The Effect of Protein-Energy Malnutrition on Immune Competence

Bill Woodward

Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

The most common relationship between protein-energy malnutrition and infection is the synergism expressed in the concept of the malnutrition-infection cycle. Recently, this important concept has been developed further by an analysis showing that primary protein-energy malnutrition increases the risk of childhood mortality from infection in a potent multiplicative manner and with no threshold effect (1). The results pertaining to wasting disease were particularly clear cut, and the impact of all degrees of wasting was revealed to be greater than previously realized. A qualitatively similar impact of the stunting form of protein-energy malnutrition was also apparent (1).

Immunodepression is accepted as a determining factor in the infection-related morbidity and mortality of childhood protein-energy malnutrition. In an extension of this concept, immune competence may be a critical but under-recognized factor in the management of malnutrition, at least where primary malnutrition in childhood is concerned (2). Nutritional deficits typically depress diverse immunologic barriers simultaneously. This is fundamental to the impact of protein-energy malnutrition on a multitiered and tightly integrated physiologic system such as the immune system. Data on stunting malnutrition and immune defenses are scant but suggestive (3), whereas information relating wasting malnutrition to immunocompetence is abundant, although skewed toward grade 3 disease. Therefore, in this chapter the term protein-energy malnutrition, when used without qualification, refers to the acute forms of malnutrition.

The focus here is on the impact of protein-energy malnutrition on the physiologically labile immune system of the prepubescent individual. For this purpose, it is assumed that extrapolation from the study of adults requires caution, particularly where an immunologic impact of protein-energy malnutrition is unapparent in the adult. Recent progress of a descriptive nature includes aspects of both innate and adaptive de-
fenses. It is important that our catalogue of descriptive information should continue to grow, but the goal is mechanistic knowledge that will permit rational, targeted interventions and dietary recommendations. Published reports on experimental animals are an invaluable source of insight for this purpose, and an objective here is to show the need to improve the relevance of animal systems to childhood protein-energy malnutrition in its various forms. Finally, the thesis is advanced that the physiologic (including immunologic) response to deficiency of dietary protein and energy is governed by endocrine-mediated metabolic priorities rather than by nonselective distribution of amino acids and energy simply in proportion to level of intake.

INNATE DEFENSES

Physical Barriers

Studies of rodents, albeit young adults, subjected to wasting protein deficiency provide formal evidence in support of the clinical observation that protein-energy malnutrition increases the risk of bacterial translocation (i.e., gut origin septicemia) (4,5). Several characteristics of the gastrointestinal barrier in protein-energy malnutrition are likely contributors to this predisposition. Overgrowth of intestinal microorganisms including Gram-negative organisms is a consistent feature of malnutrition in childhood and in experimental animals (4,6). Stomach acid exerts control over enteric microbial ecology, and hypochlorhydria may be partly responsible for the disruption of the microfloral barrier in the malnourished state (6). In addition, the intestinal mucosa shows atrophy and disruption in childhood and experimental protein-energy malnutrition (4,6), and a particularly important aspect of this phenomenon appears to relate to the integrity of the mucus barrier (5). Early histologic impressions of reduced production of intestinal mucus have been supported more recently by quantitative chemical assays of material from rats, both adolescents subjected to severe stunting (7) and young adults subjected to wasting protein deficiency (5). The water-insoluble layer adherent to the epithelial surface was reported to be the component of intestinal mucus affected (5). Moreover, a malnutrition-associated reduction in the ability of this mucus fraction to bind coliform organisms in vitro was apparent (5). This outcome was interpreted as a protective adaptation because it correlated with a reduction in the numbers of mucosa-associated bacteria in the ileum and cecum (5). However, the distribution of bacteria between the mucus layer and the epithelial glycocalyx was not determined, nor was the impact of malnutrition on the adherence characteristics of the intestinal microflora evaluated. Consequently, the opposite interpretation is equally plausible—that is, that protein deficiency in this experimental system reduced the protective blocking action of intestinal mucus (both through a reduction in quantity and through an influence on chemical composition), thereby inducing a predisposition to bacterial translocation. The impact of protein-energy malnutrition on the quantity, composition, and function of epithelial mucus deserves systematic investigation.

Further to the subject of mucous secretions in protein-energy malnutrition, the quantity of saliva (rate of stimulated flow) was found to be reduced in childhood
malnutrition, and its chemical composition was altered in a manner consistent with reduced antibacterial activity and a high risk of oral infections, including dental caries (8). These phenomena were identified in both wasting and stunting disease in their severe or even more moderate forms, and complementary findings are reported regarding the bacteria agglutinating glycoproteins of saliva in protein-deficient young adult rats (9). In another investigation, nasopharyngeal and buccal epithelial cells of marasmic children were reported to show an increased propensity to bind coliform bacteria (10). As the latter study involved washed epithelial cells, the results may reflect an impact of protein-energy malnutrition on expression of epithelial membrane glycoproteins rather than an influence on the chemistry or quantity of epithelial mucus.

The Professional Phagocytes: Cell Numbers

Studies on the neutrophil in protein-energy malnutrition are among the earliest contributions to the field of nutritional immunology. The blood neutrophil count is little affected by clinical or experimental malnutrition per se (11–13). Infection in malnourished individuals, however, produces neutropenia that has been attributed to involution of the bone marrow neutrophil pool on the indirect basis of the response of the blood neutrophil count to an injection of endotoxin (14). Recent results from a study of adult outbred mice subjected to wasting protein deficiency and sterile inflammation are instructive in this regard (12). Direct counts of femoral marrow cells revealed a modest decline in the size of the mature neutrophil pool, but inflammation-associated neutropenia in this experimental system appeared to result primarily from depressed mobilization of cells from this pool. It is fascinating that terminal differentiation was implicated in the depression of the neutrophil pool size in this experimental system, whereas early myeloid cell proliferation appeared substantially intact (12). In this connection, blood levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) in children with overt infections are reported to be unaffected by protein-energy malnutrition (13) and were increased in a model of weanling murine protein deficiency (15). This type of information is easily overinterpreted in relation to paracrine functions such as a role in myeloid cell proliferation, but clearly these results warrant further investigation.

Late stage differentiation of mononuclear phagocytes is also implicated as a point of impact of protein-energy malnutrition in studies of protein-deficient mice, both young adults (12) and weanlings (15). In the latter system, the malnourished animals showed low blood monocyte counts and Kupffer cell numbers and reduced expression of hepatic M-CSF mRNA, but responded rapidly to exogenous M-CSF (despite continued wasting disease) with a large increase in cell numbers in both compartments. These results may provide the beginnings of an explanation for the long-standing observation (14,16,17) that malnutrition results in depressed numbers of fixed and free-floating macrophages in many species including primates. Low macrophage numbers are also apparent in prepubescent rodents subjected to only moderate degrees of malnutrition that permit weight gain (18).
Clear, quantitative information is lacking to describe the impact of protein-energy malnutrition on the production and turnover of myeloid cells. Although myeloid cell proliferation is probably depressed in malnutrition (15), a basis appears to exist for focusing attention on myeloid cell terminal differentiation as a point of impact of this disease.

**Blood Clearance Activity and Phagocytosis**

As reviewed briefly elsewhere (19), clearance of colloidal particles and bacteria from the blood is consistently depressed in experimental protein-energy malnutrition, and this has been interpreted to reflect depression in phagocytic activity by hepatic and splenic macrophages *in vivo*. In fact, clearance of colloidal particles from the blood is accomplished by pinocytosis and is a function mainly of cells other than macrophages (*e.g.*, hepatic endothelial and parenchymal cells) (20). Moreover, blood clearance can provide only an indirect measure of phagocytosis or pinocytosis because factors such as blood perfusion and cell numbers also influence clearance rate. In this connection, studies of animals, including primates, collectively indicate that blood clearance in protein-energy malnutrition could be limited by the numbers of reticuloendothelial cells including macrophages (14—17,21).

Where phagocytosis is the clearance mechanism (*e.g.*, in the case of bacteria), opsonizing activity is an additional variable to consider. The opsonizing activity of the blood plasma can be depressed in childhood malnutrition, but this phenomenon is detectable only in diluted samples (22,23), an important observation corroborated by studies of weanling rats (24). Therefore, it must be anticipated that an influence on plasma opsonizing activity is a minor factor in the malnutrition-associated depression of blood clearance. However, although this may apply to particles opsonized primarily by complement fragments (25) or immunoglobulins (22,26), studies with adult rats suggest that fibronectin-dependent opsonization may be more sensitive to malnutrition (19). Moreover, studies with diluted sera may be highly relevant to the opsonizing conditions outside the vascular compartment (14,22), about which no information pertinent to protein-energy malnutrition appears to exist.

