ref. 24.)](image-url)
has been reported recently (24). One-month-old SGA infants show a marked decrease in thymic hormone activity, whereas AGA infants of comparable birth weight have titers that are near normal. These differences in thymic hormone activity in the two groups of low-birth-weight infants appear to provide a prognostic marker, since SGA infants with low thymic hormone activity continue to demonstrate depressed CMI, whereas AGA infants with near normal thymic hormone activity recover immunologically by the age of 3 months. These effects may have clinical and biological significance, particularly frequency, severity, and duration of infection.

DEFICIENCIES OF SINGLE NUTRIENTS

The effects of the deficiencies of individual nutrients have been the subject of much recent work and have been reviewed elsewhere (4,22,27,28,38).

Zinc Deficiency

Zinc deficiency is associated with marked changes in CMI. The lymphoid tissues are atrophic with preferential cellular depletion of the cortex of the thymus and thymus-dependent areas in other organs such as the spleen. Lymphopenia may be observed in a proportion of zinc-deficient subjects. Both primary and secondary antibody responses to T-dependent antigens are reduced (34,43). Recent work indicates that B-cell response to a T-cell-independent antigen, dextran, is also impaired. Delayed cutaneous hypersensitivity is impaired. Zinc-deficient patients and laboratory animals have poor lymphocyte proliferation response to mitogens, and wound healing is impaired.
Mice deprived of zinc for a few weeks show reduction in T-cell cytotoxicity against sensitizing tumor cell targets, particularly \textit{in vivo}, whereas antibody-dependent cell-mediated cytotoxicity is not altered. Recently, Bach (1) has presented evidence for zinc dependency of thymic hormones. Zinc deficiency is associated with decreased levels of thymic hormone (35). The serum thymic factor loses its biological activity after treatment with a metal-chelating agent and the function is restored by the addition of zinc. Many mechanisms may explain the interactions between zinc and the immune system. Zinc is essential for the activity of more than 100 metalloenzymes including thymidine kinase and DNA-dependent RNA polymerase. Zinc stabilizes cell membrane acting at the cytoskeletal level. It can also act as a polyclonal lymphocyte mitogen.

\textbf{Iron Deficiency}

Iron deficiency is prevalent world-wide in all age groups, including infants especially of low birth weight, women in the child-bearing age, elderly and socioeconomically disadvantaged persons. It is important to recognize that iron deficiency can affect multiple systems even prior to a drop in hemoglobin concentration. There are subtle but definite changes in cell-mediated immune responses including T-cell number, \textit{in vitro} lymphocyte proliferation in the presence of mitogens, lymphokine production, and reduced bactericidal capacity of PMN (5,10,17,28,32,46,48). Iron deficiency results in reduced activity of ribonucleotide reductase necessary for DNA synthesis and of myeloperoxidase and hydroxyl radical production. Iron also interacts with many other trace elements which may have important effects on the immune system.

\textbf{Copper Deficiency}

Copper deficiency reduces antibody response to T-cell-dependent antigens, produces neutropenia, and impairs reticuloendothelial function. There is a reduction in T-cell cytotoxicity and in concanavalin A stimulation of lymphocytes. It should be emphasized that there are marked differences between various species with regard to the effects of copper deficiency; thus it is very hazardous to extrapolate such laboratory data to man.

\textbf{Selenium Deficiency}

Selenium has important interactions with vitamin E. Deficiency in both these nutrients reduces antibody responses and changes in hydrogen peroxide levels.

\textbf{Magnesium Deficiency}

Magnesium deficiency in rats results in thymic atrophy in some animals, and thymic hyperplasia and lymphoid malignancy in others.
Vitamin A Deficiency

Vitamin A deficiency reduces lymphocyte proliferation response to mitogens and cell homing patterns, and has significant effects on the complement system.

Pyridoxine Deficiency

Pyridoxine deficiency reduces antibody responses to heterologous red cells, impairs cytotoxicity, decreases serum thymic hormone levels, and impairs lymphocyte proliferation (35,36).

Folic Acid Deficiency

Folic acid deficiency decreases lymphocyte proliferation response to mitogens (40).

Vitamin C Deficiency

Extreme deficiency of vitamin C can impair complement activity and phagocyte function.

FIG. 11. Growth, morbidity, and immunocompetence assessed periodically in a prospective longitudinal study of an infant. Impaired immune responses were noted earlier than growth failure and preceded clinical evidence of respiratory infection and diarrhea. (From ref. 26.)
Polyunsaturated Fatty Acids

Animals deprived of polyunsaturated fatty acids show reduced antibody responses, both primary and secondary, to several antigens. Cell-mediated immune responses are decreased.

FINAL COMMENTS

Malnutrition and deficits of single nutrients are the most frequent cause of immunodeficiency world-wide (60). The main effects of nutritional imbalance, deficiency and excess, on the immune system have been reviewed above. The influence of moderate PEM on immunocompetence (25,47,61), the rapidity with which immunologic tests show improvement following nutritional therapy of PEM (30), and findings from prospective longitudinal assessment of immunocompetence, growth, and morbidity have led to the suggestion that immunocompetence can be used as a functional index of nutritional status (Fig. 11; ref. 26). These observations should form the basis of multifaceted programs to reduce the intertwined problems of malnutrition and infection (22). It would be desirable to tackle both infection and undernutrition simultaneously.

REFERENCES


