Micronutrients and Infectious Diseases: Thoughts on Integration of Mechanistic Approaches into Micronutrient Research

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Results of field and laboratory studies provide convincing evidence that micronutrient deficiencies contribute to the mortality and morbidity of infectious diseases. Despite encouraging results in large trials, understanding the mechanisms by which micronutrients contribute to the outcome of the encounter between an individual and an infectious agent requires additional hypothesis-driven research. Presumably, such understanding should lead to translational studies with targeted nutritional therapy. Although these mechanistic studies are varied and complex, they must be done systematically and should include examination of the mechanisms by which micronutrients affect host-pathogen interactions, development of appropriate animal models and reliable methods for the assessment of micronutrient levels, and translation of the results of basic research findings into clinical studies. Moving the frontiers of micronutrient research from the laboratory to the field will be challenging. However, sound scientific research should lead toward better human health.

Infectious diseases remain the biggest killer of children and young adults worldwide [1] despite scientific advances in immunology, biochemistry, and molecular biology. It is generally accepted that poor nutritional status increases susceptibility to infectious diseases and that severe micronutrient deficiencies lead to specific syndromes [2–6]; this awareness has led to the conduct of several micronutrient supplementation studies. However, quantification of the contribution of nutrition to infectious disease mortality is difficult. Although there has been considerable recent progress, little is known about the impact of subclinical micronutrient deficiencies on the immune system, susceptibility to various infectious pathogens, and clinical manifestations. Further, the basic mechanisms by which micronutrients alter the immune response and subsequently the resistance or susceptibility to infection are unknown. Of more importance, micronutrient studies could benefit from better understanding of the mechanisms involved in the interrelationships between micronutrients and infectious diseases.

With this mechanistic view in mind, in September 1999 the National Institute of Allergy and Infectious Diseases sponsored a workshop [7] to examine the current cellular and molecular immune mechanisms by which micronutrients affect host-pathogen interactions. Participants included infectious disease experts, nutritionists, immunologists, and epidemiologists. At the end of the workshop, recommendations for future research were presented by the panel of experts [7]. These recommendations can be summarized into four broad categories—analytic and diagnostic methods, basic mechanistic studies, epidemiologic/field studies, and training [7]. It is clear that most of the research goals have remained the same over the years.

Here we identify and address some of the challenges in the field of micronutrient research with a strong bias toward mechanistic approaches. There is need for continued research in several areas: mechanistically driven micronutrient studies, further development of animal model systems, and strategies for the translation of basic research into therapeutic procedures.

Need for Studies on the Mechanisms of Micronutrients and Infection Interactions

Recognition of micronutrient deficiencies in the less developed world has led to large-scale supplementation and epidemiologic interventional studies, sometimes with conflicting results. The conflicting results may be caused by the lack of understanding of the mechanisms by which micronutrients modulate the host-pathogen interaction. In the last three decades, nutritionists and, to a lesser extent, immunologists have contributed significantly to basic research involving micronutrients per se but less often within the context of infectious diseases. Although some infectious disease specialists and immunologists have made significant research contributions to the field of micronutrient research, increased involvement of both specialties will enhance our understanding of the immunopathogenesis of infectious diseases and likely facilitate research on both micronutrient and infectious pathogens. In that micronutrients are not a major consideration or area of study in most infectious disease research, one must ask, “Why not?”

A plausible hypothesis is that the cause-effect relationship between micronutrient levels and susceptibility to infectious diseases is usually not accepted on the basis of available data. In
general, causation in science is based on the strength of association, consistent relationships, dose-response relationships, biologic mechanisms, temporality, and experimental proof [8]. Biologic mechanisms are not essential to establish causation, but causation is much more difficult to demonstrate without a plausible biologic mechanism. For example, vitamin A therapy has convincingly reduced the mortality of measles in clinical trials and is now standard practice. The fact that vitamin A supplementation reduces mortality in measles is generally accepted because of reproducible consistent experimental evidence, temporal association of improvement, and dose-response data. However, there is no consensus on the mechanism by which vitamin A influences the disease process and reduces mortality. Without an understanding of the mechanism by which vitamin A influences measles mortality, subsequent studies on the use of supplements in this and other diseases is challenging.