The weight of evidence points to the preservation of phagocytic capability in protein-energy malnutrition in childhood and in experimental malnutrition (14,16,19,27). Most studies provide information relating either to phagocytic capacity or to the proportion of phagocytes showing phagocytic activity. Information regarding the influence of protein-energy malnutrition on phagocytic rate concurs (27) but is scant. It is instructive to examine reports indicating a malnutrition-associated depression of phagocytic activity (17,28—31). These relate to the macrophage. In particular, phagocytosis of nonopsonized particles by macrophages is susceptible to depression in protein-deficient rodents (17,28,29,31). Interestingly, this outcome correlates with reduced expression of the macrophage mannose receptor in one experimental system (17), and probably with depressed expression of similar receptors in other rodent systems (18,29). It is questionable whether such results predict phagocytic activity *in vivo*, where opsonization is inevitable. Moreover, depression
The Professional Phagocytes: Microbicidal and Chemotactic Capabilities

Early studies established that a depressed ability to kill ingested bacteria and fungi is characteristic of neutrophils from children suffering from primary protein-energy malnutrition (14). This important phenomenon is reported even in the more moderate degrees of wasting disease (32) as well as in childhood stunting, albeit to a modest degree (3). Likewise, a large impact of malnutrition on the bactericidal activity of neutrophils from suckling rats has been reported (11). The latter results are of interest particularly because infection is unlikely to complicate their interpretation. Thus, protein-energy malnutrition per se can exert a large depressive influence on the bactericidal capacity of the neutrophil. Nevertheless, infection is an important confounding factor, as is made clear by the modest depression reported in neutrophil microbicidal activity in childhood malnutrition when overt infection is excluded (14,27). The neutropenia associated with infection in malnutrition is accompanied by disproportionately large numbers of band cells (14). Therefore, the depression reported in microbicidal activity of neutrophils from malnourished children with signs of infection may partly reflect the functional limitations of immature cells.

A reasonably consistent picture has emerged as to the impact of protein-energy malnutrition on the microbicidal (and phagocytic) capacity of the blood neutrophil when these functions are assessed in vitro. However, some caution is warranted in the interpretation of this information. Although the neutrophil is a terminally differentiated cell, studies conducted in isolation from inflammatory cytokines and endotoxin can yield false impressions of the functional capabilities of this cell at sites of infection (33).

The same caveat must apply to inconsistent reports on the influence of protein-energy malnutrition on the chemotactic responsiveness of neutrophils in diffusion chambers (14). Where a decrease in neutrophil chemotaxis has been observed in malnutrition, the effect appears to be related to overt infection (14), although aseptic stimuli (e.g., endotoxemia and chronic activation of the complement system) have also been implicated in childhood malnutrition (34). The scant evidence suggests that the chemotactic capability of the neutrophil may be preserved in the more moderate forms of wasting disease (34), and that even grade 3 wasting disease fails to exert a consistent influence (14). The same conclusion must be drawn regarding the macrophage, in which innate chemotactic responsiveness is sometimes maintained (16,24) and sometimes depressed (28) in animal models of protein-energy malnutrition.

Information on the influence of malnutrition on the innate ability of the macrophage to kill bacteria and fungi is somewhat narrowly based on studies of weanling and young adult rats and mice. In systems involving weight loss through protein deficiency, usually no effect is seen on macrophage microbicidal activity when this is assessed directly (i.e., not simply on the basis of related biochemical in-
indices) (16,24,29) and studies of rats subjected to restricted food intake concur (16,30). In one study, the microbicidal activity of alveolar macrophages was assessed in vivo and found intact following inhalant bacterial challenge (29). A dissenting report should be acknowledged, in which wasting protein deficiency induced depression in the innate fungicidal activity of resident peritoneal macrophages (17). However, in this experimental system, reduced expression of the macrophage mannose receptor was found, together with reduced phagocytic uptake of the nonopsonized Candida organisms used to test microbicidal activity (17). Thus, in general, it appears that the innate microbicidal capability of the macrophage is preserved in the face of acute wasting malnutrition. These results differ from findings relating to the neutrophil, but may reflect the capacity of the macrophage for differentiation, for example in response to the aseptic endotoxemia that can occur in protein-energy malnutrition (14). Although T-cell–stimulated actions of the macrophage are depressed (e.g., granuloma formation) (35), this is attributable to the T-cell component of the reaction system, whereas the macrophage retains responsiveness to lymphocyte-derived activation stimuli (35).

The Professional Phagocytes: the Respiratory Burst

It is instructive to examine reports regarding the influence of protein-energy malnutrition on the respiratory burst of the professional phagocytes. Eliciting stimuli have been delivered using phagocytosable particles, endotoxin, or phorbol myristate acetate, and biochemical indices used to assess this microbicide-related function include oxygen consumption, superoxide or hydrogen peroxide release, nitroblue tetrazolium reduction, fixation of iodine, and the activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or the hexose monophosphate shunt.

The results obtained with neutrophils from children, which are similar to the results of direct measurement of microbicidal activity, implicate infection as a critical confounding factor and source of variable outcomes (14,27). An additional potential source of conflicting information is methodologic and relates to the inconstant application of stimuli to elicit a respiratory burst. For example, no malnutrition-related influence was apparent on nitroblue tetrazolium reduction by unstimulated neutrophils from children with edematous malnutrition, whereas neutrophils from the same subjects showed depressed iodination activity when subjected to a procedure involving stimulation of the respiratory burst (27). Nevertheless, the simplest interpretation of available information is that the primary biochemical basis of depression in neutrophil microbicidal capacity in protein-energy malnutrition is a reduction in the ability to activate NADPH oxidase in response to the stimulus of particle attachment and phagocytosis. A cogent proposal to the physiologic basis for such a phenomenon lies in the well-known hypercortisolemia of protein-energy malnutrition and the inhibitory action of the glucocorticoids on the activity of NADPH oxidase (14). Although this idea has not been tested in relation to the neutrophil, both adrenalectomy and intervention with a glucocorticoid receptor antagonist abolished the depression of superoxide anion production by macrophages in the protein-deficient young adult rat (36).
In contrast to the neutrophil, the macrophage has almost always shown depression in the numerous biochemical indices relating to the respiratory burst in protein-energy malnourished rats and mice (17,21,24,31,36,37), although a report indicating no effect must be noted (16), and little influence was apparent in a model allowing modest weight gain in weanling animals (18). Thus, although malnutrition exerts little impact on the innate ability of macrophages of rats and mice to kill bacteria and fungi, a depressive influence is generally apparent on closely related biochemical activities. Perhaps this reflects a degree of redundancy in the respiratory burst of the macrophage. However, a second possibility must be considered. In addition to the NADPH oxidase-initiated pathway and several oxygen radical-independent mechanisms, macrophages of rats and mice possess an inducible nitric oxide synthase that initiates production of microbicidal, nitrogen-containing radicals (38). The activity of this pathway is depressed in activated macrophages from protein-deficient young adult mice (35,39), but the influence of protein-energy malnutrition on the innate activity of this biochemical pathway in the macrophage remains to be determined.

At this juncture, an important species difference must be emphasized. The professional phagocytes of humans, unlike those of rats and mice, do not possess an inducible nitric oxide synthase and, consequently, do not use nitrogen-containing radicals in killing ingested microorganisms (38). Thus, information about the innate microbicidal activity of the macrophage in protein-energy malnutrition, based as it is on studies of the rat, cannot be extrapolated with confidence to humans. Inasmuch as the tissue macrophage is not easily accessible in human subjects, it will be important to study appropriate animal species (e.g., the rabbit [38] or the guinea pig [35]) to understand the impact of malnutrition on the innate microbicidal activity of the macrophage. Presently no reason is found, on the basis of species peculiarities, for skepticism over the relevance to humans of information obtained from animal models on other functions of this cell in protein-energy malnutrition, for example phagocytosis, chemotaxis, synthesis and release of cytokines and eicosanoids, or antigen-processing and presentation.

The Complement System

Infection is a key factor connecting protein-energy malnutrition and the complement system. In fact, even the most severe forms of primary malnutrition appear to exert only a modest influence on the complement system of children in the absence of overt infection (40,41). In the face of infectious challenge, however, children with malnutrition appear unable to maintain or increase the blood complement protein (C3) concentration or serum complement-mediated lytic bioactivity (40,42). Inasmuch as infection usually accompanies protein-energy malnutrition in children, this phenomenon presumably underlies the low concentrations of blood complement proteins and reduced capacity of serum to support complement-mediated hemolysis (through either the classical or alternative pathways) that is characteristic of protein-energy malnutrition in childhood (14,40–43). By contrast, moderate degrees of wasting malnutrition in childhood have generally been reported to exert little or no influ-
ence on the complement system in terms of lytic activity and the blood concentration of C3 (41,43,44), although a dissenting report should be acknowledged (42). Likewise, the sparse information on stunting malnutrition indicates little impact on the complement system (42).

