Phenotypic and Functional Considerations in the Evaluation of Immunity in Nutritionally Compromised Hosts

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It is well established that proper nutrition is critical to the development of an effective immune system and to enhance the natural immunosurveillance and its effector mechanisms. This enhancement could be mediated either by increasing the frequency and absolute numbers of effector cells or by up-regulation of the cellular mechanisms by which these effector cells carry out their functions. Even in the Western world, large sectors of society often remain undernourished and show suboptimal immune responses, but the relationship between nutrition and immunity is best seen in developing and underdeveloped countries. Although there are many large-scale field studies that investigate the issue of nutrition and immunity, there are relatively few data that go beyond descriptive measurements and directly address how well the immune system functions. This review summarizes interactions between nutrition and immunity and focuses on practical aspects for evaluation of the immune function in the field.

One of the most frequent questions in nutrition is whether nutritionally at-risk hosts have a defect in their immune system and whether such defects can be corrected by nutritional supplementation. In fact, one can easily conclude that much of the Western world is concerned about immune function. You have only to pick up a copy of Newsweek or similar magazines to find some new claim for a nutritionally and immunologically superior product, such as a vitamin supplement, a food substance, or a novel botanical. Consumers have taken up this initiative, and there are people who consume up to 20 different multivitamins or dietary supplements per day. This is especially true of persons with AIDS, who often spend several hundred dollars each month just on over-the-counter dietary supplements. Even though the importance of nutritional status in AIDS is well established, little research exists on the immunologic benefits of dietary supplementation to AIDS patients. Because AIDS is the leading cause of death in sub-Saharan Africa, where malnutrition and individual nutrient deficiencies remain major problems, it is important to develop valid settings and methods with which to assess nutritional status and immune function in such populations. Here, we attempt to place nutrition and immunity in perspective. We also outline a strategy for reasonable field testing on nutritionally at-risk persons.

Nutritionally at-Risk Populations and Interactions between Nutrition and Immunity

The term “nutritionally at-risk” defines a person or population whose consumption and/or absorption of select nutrients is either deficient or excessive. The consumption, and particularly the absorption of nutrients, can be influenced by a variety of factors, including disease, diet-nutrient interactions, drug-nutrient interactions, and numerous lifestyle habits, such as alcohol intake and smoking. We want to highlight the point that disease states can affect nutrient uptake: Not only can nutrition influence the immune response, but immune responses can, in turn, influence nutritional status. For example, during a typical immune response, a variety of cytokines are released, and these cytokines, particularly tumor necrosis factor-α and interleukin (IL)-1, have profound influences on nutrient absorption and metabolism and on other host health parameters.

As with other animal physiologic systems, usable energy and the structural components required to build an immune system are derived through food intake. Without adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response. A few of the immunologic parameters that are often used as measures of the status of the immune system and its responsiveness to antigenic challenges include leukocyte number and mobility, oxidant balance, enzyme activities, antibody production, and IL release [1]. General malnutrition and protein deficiency, alone or in combination, can result in severe abnormalities in essentially every immune function (summarized in table 1). Deficiencies in individual vitamins have detrimental effects on a variety of natural and acquired immune responses. For example, in experimental animals, vitamins A, B6, biotin, thi-
Table 1. Results of malnutrition with or without protein deficiency.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Decrease in</th>
<th>Increase in</th>
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<tbody>
<tr>
<td>Food restriction [2, 3]</td>
<td>Immune incompetence at ≤60% of body weight</td>
<td>Circulating B cells</td>
</tr>
<tr>
<td>Caloric restriction (mice and rats) [4-8]</td>
<td>Plasma complement</td>
<td>Circulating antibodies</td>
</tr>
<tr>
<td>Severe protein and protein-energy deficiency [9-11]</td>
<td>Tumor virus expression and malignancies</td>
<td>T cell proliferative response</td>
</tr>
<tr>
<td>Protein deficiency (mice) [12-17]</td>
<td>Proliferation of autoreactive B1 cells</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Amino acid restriction (especially arginine and glutamine) [18]</td>
<td>Proinflammatory and Th1 cytokines (IL-6, TNF-α, TGF-β)</td>
<td>Th2 tolerance</td>
</tr>
<tr>
<td>Nucleic acid restriction [19, 20]</td>
<td>NK activity</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Fatty acid supplementation [21, 22]</td>
<td>Macrophage functions</td>
<td>Splenic suppressor T cells</td>
</tr>
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NOTE: All data from humans, except where indicated as in mice or rats. DTH, delayed type hypersensitivity; IL, interleukin; NK, natural killer; TNF, tumor necrosis factor; TGF, tumor growth factor.