Unfortunately, other large-scale clinical end-point trials have often yielded conflicting data. Pneumonia, for instance, improves with micronutrient supplements in children in some studies but not in others [9–12]. Despite the emphasis on mechanistic approaches, conflicting data could also result from a variety of other factors, including the micronutrient dose, the specific population and geographic location, and whether supplementation is daily or intermittent. In the vitamin A example, a team with expertise in nutrition, immunology, epidemiology, and infectious disease could develop additional hypothesis-driven studies to demonstrate the role of vitamin A in pneumonia; these protocols could then be extended to other clinical settings. There is no doubt that greater understanding of the mechanisms by which micronutrients influence immunity, disease susceptibility, disease manifestation, and host-pathogen gene expression will likely drive funding for micronutrient research in the infectious disease arena. To understand the basic cellular and molecular mechanisms of the role(s) of micronutrients in the control of infectious disease, it will be important to examine basic biologically relevant questions with clearly defined animal model systems.

**Need for Clearly Defined Model Systems**

A major challenge in developing experimental models to understand the basic cellular and molecular mechanisms involved in micronutrient research is the difficulty in integrating the several interacting variables (including host genetics, pathogen virulence, environmental factors, age, and gender) and in designing a clear testable hypothesis. Indeed, the processes by which micronutrients affect the capacity of an individual to mount appropriate defense strategies against infectious pathogens are varied and complex [13–17]. Although nutritional deficiencies can alter both innate and acquired immune responses to pathogens, the presence of microbial agents can also alter the nutritional status of the host and the host’s ability to absorb micronutrients. A number of studies have attempted to elucidate the mechanisms involved in host-pathogen interactions in a nutritionally impaired host. Understandably, it is difficult to define causal relationships between individual micronutrients and abnormalities in the immune system. Clearly, no individual micronutrient has been extensively studied with respect to all aspects of innate and adaptive immune responses.

Until recently, most investigators in this area of research have conducted separate studies to obtain an understanding of the impact of impaired nutrition on the immune response from those involving strictly host-pathogen interactions. Certainly, it is important to establish model systems that examine the significance of the genetics of host-pathogen relationships [18–20]. It is meaningless to talk about pathogen virulence or host resistance as separate entities without reference to a particular host or agent. Indeed, the clinical manifestations of infectious diseases result from an interaction between the pathogen and host. However, in “real life and real situations,” the host encounters a variety of environmental and nutritional factors that are capable of producing phenotypic changes that may override the genetic resistance to a particular pathogen [19]. As such, it is equally important to examine how other factors, specifically nutrition, affect these interrelationships.

Most studies that have been directed at understanding the interrelationship between the nutritional status of a host and the host-pathogen interactions have focused on the effects of the micronutrient deficiency on the immune response and the subsequent effects on susceptibility or resistance to infection. Little has been done to examine the hypothesis that micronutrient status may directly alter pathogen or host genes. To understand the interrelationship between host nutritional status and pathogen virulence, Beck et al. [21] used a system in which a myocarditic strain of the Coxsackie B virus (CVB3) causes active infection and disease production in mice while an amyo- cardiac, benign strain (CVB3/0) relative to the host does not produce disease even though active infection takes place. When selenium-deficient mice are injected with the benign strain, CVB3/0, they develop myocarditis. Subsequent analysis revealed that the development of myocarditis was associated with a change in the viral genome [21]. While it has yet to be demonstrated that this finding is a general biologic phenomenon, a similar question can be raised regarding the effect of nutrition on the status of the human immunodeficiency virus (HIV) in AIDS patients. Does the nutritional status of HIV-infected persons [22] contribute to a change in the mutation of the virus? Answers to such questions will be important in the development of intervention strategies for AIDS patients.

Once a well-defined model system is established, one can use the system to study cause-effect relationships, characterize factors involved in the resistance to infectious diseases for selected hosts and pathogens, and then to test the impact of micronutrients on such a system. The results of experimental studies have enabled investigators to establish immunogenetic associ-
For example, the association of resistance to a number of intracellular pathogens with the Nramp1 gene has been known for some time [20]. Likewise, although genes involved in pathogen virulence determinants are complex and difficult to clarify, some (e.g., the bacterial type III secretion system, which is required for pathogenenicity) have been extensively studied [28]. Recent advances in immunologic techniques and molecular genetics, including direct ex vivo analysis of antigen-specific lymphocyte responses by use of major histocompatibility antigen tetramer technology, the development of animal models with specific gene mutations or deletions, the availability of microarray technology, and the availability of infectious diseases databases, should make these experimental approaches feasible and enhance our understanding of the interrelationships.