Studies on experimental animals are consistent with the reports on children, for example in regard to the modest impact of protein-energy malnutrition on the complement system in the absence of overt infection (45–51). Experimental studies include investigations of weanling and young adult animals (including rats, guinea pigs, and primates) subjected to weight loss through protein or energy deficit. Nevertheless, less information appears available in experimental malnutrition than in childhood malnutrition regarding blood concentrations of individual complement proteins, particularly of the alternative pathway. Importantly, the studies of animals provide evidence that protein-energy malnutrition attenuates the response of the complement system to infectious challenge (47,48), thereby supporting a key finding relating to malnutrition in childhood. It is noteworthy that reports both on children (41) and on rodents (45) indicate that the complement system is more resistant to malnutrition than is adaptive, cell-mediated immune competence. However, this important comparison has not been made in studies examining the capacity of the complement system to respond to infectious challenge, arguably the most discriminating index of the impact of protein-energy malnutrition on this component of innate defense.

The mechanism whereby protein-energy malnutrition exerts its influence on the complement system is unclear. Indirect evidence stemming from replenishment of blood levels in children during rehabilitation has been interpreted, logically, as indicating that malnutrition reduces the capacity to synthesize complement proteins (14). Nevertheless, adult rats subjected to wasting protein deficiency from prepubescence retained the ability, both in the presence and in the absence of infectious challenge, to synthesize a constellation of complement proteins detectable on the basis of affinity for an antigen–antibody complex (47). In a similar experimental system, the malnourished animals responded to pharmacologic stimulation by increasing the blood concentration of C3 (51), the level of serum complement-mediated lytic activity (51), and the ability of serum to opsonize particles with the C3 fragment, iC3b (25). In the light of these results, quantification of complement protein synthesis rates in malnourished prepubescents would be of great interest. This would require investigations that account for pool sizes of specific complement proteins and of the amino acids used as tracer compounds.

Other evidence suggests that protein-energy malnutrition may influence the complement system partly through enhanced turnover. Thus, early studies revealed a high prevalence of presumptive C3 activation fragments, as well as high concentrations of immunoconglutinin, in the blood of children suffering primary malnutrition (14,40). These observations were recorded in subjects not showing signs of overt infection. Therefore, the reasonable suggestion has emerged that a high rate of consumption of complement proteins is a feature of protein-energy malnutrition, and that this characteristic originates with the aseptic endotoxemia that has been reported in this disease (14). Thus, the well-known attenuation of the inflammatory response in protein-
energy malnutrition (14) may result, in part, from a chain of events including hypochlorhydria, intestinal Gram-negative overgrowth, increased entry of endotoxins into the portal blood, reduced clearance of these potent molecules by Kupffer cells, and consequent activation-related losses of complement proteins.

The complement system was among the first components of immune defense to be studied in connection with protein-energy malnutrition, and a sound database was established. During the last 15 years, however, little interest has been seen in pursuing this subject and very little progress has been made. In particular, rigorous information is needed to describe quantitatively the impact of malnutrition and rehabilitation strategies on the synthesis and turnover of complement proteins, especially in the face of infectious challenge.

The Acute Phase Response

A triad of cytokines—tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6—is produced by the macrophage as a cascade of hormones mediating the acute phase response, although other cytokines may also be involved and IL-6 may be the principal endocrine mediator of the group (52). Recent evidence shows a depression in release (and presumably synthesis) in vitro of TNF-α, IL-1, and IL-6 by endotoxin-stimulated monocytes from children with wasting malnutrition (53,54), of TNF-α by macrophages of protein-deficient guinea pigs following stimulation by mycobacterial products (35), and of IL-6 by endotoxin-stimulated macrophages of wasting, protein-deficient young adult rats (36). These results are based on immunoassays and, thus, significantly extend reports of attenuated production of macrophage cytokine bioactivities in comparable systems (55,56). A report must be noted that endotoxin elicited a normal increase in IL-6 production by peripheral blood mononuclear cells from a group of children in whom severe stunting was the prevalent form of protein-energy malnutrition (57). However, the weight of evidence is that malnutrition reduces the capacity of the mononuclear phagocyte to release the cytokines that mediate the acute phase response. A mechanistic clue in this regard derives from studies revealing increased levels of transforming growth factor-β (TGF-β) in the blood of protein-deficient guinea pigs (35). This cytokine can induce depression in TNF-α production by activated macrophages (35).

Despite the foregoing results, children with protein-energy malnutrition and overt infection are reported to sustain blood concentrations of IL-1 that are either normal or only modestly reduced (13), and a small sample of subclinically infected children with malnutrition showed blood levels of IL-6, TNF-α, and (antagonistic) soluble TNF receptors that did not differ from controls with similar laboratory evidence of infection (58). Results of experimental animal studies appear consistent with these findings. Thus, the blood concentration of IL-6 was raised in protein-deficient weaning mice (15), and did not differ from well-nourished controls (except for a modest delay in response) in protein-deficient young adult rats subjected to aseptic inflammatory challenges (59). Presuming blood IL-1, TNF-α, and IL-6 to be produced mainly by mononuclear phagocytes (52), the results suggest that reduced turnover
compensates for the impact of malnutrition on the synthesis and release of these cytokines, thus maintaining their circulating concentrations during infectious challenge. The scant information base relating to blood concentrations of the acute phase cytokines and their antagonists in protein-energy malnutrition must be increased if more than a descriptive understanding of the acute phase response is to be achieved in relation to this disease.

Protein-energy malnutrition in children consistently attenuates both the febrile response (13,26) and infection-related neutrophilia (13,14), and studies with experimental animals concur (11,12,60,61). Likewise, an attenuated increase in blood concentrations of acute phase proteins is reported in childhood malnutrition during natural infection (62) or following vaccination (63), as well as in weanling rats subjected to protein deficiency or to food intake restriction together with a sterile inflammatory challenge (64,65). In the studies of malnourished rats, both stunting and wasting deficiencies exerted a measurable impact on the peak response using α2-macroglobulin as the indicator protein. Likewise, an attenuated acute phase protein response has been suggested even in mild childhood malnutrition without wasting (63). Despite these results, an important study of marasmic children emphasized their ability to mount a comprehensive acute phase protein response to infection at a level anticipated among comparable well-nourished subjects, albeit historical controls (66). Similarly, malnourished children with subclinical infections had high blood concentrations of C-reactive protein (58), and wasting protein deficiency did not affect the ability of young adult mice to maintain high blood concentrations of acute phase globulins in response to a sterile inflammation (12). Thus, a substantial increase in blood concentrations of acute phase proteins can be sustained even in the most severe forms of wasting protein-energy malnutrition (although this can be reduced relative to the response mounted by well-nourished subjects), whereas other components of the acute phase response appear to be more severely and consistently affected. It is important to note that the acute phase protein response to infection in childhood malnutrition is reported to be achieved through reduced turnover rather than, as in well-nourished subjects, through increased hepatic synthesis (66).

Collectively, the available information suggests that protein-energy malnutrition usually reduces the capacity of the macrophage to produce the triad of cytokines that regulates the acute phase response. Nevertheless, the ability to sustain high blood levels of these cytokines appears to be maintained in the malnourished state, presumably by way of an influence on turnover, although the database is scant and mechanistic evidence is lacking. The prediction follows that target cell responsiveness to the acute phase cytokines is depressed in protein-energy malnutrition. In this connection, protein-deficient guinea pigs responded to purified IL-1 with a reduction in serum iron and zinc concentrations, but showed neither neutrophilia nor a febrile response (61). In other work, protein-deficient rabbits had a normal fever response when injected with supernatants of activated monocytes (60). Thus, some responsiveness to acute phase cytokines appears to be retained in protein-energy malnutrition, and the level of responsiveness may be target specific. This proposition provides a framework within which to pursue findings such as the report implicating depressed mobiliza-
tion of neutrophils from the bone marrow in response to inflammatory stimuli in malnutrition (12). Precise, quantitative information is needed as to the impact of protein-energy malnutrition, in its different degrees of severity and various metabolic forms, on the blood concentrations of acute phase cytokines and their soluble inhibitors, and on the response capacity of target cells.

The Natural Killer Cell

The natural killer (NK) cell is considered particularly important in defense against viral diseases, but also may be important in resistance to some prokaryotic intracellular parasites (67). Early findings of depressed NK-cell lytic activity in blood mononuclear cells of children with grade 3 protein-energy malnutrition (68) could not distinguish between an effect on cellular numbers and an effect on cellular activity. These results have been extended recently in a study of protein-deficient weanling mice (69). In this investigation, splenic NK-cell lytic activity declined on a per cell basis, and NK cell numbers declined in proportion with the atrophy of the splenic mononuclear cell compartment.

The mechanism of depression in NK-cell activity is unknown, although overproduction of prostaglandin E$_2$ (PGE$_2$) is reported in adolescent (16) and young adult (37) experimental animals subjected to wasting protein deficiency. In addition, the influence of protein-energy malnutrition on $\gamma$-interferon production by NK cells has not been investigated despite the probability that this function is a key initiating factor of the microbicidal action of macrophages and of the primary cell-mediated adaptive immune response (67). With regard to NK cell numbers, nothing is known of the impact of protein-energy malnutrition on NK-cell progenitor activity. Moreover, it remains unknown whether a redistribution of NK cells occurs in malnutrition away from sites such as the spleen wherein these cells are normally located.