* Mice fed even moderately protein-deficient diet will reduce their food intake, resulting in protein-energy malnutrition.

The nutritional deficiencies that have all been associated with reduction in thymic weight, which is often accompanied by a decrease in lymphocytes [2, 23, 24]. Reduced lymphocyte proliferative responses have also been reported in deficiencies of vitamins E, B6, B12, and biotin [2, 23–25]. Delayed-type hypersensitivity (DTH) reactions are depressed in animals fed diets deficient in vitamins A, B6, B12, C, and E [24, 26, 27], and antibody responses are compromised by diets low in vitamins A, B6, E, pantothenic acid, thiamin, and riboflavin [26, 28]. In addition, natural killer (NK) cell activity is diminished by vitamin A deficiency [29, 30], and reduced phagocytosis has been observed in deficiencies in vitamins A, C, E, and B12 [2, 24, 27]. Furthermore, numerous cytokine abnormalities (e.g., increased interferon-γ and IL-12 in vitamin A-deficient mice [26, 30, 31]) have been reported in various vitamin deficiencies [2]. Repletion of animals deficient in a particular vitamin restores their impaired immune responses. Similar findings have been reported in human subjects. It is well established that immune function declines with age and that many elderly people have micronutrient deficiencies. In this population, supplementation with various vitamins [32], and particularly with vitamin E [33–35], significantly enhances DTH and proliferative responses and antibody titers after vaccination. Similar lack of particular vitamins, deficiencies in trace elements and minerals can also affect a variety of immune responses (summarized in table 2).

The nutritional deficiencies that are of particular interest here involve those that compromise an individual’s ability to resist infectious microorganisms or cancerous growths. Decreased leukocyte proliferation and phagocytic activity, as observed in many nutrient deficiencies, could result in less clonal expansion of microbe-specific clones of lymphocytes and less vigorous microbial elimination. Proteins play vital roles in immune responses as antibodies, cytokines, acute-phase proteins, components of the complement pathways, transcription factors, and enzymes. Alterations in proteins could, therefore, lead to immunologically important changes in enzyme-dependent antioxidant protection (selenium–glutathione peroxidase), transcription regulation (zinc finger proteins), complement activation, antibody-mediated virus neutralization, and intercellular communication via cytokines.

Shifts in oxidant balance either due to low antioxidant enzyme levels or deficiencies in antioxidant vitamins will have repercussions on cell function and survival. In addition, infections, particularly viral infections, are accompanied by increased production of reactive oxygen species, further depleting the host of antioxidants [47]. Furthermore, vitamin E– or selenium-deficient animal hosts provide an environment in which a benign coxsackievirus can mutate into a virulent strain [48]. Similar findings were made in glutathione peroxidase knockout mice. Taken together, these results suggest that oxidative stress due to either dietary or enzymatic deficiencies can influence the virulence of a virus.

Other alterations in immune responses due to dietary deficiencies, as described above, could also have a myriad of effects on immune responsiveness and homeostasis through the disruption of cooperative leukocyte activity. Thus, the immune problems related to nutritional deficiencies could range from...
increased opportunistic infections and cancers, to suboptimal responses to vaccinations, and perhaps to other immunologic disorders such as allergies.

The critical role that nutrition can play in a disease is illustrated by AIDS. It is well established that the progression of the disease is influenced by the patient’s nutritional status [49]. Optimal nutrition plays a preventive role by supporting T cell development. However, one must not forget that asymptomatic human immunodeficiency virus infection is accompanied by heightened proinflammatory cytokine production, which can significantly impact the host and ultimately interfere with host protective mechanisms. In fact, if one studies patients with AIDS with respect to nutrition, clearly one must take into account far more than CD4 cells (e.g., the study of leukocytes, oxidant balance, enzyme activity, antibody production, and soluble factors). All of these parameters and more are affected by nutrition; however, it is difficult to find studies in the peer-reviewed literature in which a comprehensive attempt at studying nutrition and AIDS was made. Regrettably, it can be argued that at present a problem with most nutrition-immunology studies is that they are done by nutritionists.