To demonstrate the effect of micronutrients on host-pathogen interactions, it will be necessary to develop reliable and reproducible methods for the assessment of micronutrient levels. It was clear from the September workshop [7] and from others that the lack of reliable methods is a major obstacle in this area of research. For example, the measurement of micronutrients by serum or plasma levels may not be adequate. In some cases, it may be more appropriate to use levels in blood leukocytes and in immune organs. It will also be important to establish biochemical cut-off points for micronutrient deficiency. In that the production of acute-phase proteins is common in malnutrition, it will be necessary to include markers for acute-phase proteins such as C-reactive proteins. Thus, the availability of reliable methods for the assessment of the role of a micronutrient in host-pathogen interactions as well as an understanding of the molecular events involved in the process should pave the way for future therapeutic applications.

**Need for Translation of Basic Research into Clinical and Therapeutic Applications**

Micronutrients offer a potentially inexpensive feasible means of altering the outcome of infectious diseases in the developing world. However, any rational intervention could benefit from, among other issues, well-controlled basic and clinical studies designed to understand the mechanisms by which micronutrients influence the manifestation of infectious diseases. These approaches must consider the issues raised above in terms of host-pathogen interactions and other social and economic parameters. Thus, it would be important for recent methodologies in immunology and molecular biology to be incorporated into the development of intervention strategies (e.g., vaccines), to be utilized in the identification of target populations (e.g., by use of immunogenetic markers), and for design of trials involving multiple micronutrient supplements. It is possible that micronutrient intervention could improve vaccine response rates, shorten the course of infectious diseases, reduce mortality, and prevent pathogen mutation. Vaccines are potentially useful in light of emerging antibiotic-resistant strains and because of the difficulty in treating latent and persistent infections. Recent advances in molecular technology and the availability of several microbial genomes within the past 5 years should enhance progress in the development of microbial vaccine candidates.

Because micronutrients can influence a variety of diseases including chronic diseases with infectious etiologies, the nutritionally at-risk host must be clearly defined before any attempts are made at correcting these defects by supplementation. The results of recent compelling studies results indicate that infectious agents may be associated with the development of atherosclerosis and other cardiovascular diseases [29, 30]. For example, Chlamydia pneumoniae, a common respiratory pathogen, has been implicated in the development of atherosclerosis [29]. Studies suggesting that dietary factors are risks for cardiovascular disease have led to the conduct of intervention trials designed to examine the effect of vitamin E supplementation in heart disease. Results have shown both positive and negative effects [31, 32]. It would be prudent to target supplementation at clearly identified risk groups in the population and to base experimental designs on sound scientific principles with an understanding of the multifaceted issues.

Because no single nutrient can solve the problem of micronutrient deficiency, it will be necessary to examine combined nutrient programs as suggested by previous studies [33]. Although epidemiologic studies indicate a need to invest in nutrition programs, an understanding of the basic mechanisms by which nutrition alters the immune response and ultimately affects the infectious disease burden would provide useful guidelines for future investments. It will also be important to conduct studies to clarify which micronutrients are required, the dose that will influence the immune response, and to identify possible common intracellular pathways involved in the functional activity of the micronutrient. Thus, it will be important to bridge the gap between molecular biology and nutritional epidemiology. Further understanding of the combined use of nutritional supplementation programs and vaccination strategies on defined target populations will be critical in developing cost-effective health interventions.

In summary, optimal clinical use of micronutrients in the reduction of mortality associated with infectious diseases will be facilitated by a greater understanding of the cellular and molecular mechanisms by which micronutrients modulate the immune system. Well-established animal models and the use of state-of-the-art molecular biology and immunologic technologies will facilitate both the exploration of mechanisms of immune modulation and the potential effect of micronutrients on pathogens. Focused clinical studies, based on results from well-established experimental models, can bring micronutrient research from broad supplementation programs to potentially targeted therapeutic adjuvants in specific infectious diseases or in the context of vaccination. Approaching the study of micronutrient supplementation with mechanistic rigor in the con-
text of hypothesis-driven research will enhance understanding and hopefully utilization of an inexpensive intervention that may be of global health importance.

References