A synthesis of evidence developed elsewhere (70) emphasizes the resistance of NK-cell activity to diverse nutritional deficiency conditions. Thus, NK-cell activity is reported to be preserved in stunted children (3). Moreover, adult mice subjected to stunting initiated at weaning showed preservation (71) or only modest depression (72) of splenic NK-cell lytic activity. Although depressed lytic activity of NK cells from children suffering grade 3 protein-energy malnutrition failed to respond to interferon in vitro (68) and NK cells of protein-deficient young adult mice likewise failed to respond to IL-2 in vitro (73), injections of a drug that generates interferon in vivo produced activation of splenic NK-cell lytic activity in stunted, energy-restricted mice (72). Systematic analysis is warranted regarding the responsiveness of NK cells to regulatory signals in protein-energy malnutrition.

ADAPTIVE DEFENSES

Systemic Immune Competence: Humoral and Cell-Mediated Responses

It is a longstanding and firmly established concept that childhood protein-energy malnutrition consistently reduces cell-mediated immune competence but exerts a less
predictable impact on the systemic antibody response. As reviewed elsewhere (14,74,75), this is supported by a large weight of evidence that includes studies in which measurements of both humoral and cell-mediated immune competence were made concurrently in the same children or experimental animals. In clinical studies, such comparisons can be complicated by the influence of rehabilitation. This is because cell-mediated responses are commonly examined as delayed hypersensitivity reactions to recall antigens, whereas antibody production—commonly studied as the more slowly developing primary response—is usually examined only after a longer period of rehabilitation. This concern is reinforced by early studies showing more rapid recovery of humoral immunocompetence than of the capacity to generate a cytotoxic T-cell response during rehabilitation of mice from experimental protein-energy malnutrition (76). However, the concern appears to be addressed satisfactorily by several types of investigation, including population-based studies in which clinical intervention was not offered, investigations of experimental animals, and clinical studies in which both humoral and cell-mediated immune responses were assessed as primary reactions (14,74,75). Perhaps the most direct evidence that adaptive cell-mediated and humoral responses differ in sensitivity to protein-energy malnutrition derives from a study of weanling mice in which primary delayed hypersensitivity and antibody responses were each elicited by intraperitoneal administration of sheep red blood cells at the same stage in development of protein deficiency (75).

The delayed hypersensitivity skin test is a critical component of the database relating to cell-mediated immune competence in childhood protein-energy malnutrition. Both primary and recall responses are consistently depressed in this disease (14,26), even with only moderate degrees of wasting (77,78). By contrast, the recall response is reported to be unaffected by stunting (77,78), although other investigators do not concur with this (3) and the point clearly deserves further investigation. Infection has an important depressive influence on delayed cutaneous hypersensitivity in malnutrition (74), but carefully designed studies show that this response is depressed independently of overt infection in childhood malnutrition (77-79). In addition, concurrent infection is unlikely to be a factor in studies of experimental animals; yet primary and recall cutaneous delayed hypersensitivity responses are consistently depressed in diverse species, including primates, subjected to wasting malnutrition (14,45,49,80).

Interpretation of skin test results is also complicated by the depression of the cutaneous inflammatory response that often accompanies malnutrition (74). Thus, a depressed skin test response is not unambiguous evidence of an effect on adaptive cell-mediated immune competence. The same limitation must be recognized in relation to results showing depression in the skin allograft response in experimental protein-energy malnutrition (14,49,81). However, polyclonal mitogen tests in both clinical and experimental settings (14,74), as well as studies of antigen-driven proliferation of T cells from experimental animals (35,56,73,80), adoptive transfer experiments (35,74), and studies of the cytotoxic T-cell response in vitro (73,76), show a depressive impact of malnutrition on T-cell functions relating to cell-mediated immunity. In addition, the course of viral, mycobacterial, and protozoan infections in protein-energy malnutrition strongly suggests depression of cell-mediated immune compe-
tence through an influence on the T-cell system (74), and an important series of studies with tuberculous guinea pigs (35) has provided clear evidence of this. The latter experimental system has been used to show that effective antimycobacterial resistance, including the capacity to generate a granulomatous reaction, can be restored to protein-deficient animals by adoptive transfer of syngeneic immune T cells (35).

The best evidence pertaining to humoral immunocompetence in childhood malnutrition derives from studies of the serum antibody titer generated in response to vaccination procedures. Even in the most severe forms of wasting disease, outcomes range from depression through normal, and their most outstanding feature appears to be unpredictability (14,26,82). This point is illustrated with clarity in a recent summary of the T-cell–dependent response to tetanus toxoid in malnourished children (82). In this connection, coculture experiments using blood T and B cells from well-nourished and malnourished children indicate that, when this type of response is depressed in protein-energy malnutrition, the phenomenon relates to the competence of T cells rather than of B cells (83). Likewise, a predominant influence of protein-energy malnutrition on T cell help rather than on B cell competence is suggested by early experiments in which adoptive transfer of thymocytes improved the humoral immune competence of protein-deficient weanling mice (14). Thus, studies of humoral immune competence that pertain to regulatory T-cell activities have extended the substantial information base showing depression in both effector and regulatory T-cell actions in protein-energy malnutrition.

The T-cell–independent type of antibody response (e.g., to typhoid O antigen) is also often depressed in malnourished children (26). Although no clear evidence points to a cellular basis for this phenomenon, ontogeny is potentially a variable in the resistance of the B-cell system to the effects of murine protein-energy malnutrition (75,84). Antigen dose can also influence the impact of malnutrition on the antibody response. This was demonstrated in early studies of the T-cell–independent response of protein-deficient weanling rodents (85), and is a phenomenon that should be pursued in exploring the mechanisms of immune depression in protein-energy malnutrition. Concurrent infection often exerts a depressive influence on humoral immune competence in malnutrition (14), and may contribute to the unpredictability of the antibody response in this situation. However, numerous studies of experimental protein-energy malnutrition show depression of T-cell–dependent (84,86,87) and T-cell–independent (85,88) antibody responses, apart from the confounding influence of infection.

Blood concentrations of IgM, IgG, and IgA are generally unaffected or modestly increased in childhood protein-energy malnutrition (14,26). This appears to be the case even in the absence of overt infection, an observation supported by results obtained with weanling mice (89). Nevertheless, concurrent infection is clearly associated with high blood immunoglobulin levels in protein-energy malnutrition (14). In the case of IgG, this has been attributed to increased rates of synthesis in a study of patients with kwashiorkor syndrome (14), thereby providing evidence compatible with humoral immune competence despite the pathology of wasting protein-energy malnutrition.
It is important to note that blood IgM is a mainly polyreactive, low affinity (natural) antibody, probably produced by the B1 subset of B cells (90). This subset is also thought to release significant quantities of IgG and IgA class natural immunoglobulin in the absence of high dose antigen exposure, and so it is distinct from the conventional B2 subset that generates the classical high affinity, high specificity antibody response (90). Thus, the malnutrition-associated phenomenon of high or normal blood immunoglobulin levels despite low or normal antibody responses to specific immune challenge could occur if the conventional B2 subset were affected more severely than the B1 subset. In fact, B1 cells appear to participate in the primary antibody response (90), and longstanding reports indicate that malnutrition results in production of low affinity antibody to specific antigenic challenge (14). However, no information is available to address directly the proposition of a subset imbalance between B1 and B2 cells under these conditions.

Mucosal Humoral Immune Competence

Most information on the adaptive defenses in protein-energy malnutrition pertains to systemic responses, despite the fact that most infections in wasting disease are opportunistic mucosal infections. The mucosal secretory IgA antibody response has been widely accepted as more sensitive to wasting disease than systemic humoral immunity. The main reason for this is that IgA concentrations of diverse mucosal secretions, including those of the gastrointestinal tract, salivary glands, lacrimal glands, and nasopharynx, are almost always depressed in childhood malnutrition. This has been reviewed briefly elsewhere (14,89), and is an outcome with which studies of weanling rodents concur (89,91–94). Even grade 2 protein-energy malnutrition is reported to result in low secretory IgA levels in the lacrimal secretions of children (95), although occasional inconsistencies in published reports relating to grade 3 disease are important to acknowledge (95). In addition, the few studies reporting mucosal IgA responses to defined antigens in protein-energy malnutrition consistently demonstrate depression. These reports include only one study of children (96), but several studies of protein-deficient weanling rodents (91,93,97,98).