In addition to providing an example of how host nutritional status affects disease progression, AIDS also illustrates that disease can, in turn, influence nutrient intake, absorption, and metabolism. Malabsorption due to enterocyte injury resulting from protozoan infection or due to ileal dysfunction caused by Escherichia coli infection is common in AIDS patients and is often accompanied by undereating and weight loss [50]. Diarrhea is another important contributing factor to this wasting syndrome [50]. It was recently shown that the micronutrient deficiencies associated with this wasting syndrome could not be significantly corrected by supplementation [51], possibly because of both malabsorption and increased loss due to diarrhea. Thus, in evaluating a person’s diet with respect to the identification of potential deficiencies, it is critical to note that a suboptimal nutritional status can arise through multiple mechanisms.

While malnutrition and individual micronutrient deficiencies can contribute to disease susceptibility and progression, excessive intake of nutrients also is a risk factor. Thus, an obese person is potentially another example of a nutritionally at-risk host. Despite epidemiologic associations between obesity and decreased immunocompetence, there have been only a handful of studies concerning the effect of obesity on the immune system, and the results have been in conflict [52–54]. Finally, various lifestyle habits can either directly or indirectly—by influencing nutritional status—affect immune function. There is increasing evidence of a relationship between smoking, abuse of drugs, nutritional status, and immune function. Although we cannot begin to discuss these issues in detail, we direct the reader to several recent reviews of these topics [55, 56].

### Assessing the Immune Status of an Individual or a Population

With all of the above issues in mind and with the knowledge that we are not attempting to present a complete overview of nutrition and immunity, we turn to the issue of field evaluations. Public health management requires the constant monitoring of human populations and the resources on which they depend, including food. Regrettably, numerous populations worldwide are not receiving diets that allow them to reach or maintain minimal health standards. The morbidity and mortality characteristics of these underserved populations are in part related to underlying properties of their immune systems. The typical measurements in the field are serum immunoglobulins, levels of CD4 and CD8 cells, complement, and sometimes autoantibodies. These are descriptive measurements of phenotypic im-
mune responses that do not always provide significant information on how well the immune system functions. In fact, the ideal response is determined by what a human will do when exposed to an antigen, since the immune response was developed in evolution to protect us from infections. In our opinion, it is critical that monitoring of immunologic function be integrated into public health management systems to better define the health problems of the undernourished.

Because nutritional deficits are often associated with societal disruptions, poverty, and inadequate public services, the materials and personnel needed to obtain and interpret immunologic information are often limiting. The types of immunologic studies and tests that can be done on selected individuals or populations depend on a number of factors, including the ability to store and transport biologic samples and test equipment, test site location (a major city or an isolated township or village), and available personnel and their level of expertise. Thus, the conditions under which immunologic information can be obtained varies widely.

A flexible system that can adapt to a variety of situations, but still achieve the goal of obtaining useful immunologic information, is desired. We have proposed that a practical testing structure for nutrition and immunity include a tiered system [57]. In this system, one applies increasingly sophisticated examinations of the study materials and subjects in moving from provincial and isolated sites to metropolitan centers. It must be kept in mind, however, that even in countries with similar economic conditions, there will be great variability in facilities, equipment, and personnel available for immunologic analysis in the different settings.

The Tier System

We provide the following description as an example of the different conditions that one might confront and the studies that could be done within these circumstances (table 3). In real situations, the feasibility and appropriateness of the methodologies will depend on the actual working environment, the particular needs of the public health personnel and the treated population, and the available resources.

When deemed necessary, biologic samples could be sent to a centralized facility (e.g., from tier one or two regions to a tier three region) in order to do tests in a more controlled environment or to gather information on additional immunologic parameters. However, the practicality of preservation must be considered (e.g., cryopreservation), and transport of samples would depend on factors such as distance between sites, the condition of roads or airfields, temperature-controlled storage, and the ability to communicate and transmit information. While many isolated sites may have airfields and planes, the expense of their use needs to be well justified or compensated. The parties must also coordinate the arrival of samples with the readiness to deal with them. Nevertheless, several immunologic tests are feasible even under conditions that preclude sophisticated analyses. Below we outline the conditions likely to be encountered in tier one, two, and three areas and the immunologic assessments possible under these conditions.

**Tier one.** The first tier is embodied by a working situation in which a permanent health facility is not available. In this case, public health workers may travel into extremely rural or undeveloped regions and be required to perform examinations from temporary quarters such as tents. The population being examined may reside in relatively permanent settlements (i.e., villages) or be migratory and live in impermanent shelters. Water will come from a well or neighboring stream and electrical power should not be expected to exist. As a result, these on-site tests should not require temperature-controlled or sterile environments or constant electricity (unless a portable generator is available).