Nevertheless, the concept that systemic and mucosal antibody responses differ fundamentally in sensitivity to malnutrition deserves renewed attention (99). Recent investigations of weanling mice emphasize the resistance of both systemic and mucosal antibody-producing cellular compartments, even to profound protein deficiency (89). Thus, increases were recorded (per organ) in the numbers of IgG-containing splenic plasma cells and in the numbers of IgA-containing intestinal plasma cells, despite weight loss and atrophy of the inductive compartment of lymphocytes in the two anatomic sites. In addition, indirect evidence has suggested that isotype switching is substantially intact, both to IgG class immunoglobulins in the spleen and to IgA in the lamina propria in experimental weanling protein-energy malnutrition (89). As a further point of similarity between systemic and mucosal humoral competence, malnutrition-associated depression in the intestinal IgA response to oral immunization appears likely to reflect an impact on the action of regulatory T cells (93,98).
In studies of weanling mice subjected to wasting protein deficiency (100), or to a marasmus-like condition (94), expression of the hepatic polymeric immunoglobulin receptor (pIgR), the epithelial IgA-transporting protein, was depressed sufficiently to account for the low concentrations of secretory IgA found in intestinal secretions. These results extend reports that the concentration of free secretory component is low in tears of wasted children, although not in grades 1 and 2 stage disease (95), and in lacrimal secretions, saliva, bile, and intestinal washings of weanling rats and mice subjected to protein-energy malnutrition (92,94,100). Thus, an experimental basis is forming for the proposition that epithelial IgA transport is a focal point of the influence of malnutrition on mucosal immunity, and that immunoglobulin production responds similarly to malnutrition at systemic and mucosal sites. An implication with regard to mucosal defense is that clinical interventions should be aimed at the synthesis and function of the pIgR.

These recent studies relating to mucosal defenses highlight an issue regarding the use of animal models. According to present information, transport of IgA into intestinal mucus is primarily a function of the intestinal epithelium in humans, but is substantially a function of the liver in rats and mice (99). In view of evidence that control of pIgR synthesis differs among anatomic sites (101), these species differences are potentially critical. Consequently, the use of rats and mice may permit identification of the pIgR as a point of impact of protein-energy malnutrition on mucosal immunity, but is arguably unsuitable for mechanistic analyses relevant to the human intestine (99).

Mechanisms of Depression in Adaptive Immunocompetence: Lymphoid Involution

Lymphoid involution, as indicated by the size and cellularity of the thymus and secondary lymphoid organs, is characteristic of wasting malnutrition and is of such magnitude that it must contribute to depression of adaptive immunocompetence (99). The thymus is affected particularly severely, and loss of cortical thymocytes is the most obvious aspect in observational histology. The extent of thymic involution in protein-energy malnutrition has been used in studies suggesting that immunologic recovery from childhood malnutrition (assessed by the size of the thymic shadow) may require more time than recovery of the standard anthropometric indices (2). With regard to the periphery, an estimate of the size of the recirculating pool of lymphocytes in the protein-deficient weanling mouse indicates that secondary lymphoid organ size may yield an inflated impression of the extent of lymphoid involution in malnutrition (86). Likewise, peripheral lymphoid organs can show pronounced differences in the extent of cellular losses in malnutrition, as reported in the weanling mouse (102). Thus, compartment-specific complexities are emerging with regard to malnutrition-associated lymphoid involution, and our inability to predict such outcomes highlights a surprising dearth of mechanistic information. Indeed, a recent study of young adult mice (103) allows the speculation that at least some forms of malnutrition can increase the propensity of T cells to
undergo apoptosis following engagement of the T-cell receptor. Depression of proliferative capacity is clearly a feature of lymphocytes in malnutrition (35) but is, logically, a less satisfactory basis than cell death on which to build an understanding of peripheral lymphoid involution. Early studies using adrenalectomized rodents implicated the high levels of glucocorticoids characteristic of protein-energy malnutrition in the involution of lymphoid organs in this disorder (14). It will be important to pursue these threads of evidence and their potential connection. As pointed out elsewhere (99), the thymocyte in malnutrition would be studied more profitably using a species such as the guinea pig rather than other rodents or rabbits. Guinea pigs, like humans and unlike rats, mice, or rabbits, possess cortical thymocytes with a high degree of resistance to glucocorticoid-mediated lysis.

Information from observational histology and blood leukocyte counts is widely interpreted as showing that malnutrition causes a greater reduction in T-cell numbers than in B-cell numbers (104). To the extent that the blood lymphocyte compartment is informative in this regard, more moderate forms of wasting disease in children may exert a similar disproportionate impact on the T-cell system (78), whereas this is not the case in stunted children (3). Despite a low blood T-cell count, lymphopenia is relatively rare in childhood malnutrition (14,26). This may be attributed, at least in part, to the large numbers of immature T-lineage cells that are found in the blood in this condition (14,26) and which have most recently been quantified by means of the surface marker, CD1a (105). It is of interest that in vitro exposure of blood mononuclear cells from malnourished children to thymic hormones, or to extracts containing such peptides, effects a rapid increase in the numbers of phenotypically mature T cells and a corresponding decrease in the numbers of immature cells (104,105). Thus, depression in adaptive immune competence in malnutrition may result, in part, from release of thymocytes that have received insufficient maturational stimuli. This concept may be extended to include the B-cell system on the basis of indirect phenotypic evidence suggesting premature release of B-lineage cells from the bone marrow of protein-deficient weanling rats (93).

Despite the potential importance of primary lymphoid organs in relation to the capacity of wasted subjects to respond to therapeutic interventions, little is known about lymphopoiesis in protein-energy malnutrition, and no information is available on the impact on the T-cell or B-cell repertoire. However, an immunohistochemical analysis produced evidence of depressed production of thymulin by the thymic epithelium in children suffering various forms of grade 3 wasting malnutrition but without overt infection (106). Even stunting malnutrition in children is reported to produce a modest depression in serum thymulin bioactivity (3), albeit much less marked than in wasted children and weanling rodents (104). Moreover, studies of weanling mice subjected to wasting through either protein or energy deficiencies revealed profound atrophy of the thymic epithelium, together with ultrastructural evidence of derangements in the secretory vacuolar apparatus of this tissue (104). The impact on the vacuolar apparatus was detectable sufficiently early in the progress of malnutrition to permit the proposition that the thymic epithelium is a primary point of impact of protein-energy malnutrition on adaptive immune competence (104).
Mechanisms of Depression in Adaptive Immunocompetence: T Cell Subset Imbalances

Imbalances between or among critical subsets of lymphocytes may also contribute to the initiation or the continuation of malnutrition-induced immunodepression. This important concept originates with the discovery of a low CD4:CD8 ratio in the blood of malnourished human subjects (99). The same phenomenon is reported in weanling mice (102) and has become widely accepted as a key factor in the depression of T-cell–dependent immunity in protein-energy malnutrition (99,104). In fact, however, a low blood CD4:CD8 ratio sometimes fails to develop in malnutrition, both in children (105) and in weanling rodents (102). Moreover, diverse rodent models of weanling malnutrition show profound depression of T-cell–dependent adaptive responses in the absence of a low CD4:CD8 ratio, either within the circulating surveillance pool of T cells or within the secondary lymphoid organs in which immune responses are initiated (86,87,102). Recent studies of tuberculous, protein-deficient guinea pigs reveal an extremely low CD4:CD8 ratio in the lymph nodes draining the infected lungs (35). However, this phenomenon appears to reflect an influx of CD8+ (presumptive) effectors rather than a paucity of CD4+ T cells. A low CD4:CD8 ratio, therefore, commonly occurs in the blood in protein-energy malnutrition but appears unnecessary and generally irrelevant to immunodepression. In fact, the CD4:CD8 ratio is not reliable as a helper-to-suppressor index, or even as a helper versus suppressor/cytotoxic index. Many recirculating suppressor T cells, for example, may show a CD4+ phenotype (107). In addition, CD8+ T cells are a major source of cytokines that serve in a helper capacity (108).

In this context is seen an important caveat relating to the interpretation of blood lymphocyte data. The blood is a small and unique lymphoid compartment in which disease-related disturbances among lymphocytes are often unrepresentative of events within the secondary lymphoid organs (109). Therefore, although the use of blood lymphocyte data in studies on human subjects is fully understandable, it is subject to overinterpretation, as has occurred in relation to the CD4:CD8 ratio.

An overabundance of the quiescent, CD45RA+ (naive) phenotype has been identified recently within the involuted T-cell compartment of the blood and secondary lymphoid organs of weanling mice subjected to either energy restriction or protein deficiency (87,110). This naive shift was apparent in both CD4+ and CD8+ T-cell subsets, and similar results are reported in the blood of elderly humans with protein-energy malnutrition (111). At this stage, the phenomenon appears likely to contribute to immune depression only in the advanced stages of protein-energy malnutrition (110). In addition, information on this imbalance is confined to analysis of surface phenotype and, thus, relates only indirectly to cellular function. Nevertheless, the phenomenon is interesting in the light of the recent proposition that T cells of protein-deficient, tuberculous guinea pigs home preferentially to the lymph nodes draining the infected lungs (35). Such trafficking behavior would be expected of naive type T cells which show a migratory preference for lymphoid rather than nonlymphoid sites (112). Likewise, homing to the small intestine was depressed on the part
of lymphoblasts adoptively transferred from protein-deficient rats, whereas localization within the associated mesenteric lymph nodes was not affected (113). Finally, it is noteworthy that experimental protein-energy malnutrition has occasionally been reported to impose immunodepression without affecting the ability of T cells to show antigen-driven proliferation (114,115). This is interesting because naive phenotype T cells are capable of matching memory phenotype cells in terms of proliferation if their stringent activation requirements are met (116). Thus, an understanding of the real significance of an overabundance of naive phenotype T cells in protein-energy malnutrition requires precise information on the cytokine and antigen presentation microenvironments within which these cells must function in this disease.

Mechanisms of Depression in Adaptive Immunocompetence: Antigen Processing and Presentation

The influence of protein-energy malnutrition on antigen processing and presentation has been studied only in rodent systems, and information is scant. One study has addressed the influence of malnutrition on antigen processing and presentation for the primary response (114). This is accomplished by the dendritic cell, which is uniquely capable of meeting the activation requirements of naive type T cells (117). Thus, the ability of spleen cells to stimulate a mixed leukocyte response, the classic assay of dendritic cell competence (117), was depressed in mice subjected to protein deficiency from weaning through young adulthood (114). The results did not distinguish between effects on cellular numbers and cellular function. However, a recent report shows that the numbers of mature splenic dendritic cells are much reduced in the advanced stages of weanling protein or energy deficiency in the mouse (118). Presently, no information is available on the impact of malnutrition on the functional capacity of dendritic cells. In addition, information about dendritic cells in malnutrition is at present confined to the spleen, an important limitation in view of the heterogeneity of these cells in diverse lymphoid sites (117). Studies of the development and function of dendritic cells in malnutrition are needed to improve our understanding of the primary adaptive immune response in this disorder.

Some information is available on antigen processing and presentation in the secondary response in protein-energy malnutrition. Thus, in a study of weanling mice subjected to energy restriction sufficient to produce stunting and modest lymphoid involution, splenic cellular suspensions containing the three professional antigen-presenting cells (B cells, macrophages, and dendritic cells) showed a reduced capacity to stimulate antigen-induced proliferation and production of γ-interferon by memory T cells (115). These results appear likely to reflect reduced function on a per cell basis. Comparable findings derive from a study of protein-deficient mice in which antigen-presenting cells—undoubtedly a mixture of macrophages, B cells, and dendritic cells—were recovered from the peritoneum (119). As in a report pertaining exclusively to dendritic cells (114), the latter study suggested that antigen presentation (rather than uptake and processing) is the main point of impact of malnutrition on the cellular functions that prepare antigen for recognition by T cells. Although reduced
production of IL-1 has been implicated mechanistically (114), systematic analysis of the competence of antigen-presenting cells in malnutrition is lacking. In view of results showing that T cells from rodents subjected to either protein or energy deficiencies sometimes retain their capacity to respond to appropriately presented antigen, for example in terms of proliferative capacity (114,115), improved knowledge of antigen processing and presentation in malnutrition is important.

Mechanisms of Depression in Adaptive Immunocompetence: Cytokines

Early reports, summarized elsewhere (55), indicated depression in the production of several cytokine bioactivities—including macrophage migration inhibition factor, viral interferons, and leukocyte inhibition factor—by blood leukocytes in childhood malnutrition. More recent results showed that the production of IL-6 by mitogen-stimulated blood T cells was unaffected by malnutrition in children from a predominantly stunted population (57). In addition, severe protein-energy malnutrition, but not more moderate forms of the disease, caused depression in the capacity to produce IL-2 on the part of blood T cells of elderly human subjects (111). A scant and disperse literature, therefore, documents the impact of protein-energy malnutrition on cytokine production by lymphocytes in humans, and the results suggest that the degree and type of malnutrition is important to the outcome.

Current understanding of lymphocyte-derived cytokines in prepubescent malnutrition is based mainly on studies of experimental animals. Although not extensive, these results are clearly germane to the depression in various indices of cell-mediated immunocompetence that characterize protein-energy malnutrition, including reports of an inability to generate a granulomatous response to infection with facultative intracellular parasites (35,120). Thus, depressed production of IL-2 is reported in studies of mitogen- and antigen-stimulated blood and splenic T cells from protein-deficient guinea pigs (35,80) and adolescent rats (121). Together with evidence of reduced responsiveness to this cytokine in the protein-deficient guinea pig system (35,80), these findings initiate an explanation of the characteristically low T-cell proliferative capacity in malnutrition. Depressed γ-interferon production is also reported consistently in experimental protein-energy malnutrition. Thus, γ-interferon production was low in concanavalin A-stimulated splenic mononuclear cells from adolescent rats subjected to wasting protein deficiency (121). In this system, the mRNA level for γ-interferon was depressed in parallel with the level of functional protein assessed by bioassay, and γ-interferon was affected more severely than IL-2 in terms of both protein and transcript levels. In addition, modest energy restriction was sufficient to depress antigen-stimulated γ-interferon production by splenic mononuclear cells from nematode-infected weanling mice (115), and the level of mRNA for this cytokine was depressed in the lungs of protein-deficient young adult mice during the early stages of tuberculosis infection (120). In other work with mice subjected to protein deficiency from weaning to young adulthood, depression was noted both in production of macrophage migration inhibition factor by mitogen-stimulated splenic T cells and in responsiveness to this cytokine by peritoneal macrophages, although an
impact on responsiveness could not be confirmed in studies of protein-deficient guinea pigs (35).

It is important to recognize that cytokines function in networks and show redundancy. Consequently, full appreciation of the involvement of these regulatory molecules in the immunopathology of protein-energy malnutrition requires that they be studied simultaneously as networks. Although this has not yet been done in the context of malnutrition, three examples of the study of abbreviated panels of T-cell cytokines in this disorder serve to illustrate the potential of such a research strategy. Thus, the ability of splenic T cells to produce IL-4, IL-5, and IL-10 in response to antigenic stimulation in vitro was unaffected by energy restriction of nematode-infected weanling mice, whereas production of γ-interferon was depressed (115). Likewise, a protein-deficiency protocol that produced depressed resistance to Candida in young adult mice also produced depression in the production of γ-interferon while exerting no effect on the production of IL-4 or IL-10 by mitogen-stimulated splenic T cells (39). These important outcomes are suggestive in relation to the sensitivity to malnutrition shown by cell-mediated immunocompetence relative to antibody responses. Similarly, recent results point to cytokine balance as a basis for understanding the enhancement in cell-mediated immunocompetence that occurs in some rodent models of stunting protein deficiency. In such an experimental system, the capacity of murine splenic T cells to release IL-2 and γ-interferon when stimulated with anti-CD3 in vitro was preserved or increased, whereas the capacity to release IL-4 was depressed (122).

Both cytokine production and the responsiveness of cytokine targets in protein-energy malnutrition deserve systematic investigation, with attention to the impact of malnutrition on the balance between Th1- and Th2-type cytokines. Proposals about the physiologic basis for altered cytokine production in malnutrition include a shift in eicosanoid metabolism favoring cyclo-oxygenase products (16) and glucocorticoid-mediated depression of the synthesis of numerous cytokines (99). In addition, an intriguing recent study reports increased bioactivity of the broadly immunosuppressive cytokine, TGF-β, in the blood of protein-deficient guinea pigs (35). This could downregulate the synthesis of numerous cytokines (123) and might underlie early reports (124) that blood plasma from children with protein-energy malnutrition shows a reduced capacity to support T-cell functions (e.g., blastogenesis) in vitro. Knowledge relating to the control of cytokine activities in malnutrition requires much refinement to accommodate the complex variation in the effects on these regulatory proteins in different forms of malnutrition.

IMMUNODEPRESSION: A COMPONENT OF THE SYSTEMIC PATHOPHYSIOLOGY OF PROTEIN-ENERGY MALNUTRITION

It appears widely accepted that synthesis of proteins and other amino acid-containing compounds in protein-energy malnutrition is nonselectively depressed because of a general shortage of amino acid substrate or energy. This model is often invoked as underlying the malnutrition-induced depression of immunologic functions (e.g., antibody or cytokine synthesis and antigen-driven clonal expansion of lymphocytes).
Fine control at the level of transcription, however, has been reported in relation to hepatic protein synthesis when wasting protein deficiency was imposed on weanling rodents (125). Thus, transcript levels were low relative to total cellular RNA for some proteins (e.g., albumin), but were either unaffected or even increased for others (e.g., ubiquitin). A similar effect is seen in the control of leukotriene (LT) synthesis in kwashiorkor. Although amino acids and glutathione are in limited supply in this condition, leukotriene synthesis has been reported to shift from LTB\(_4\) toward the synthesis of the cysteinyl compounds LTC\(_4\) and LTE\(_4\) (126). Thus, the classical model connecting low intake of amino acids and energy directly to a generalized reduction in protein synthesis does not accommodate available information on malnutrition in childhood or on experimental protein-energy malnutrition. This point has occasionally been made elsewhere (12,99).