Skin tests are appropriate for the first tier. These would include dermatologic testing for contact hypersensitivity to various immunologic stimuli and tuberculin-type assays [58]. The subjects could either have a history of known contact with immunogenic materials or have received past vaccinations. Subsequent skin testing could be used as an overall measure of the person’s ability to generate an immunologic response. Immunodeficiencies may be evidenced by lower than normal or absent responses to antigenic challenges. The length of time needed for an observable skin reaction will depend on the underlying immunologic mechanism in progress: That is, antibody IgE-mediated “immediate hypersensitivity” will be evident in minutes while cell-mediated “delayed hypersensitivity” must be followed over several days. Blood smears can also be done in the field. Staining and microscopic analysis of blood cells for hematologic counts will give general information on the numbers of different white blood cells.

### Table 3. Hypothetical tiering system for assessing populations by settings.

<table>
<thead>
<tr>
<th>Tier</th>
<th>Characteristics</th>
<th>Methods</th>
</tr>
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<tbody>
<tr>
<td>One</td>
<td>Absence of permanent public health facility</td>
<td>Dermatologic hypersensitivity tests, Hematology</td>
</tr>
<tr>
<td>Two</td>
<td>Rural medical clinic</td>
<td>Immunodiffusion, ELISA, and electrophoresis for immunochemical analysis, Short-term cell culture for chemotaxis, phagocytosis, antibody production, Immunofluorescence staining with fluorescent microscopy for cell phenotyping</td>
</tr>
<tr>
<td>Three</td>
<td>Medical center</td>
<td>Flow cytometry for cytokine analysis, cell phenotyping, cell cycle analysis, In situ hybridization and reverse transcription polymerase chain reaction for cytokine analysis, Longer-term cell culturing for cytotoxicity, proliferation, antigen-presentation</td>
</tr>
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Tier two. The tier two scenario may be a rural clinic. There may be running water, although its quality may not be equal to that of a modern city in a developed country. Portable filtering devices and boiling are probably desirable. Electric power is fairly dependable but subject to occasional (maybe once-a-week) outages. There may be considerations that limit the feasibility of some types of research and diagnostics work. For example, depending on the location and time of year, the ambient temperature may be higher than incubator temperature. An air-conditioned workroom is an improbability (but not impossibility), but there will be no ultra-cold (−70°C) freezers for storage and no cold rooms. Sterility for cell culturing may be problematic. This type of site would not have facilities for the responsible disposal of biohazardous or radioactive wastes, thus precluding the use of assays that generate such waste. On the other hand, equipment such as fluorescent microscopes and ELISA readers are conceivable in this setting, assuming there are sufficient study subjects to justify such procedures. These types of materials need not be permanent but could be brought into the clinic on a temporary basis when needed.

A variety of the immunochemical tests described are feasible in a tier two setting. Immunodiffusion assays probably constitute the least complicated and least expensive way of determining the classes and subclasses and antigen specificity of antigens from peripheral blood. ELISAs are more quantitative and sensitive than immunodiffusion tests and might be appropriate for the second tier. The feasibility of these assays may be limited by the ability to obtain necessary materials including plasticware, ELISA reader, pipettors, and immunologic reagents. However, there are no significant obstacles to their safe storage or competent use in a rural setting. The same may be said for other biochemical methods (e.g., ELISAs) for the concentrations of serum complement proteins or SDS-PAGE. The materials for these analyses are easily stored, and the temperature (ambient, domestic refrigerator/freezer) and utility (electricity and water) requirements can be met by most rural clinics. Determining the activity of complement proteins from patient sera may represent somewhat of a problem since the shelf-life of commercial sheep blood cells is limited. Other assays that could be feasible in tier two facilities include the measurement of phagocytic and chemotactic activity, the quantitation of antigen-specific B lymphocytes by the ELISPOT assay, and the staining of cells with fluorescent antibodies and counting by fluorescent microscope to determine the relative numbers of different types of peripheral blood lymphocytes.