The physiologic response to malnutrition is orchestrated by endocrine hormones that govern the systemic distribution of substrates and energy (99). Thus, it is critical that the immunobiology of protein-energy malnutrition is understood in the context of the hormonally mediated reorganization of metabolism in this disorder (99,104), and much current interest focuses on the glucocorticoids. For example, depressed synthesis of IL-6 and of the superoxide radical by macrophages is prevented by either adrenalectomy or the administration of a glucocorticoid receptor antagonist in a model of protein-deficient young adult mice that features the high blood glucocorticoid concentrations characteristic of protein-energy malnutrition (36). As a second example, in systems of either protein- or energy-deficient weanling mice, administration of triiodothyronine can prevent the development of depression of primary adaptive immunocompetence (81) as well as depression of splenic NK-cell lytic activity (69). In the cited examples, the hormonal interventions achieved immunomodulation in the presence of unabated weight loss and profound lymphoid atrophy. Some experimental evidence, therefore, points to the concept that the endocrine microenvironment is likely to define the limits of immunocompetence in protein-energy malnutrition. Recent evidence, including results from studies of short-term murine starvation, implicates leptin as an endocrine link between nutritional status and immune competence (127). Perhaps this new clue will stimulate research leading to a cohesive knowledge of the immune-endocrine nexus in protein-energy malnutrition.

KEY FEATURES OF DESIGN IN STUDIES OF EXPERIMENTAL PROTEIN-ENERGY MALNUTRITION

An urgent need exists to establish criteria and standards for assessing the nutritional status of animals in studies of experimental protein-energy malnutrition. At present, it is difficult to assign most experimental animal systems to any particular category of human malnutrition, and this problem limits the relevance of experimental studies to the human disease. Two critical variables are stage of life and the nature of the diet-induced disease (i.e., whether the form of protein-energy malnutrition imposed is stunting or wasting and whether it should be classified as moderate or severe). These points have been discussed elsewhere (99).
Stage of life appears to have a critical influence on the impact of both wasting and stunting forms of protein-energy malnutrition on immune function (99). For example, the rejection response to a completely MHC disparate skin allograft was profoundly depressed in wasting protein-deficient weanling mice (81). This reflects a depression in the competence of naive T cells of both CD4$^+$ and CD8$^+$ phenotypes. In contrast, a comparable primary one-way mixed leukocyte reaction revealed functional sufficiency on the part of naive CD4$^+$ and CD8$^+$ T cells from wasted, protein-deficient adult mice (56). Also in connection with adaptive immunity, the ability of murine macrophages (in a preparation probably also containing dendritic cells) to present antigen in vitro to memory T cells was unaffected even by severe wasting protein deficiency when imposed at the adult stage of life (56), an outcome contrasting with results of similar studies of antigen presentation in weanling protein-energy malnutrition (115,119). In relation to innate defenses, the capacity of macrophages to synthesize and release IL-1 and TNF-α bioactivities was preserved in young adult mice subjected to nonedematous (i.e., marasmic type) protein-energy malnutrition (128), and comparable findings are reported relating to the production of IL-1, IL-6, and TNF-α by monocytes from elderly human subjects with nonedematous protein-energy malnutrition secondary to a variety of noninfectious, nonmalignant conditions (129). These results contrast with the depressive influence of marasmus on the production of these cytokines by monocytes from children (53,54).

Apart from the factor of physiologic age, diet composition is a critical point of misunderstanding and misinterpretation relating to studies of experimental protein-energy malnutrition. It is important to duplicate the critical features that characterize a human pathology, but less important to reproduce the details of the human diet that produced the pathology. Currently, much skepticism as to the value of studies with experimental animals centers on the details of dietary composition. However, it is not surprising that species differences exist relating to the details of the diet required to produce a particular pathology. For example, it is reasonable to expect that a lower dietary protein content would be required to produce the signs of kwashiorkor in a coprophagous animal such as a rodent than in children. As a separate but related point, it is desirable that growth and physiologic indices should be applied as rigorously in studies of animals as they are in studies of children. However, to render this possible, it is necessary to establish standards that can be used to connect experimental models with specific categories of wasting and stunting malnutrition in childhood. Currently, this essential information base is entirely lacking.

A third point regarding experimental design relates to the need for a zero-time control group in order to identify diet-induced immunologic change unambiguously. This design feature is rare, but is particularly important where diet-related influences can be confounded by ontogeny. For example, in a recent study of protein-deficient weanling mice (89), comparison with an age-matched control group emphasized the small sizes of both the splenic IgG-containing cell compartment and the intestinal IgA-containing cell compartment in the deficient animals. It would be easy to interpret these results as reflecting compartmental atrophy. Comparison with a zero-time control group, however, revealed a malnutrition-associated attenuation of the onto-
genetic expansion in splenic and intestinal plasma cell numbers. This feature of
design, thus, provided an important additional perspective by highlighting the remark-
able resistance of both mucosal and systemic antibody-producing effector compart-
ments to wasting disease, even in the weanling animal.

CONCLUDING PERSPECTIVE

A substantial catalogue of clinical and experimental information documents the in-
fluence of protein-energy malnutrition on immune defenses in the young. This body
of immunologic knowledge must continue to grow, particularly in relation to stunt-
ing disease and the more moderate forms of wasting malnutrition. Research direc-
tions with a mechanistic focus have also emerged. These activities include research
into cellular and molecular mechanisms and, at least as importantly, investigations
aimed at a metabolically integrated understanding of malnutrition-associated im-
mune depression as a component of the systemic, endocrine-mediated attempt to
adapt to dietary deprivation. Thus, even in wasting malnutrition, it is probable that
the associated immunodepression has adaptive value, although it imposes an unac-
ceptable cost in terms of risk of opportunistic infection (104). A key factor limiting
opportunities to develop depth of knowledge on immunocompetence in protein-en-
ergy malnutrition is the paucity of thoroughly characterized experimental animal
models and the complete absence of growth and physiologic standards that would al-
low animal systems to be clearly related to established categories of childhood mal-
nutrition. Finally, although research on the immunologic response to protein-energy
malnutrition must continue, recent evidence suggests that microorganisms may also
respond to the nutritional status of the malnourished host (130). Thus, a diet-induced
compromise of antioxidant defenses can promote mutation to pathogenicity on the
part of normally avirulent microorganisms. As antioxidant status appears to be com-
promised in some forms of protein-energy malnutrition, a new dimension may be
emerging in the host–microbe interaction in this disease.

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DISCUSSION

Dr. Chandra: I would emphasize the age of the subject—whether human or an animal model, because obviously there are differences between immune responses at baseline and during nutritional stress. In addition to that, I think it would be useful to know the severity and the speed of induction of nutritional deprivation, both in animals and in humans. For instance, adults who are subjected to acute energy deprivation over 5 to 7 days of complete fasting have
a very different immunologic profile from a more chronic energy deprivation. Finally, I think that replenishing malnourished animals or humans with appropriate nutrients and looking at the immunologic profile afterward should also be done. It is only then that you can say that the results are truly related to the nutritional insult.

You raised the issue of what animal model might be most appropriate. For obvious reasons, subhuman primates are ideal, but they are very expensive and it is often difficult to get ethics approval to do experiments on them.

My final comment is that we also need to look more deeply into the question of movement of cells. We have all looked at blood cells, some have looked at tissue cells, but how cells or their components traffic from one site to another may also be important. From the limited information that is available, both for protein-energy malnutrition and perhaps for vitamin A deficiency, the cell surface proteins of lymphocytes are changed in a way that suggests they traffic differently to different sites, particularly to the gastrointestinal tract. I think that needs to be looked at in greater depth.

Dr. Woodward: Dr. Chandra's point on trafficking is an interesting one. I think you may be referring to the work of McDermott and Bienenstock on the mouse (1), showing that adoptive transfer of lymphocytes from malnourished into well-nourished animals resulted in failure of these cells to distribute in normal ways; in particular, they did not home in on the gut, where you would have anticipated they would go. Likewise, McMurray (2), who uses a very relevant model of the protein-deficient tuberculous guinea pig, has recently shown that CD8⁺, antigen-specific T cells in this system appear to home in on the lymph nodes draining the infected lungs, but cannot get into the lungs and hence cannot interact with the macrophages in the lungs; this provides some basis for an understanding of the inability of malnourished individuals to generate a granulomatous response. I see a real parallel between those results and what we have found in our own system with regard to an overabundance of naive phenotype, quiescent T cells, which one would expect to do exactly what McMurray found—that is, they would home in on lymph nodes but not on the nonlymphoid sites where infections are occurring, because that is how naive-type cells recirculate. So I suspect there really are, as Dr. Chandra has just said, some very important changes in the trafficking of T cells (and I am sure the changes are not limited to the T cells) that are induced by various forms of malnutrition.

Dr. Suskind: Some years ago, we did some studies where we put normal lymphocytes into media where one specific nutrient was deficient. We made up a series of media, leaving out a different nutrient in each experiment, then adding it back to give a dose-response curve (unpublished data). Interestingly, every single nutrient affected the cellular immune response. So, with the lymphocyte, we have a very sensitive cell that needs all those nutrients to function effectively. I think that was an important observation. I would be interested in your comments.

Dr. Woodward: I agree, I think that is an important observation and I doubt whether it is confined to the T cell. I would suspect that would be the case for any cell that might be cultured, immunologic or otherwise. The traditional nutritionist's perspective on any nutrient deficiency condition in an intact animal or human, let alone in tissue culture, would be that there is no such thing as a simple nutritional deficiency. So, for example, the idea that the nutritional deficiency produced with zinc deficiency or vitamin A deficiency is somehow a simpler condition than the one produced with protein-energy malnutrition, I think, is mistaken. In the tissue culture work you referred to, you may come closer than you can in an intact animal to seeing what a simple nutritional deficiency condition can produce, but I doubt that there is such a thing in the intact animal. That is the principal reason why I think in terms of the hormonal microenvironment within which cells have to operate as being the underlying physiologic determinant of the immunologic changes we see, rather than the availability of any particular nu-
trient. I believe that the idea that there is a generalized decrease in protein synthesis in response to decreased intake, and that this is responsible for the immune depression, is now untenable. The metabolism of the malnourished is every bit as complex and every bit as well-controlled as the metabolism of the well-nourished; it is just different. Different metabolic priorities are established, I am sure, by endocrine hormonal means, so what we are doing is studying an immune system that has come off the metabolic priority list.

Dr. Ashworth: In commenting on the diets fed to animals, you drew attention to the very low protein of your laboratory diet. It would also have been helpful to bring out the fact that the animals have complete mineral and vitamin supplements. That is in contrast to the reality of malnourished children, whose diets are low in micronutrients. I would support your emphasis on protein-energy malnutrition being a very complex entity; we need always to bear in mind that this is a multideficiency state.

Dr. Woodward: We did some work in the mid-1980s where we doubled and tripled the micronutrient package that we include in our experimental diets. This never made any difference. Regardless, for example, of the level of dietary zinc that we included, serum zinc levels were always profoundly depressed. We can put as much as 200 μg/g of zinc in the diet and it just does not touch serum zinc levels at all (3). I think there is a sense in which the protein-energy malnourished animal, and I suspect human too, is not formally deficient in micronutrients. That is not to say that they are in an admirable nutritional state, but I do not think they can respond to micronutrient supplements in the way that you and I can, because their metabolic priorities are different. So, giving a protein-energy malnourished animal a supplement of zinc is not going to do anything immunologically. In human populations where there appears to have been a response to zinc supplements, I think you will find the subjects were stunted, not wasted.

Dr. Cochran: Older studies have shown that if you subject rodents to malnutrition during pregnancy, the offspring are smaller, and it takes several generations for them to come back to the normal size, although they are well nourished. Has anybody looked at the effects on the immune system in that kind of a setting?

Dr. Woodward: There are studies of an intergenerational nature like that. The first one of which I am aware was done by Dr. Chandra, and then Lucille Hurley's group picked up on that in the early 1980s (4,5). In the United Kingdom, Barker's work on the impact of fetal malnutrition (although I think that is a term many would dispute) and its long-term impact on the ability of the endocrine system to develop properly (6), is a rather broad paintbrush view of what I suspect is the underlying mechanism. What we are seeing are long-term impacts on endocrine development, on the development of the heart and the pancreas and on the immune system as well. I suspect that the impact on reproductive hormones, whatever it may be, is such as to carry over and influence the development of other organ systems in the F2 and even the F3 generation.

Dr. Gershwin: In the early 1980s, we fed animals on diets deficient in zinc and we saw abnormal responses for the second and even the third generation, although the abnormality decreased significantly. We went back to those studies some years later, and found if the animals were housed in pathogen-free environments we did not see those multigenerational effects (5,7). So, although we never explained the mechanism, we felt that it had a lot to do with the flora and whatever organisms the animals happened to carry.

Dr. Griffin: In relation to early priming effects on development, some very interesting studies are coming from the Medical Research Council unit in Gambia. In that region, there is cyclical malnutrition in the dry season followed by good nutrition in the wet season. When children born in the dry, malnutrition season and in the wet, good nutrition season were fol-
lowed for 20 years, there was a profound increase in death from infection in the children born in the malnutrition season (8,9). The mechanisms are not known, but clearly some very early priming goes on which results in a relatively deficient immune system in later life.

Dr. Keusch: It does not necessarily make sense to me to take cells from a deficient environment in the host and put them into a nutritionally complete medium in the in vitro setting, but we will continue to need culture experiments to dissect out aspects of the immune response—we cannot do it on cells as they are removed immediately from the host. The environment in the host is not medium 199 or some other artificial mixture; in fact it is serum or plasma. So, from a standardization point of view, should we not be using autologous plasma for the cultivation of the cells? Analogous to many kinds of experimental situation where we are trying to standardize results across geographic regions and between laboratories, should we not establish reagent repositories of standardized reagents, and perhaps in this case standardized plasma? You asked for challenges for the next century. I put this challenge to Nestlé, to take on the development of a reagent repository that people could use in their own laboratories, so that they can compare autologous plasma with a reference sample in different situations. When we are able to characterize the nature of malnutrition using a standardized protocol to collect clinical information and to define the nutritional state of the host, I think we will make a lot of progress in understanding what we are talking about.

Dr. Kennedy: We looked at that issue in a lot of our experiments. We were puzzled by published reports on experiments in which lymphocytes or blood were placed in Roswell Park Memorial Institute (RPMI) or various types of media and still showed the biological effects under study. We felt that if these effects were related to micronutrient deficiency they should be reversed in RPMI media. We found that it did not matter whether we used autologous plasma or whether we supplemented with RPMI media, and this led us to believe that in our studies at least the nutritional deficiency may not be playing the primary role in modulating the immune response. I am not saying that it plays no role at all, but perhaps the nutritional deficiency sets up a series of neuroendocrine changes that are what really affects the immune cell, and that repleting nutrition as a single entity is probably not going to alter the immune deficit.

Dr. Woodward: Some old studies have indicated that unidentified serum factors (still unidentified in fact) in malnutrition can exert influences such as reduced T-cell blastogenesis, whereas serum from otherwise comparable, well-nourished controls does not exert such suppressive influences. I am quite taken by the idea of using autologous serum as part of an experimental design. At the same time, there clearly are hormonal influences that program cells, so that once you take them out of the environment in which they have been programmed, they continue to function as if they were still in the old environment.

Dr. Tontisirin: Can you summarize the intercellular communications in malnutrition? What are the hormonal or endocrine mechanisms?

Dr. Woodward: There have been a number of suggestions of what might influence down-regulation of cytokine production on the part of T cells. One is glucocorticoids. High levels of cortisol can be downregulatory for many cytokines (IL-1 to 6, IL-11, TNF-α, GM-CSF, and so on). I am sure there would be individual dose-response curves that we would need to sort out for each of those. Another really interesting suggestion is that the eicosanoid metabolism of the malnourished is shifted toward prostaglandin synthesis, and everybody focuses on PGE2, which will do some specific things such as reducing the ability to produce IL-2. Thirdly, an interesting possibility has come out of McMurray’s work at Texas A&M, in which he found in his model of low protein tuberculous guinea pigs a high level of transforming growth factor-β in the blood (2). That is a broadly immunosuppressive, although not uniformly immunosuppressive, cytokine; in fact, I even wonder if it might be a contributor to the long-
standing observations of what autologous serum from the malnourished can do to blast cell transformation. So, several possibilities exist.

**Dr. Suskind:** I think we forget that a number of years ago the real emphasis was on the influence of nutrition on the endocrine system. I wonder if somehow we could bring together all that information and gain new insights into those changes that occur in the immune system that can be modulated by nutrition, or by nutrition via the endocrine system.

**Dr. Woodward:** The amount of experimental work directly demonstrating that you can influence immune function by intervening with a hormone or by removing a hormone is really quite slight. It is one thing to say that A results in B, and B results in C, but you cannot then say A causes C. That is why I would say the information base we have right now is sufficient for hypothesis generation but nothing more.

**Dr. Chandra:** In relation to the possible role of sex hormones in modulating the immune response in nutritionally deprived individuals, some studies show that antibody affinity and phagocytic abilities, when tested in protein-deficient or amino acid-deficient mice, show different results in males and females. As the rest of the protocol is similar, there is an obvious need to investigate this further to explore the role of the sex hormones.

**REFERENCES**