Tier three. The third tier envisions a modern medical center in a capital city. This facility provides the personnel, expertise, materials, storage facilities, and laboratory equipment needed for more expensive, highly specialized, and probably less frequently done tests. In such a facility, the proliferative capacity of lymphocytes may be measured by either the mixed leukocyte reaction or by mitogen stimulation. The cytotoxic capacity of peripheral blood T lymphocytes and NK cells are also more appropriate for tier three than for tier two since cytotoxicity must be demonstrated on live target cells. This will require facilities for the culture of target cell lines and possibly the in vitro generation of target-specific cytotoxic T cells [59]. Also, proliferation and cytotoxicity are both commonly measured with radioactive isotopes, and the cost and size of radioactivity counters combined with the need for safe disposal of radioactive waste probably preclude the use of these methods in tier two areas. However, nonradioactive methods, such as the MTT assay for proliferation [60], require only a microtiter plate reader and no disposal of radioactive waste.

A Simplified Practical Approach

The clinical association of particular interest is between malnutrition and an individual’s ability to respond to infectious microorganisms of their antigenic constituents. Mechanisms include reduced phagocytic activity and decreased leukocyte proliferation which, respectively, result in less vigorous microbial elimination and poor clonal expansion of microbe-specific lymphocytes. In addition, cell cycle, transcription regulation, antibody production, cytokine secretion, and antioxidant protection may also be altered. Thus, the immune problems related to nutritional deficiencies vary from increased opportunistic infections to suboptimal responses following vaccination. In such cases, dietary supplementation is desirable, but the key questions are which patients should be selected and how efficacy can be determined after intervention.

Practical issues play a major role in the assessment of immune function in a local population. In a simplified paradigm in which small volumes of sera are used, the information in the tiered system can be applied to the role of nutritional intervention to improve immune competence based upon data of either the local pathogens or the immunization record. This can take advantage of already banked serum specimens, eliminating the need for additional blood drawing. As a field assay, ELISA measurements of antigen-specific circulating antibodies may provide a “snapshot” of immune status. Antibodies are associated with virus neutralization and complement activation to neutralize pathogens such as bacteria and atopy and should be produced by all residents against indigenous pathogens such as malarial antigens, rotavirus, trypanosomes, arboviruses, flaviviruses, and E. coli. Titer and isotype analysis of antibodies against regional pathogens could provide an indication of a person’s ability to respond to infection and would be the first simple outcome measure for assessing immune status and the effect of nutritional supplementation. For example, the antigens chosen could be panels of high-quality recombinant malarial antigens in the following nine groups: circumsporozoite protein; major surface proteins (MSP)-1, -2, -3, -4, -5; RAP1, RAP2; recombinant human osteogenic protein H3, antigen 512, AMA1, endothelial barrier antigen 175; acidic basic repeat antigen, rC1-2; ring-infected erythrocyte surface antigen, MESA;
knob-associated histidine-rich protein, ASL; BIP, lactate dehydrogenase; and Pfs25 and Pfs28.

One would use a pyramid evaluation of these antigens. If there are restricted serum volumes, we recommend testing only for MSP, MSP-2, rcl-2, and RAP1 and RAP2. A similar scheme can be organized for rotavirus or for any antigens for which the persons under study were previously immunized. Technically, one would draw blood and separate the serum in the field and then use the serum in a simple ELISA against a panel of antigens specifically chosen for the geographic region. Once sera have been obtained, samples can be easily transported to a nearby facility with the basic reagents and equipment needed to perform an ELISA. The plates used for analysis can be prepared elsewhere ahead of time and stored until needed. Thus, as a field analysis of immune status, a screening of antibody titer and isotype by ELISA against regional pathogens before and after immune supplementation would yield valuable information regarding the effects of nutritional intervention. We estimate that the subjects should be as homogeneous as possible and thus stratified by age and sex; statistical estimates for this work would be 50-100 subjects/group with blood volumes of 0.25–2 mL of serum. If needed, the volumes can be miniaturized further. Much useful data can be developed from this very simplified outline.

Summary and Conclusions

We have discussed some of the ways in which nutrition-immune interactions can come about and their implications for human health. We also proposed a possible multitier system that can be used to study nutrition-immunology issues in developing areas. With respect to such populations, nutritional deficiencies are often associated with social disruption and lack of essential services. Thus, we think the collection of sera in endemic areas, before and after dietary supplementation would yield valuable information regarding the effects of nutritional intervention. We estimate that the subjects should be as homogeneous as possible and thus stratified by age and sex; statistical estimates for this work would be 50-100 subjects/group with blood volumes of 0.25–2 mL of serum. If needed, the volumes can be miniaturized further. Much useful data can be developed from this very simplified outline.

References


