

Symposium: Nutrition and Infection, Prologue and Progress Since 1968

The History of Nutrition: Malnutrition, Infection and Immunity^{1,2}

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ABSTRACT The relationship between nutritional status and the immune system has been a topic of study for much of the 20th century. Dramatic increases in our understanding of the organization of the immune system and the factors that regulate immune function have demonstrated a remarkable and close concordance between host nutritional status and immunity. This report traces the increasing sophistication of our understanding of these relationships and their impact on susceptibility to infection through six stages to the present time. The cyclical relationship between poor nutrition, increased susceptibility to infectious diseases, leading to immunological dysfunction and metabolic responses that further alter nutritional status is described and, wherever possible, related to physiological mechanisms. In addition, the particular role of Nevin Scrimshaw in guiding the progress over the past 50 y is discussed. *J. Nutr.* 133: 336S–340S, 2003.

KEY WORDS: • *malnutrition* • *protein-energy malnutrition* • *micronutrients* • *immune system*
• *cell-mediated immunity* • *humoral immunity* • *complement system* • *host defense mechanisms*
• *infectious diseases*

This symposium on the history of nutrition, with particular emphasis on our increasing understanding of the interactions of nutritional status with the immune system and its impact on host susceptibility to infection, is also a history of the career and contributions of the convener, Nevin Scrimshaw. My own abiding interest in this field is a direct consequence of meeting Nevin in 1968. I was, at the time, an infectious diseases fellow in Boston, with the opportunity to take courses at Massachusetts Institute of Technology, where Nevin was chair of the Department of Nutrition and Food Science; and so I registered at MIT for his course on nutrition. I was struck by his boundless enthusiasm for the subject as well as his willingness to engage with students with little to contribute except questions. His great cheer was also in part because he had finished writing the now world-famous 1968 WHO Monograph on nutrition–infection interactions together with Carl Taylor and John Gordon (1). Timing is, of course, a critical element in science, and, for the field he has done so much to create, the timing of this colossal work was just about perfect. The remainder of this presentation serves as the evidence for this statement. I describe six discernible stages in the history of our

knowledge of nutrition–immunity–infection interactions. As we have learned of late, history as written by some contemporary historians is sometimes indistinguishable from plagiarism. In this brief account of the history of this field, I acknowledge up front that I will plagiarize ideas from many who have contributed to the development of the field, many of whom are in this room today, without worrying about citations. This talk is not about my work, or the work of any one individual, including Nevin; rather it is about our collective work and the generation of big new concepts from kernels of insight, most of which were nurtured by Nevin Scrimshaw over the past 50 y of his amazing career.

Stage I: the dark ages, before 1959

In the beginning it was dark, although looking back, occasional isolated points of light can be discerned before 1959. Some of these points of light came from the epidemiological work Nevin Scrimshaw and colleagues carried out during the first 10 y of his tenure as the founding Director of the Instituto de Nutricion de Centro America y Panama (INCAP) in Guatemala. This body of careful field and clinical research center-based metabolic studies documented the close relationship between infectious diseases and the progressive malnutrition so common in that population. During the decade of the 1950s, our knowledge of the immune system was primitive, and much of what was known focused on the development of antibody humoral immunity, probably because antibodies could be measured functionally in vitro and were clearly important to host defenses in vivo. Although it was known at that time that plasma cells were responsible for producing antibodies, there was no clear understanding of the relationship of plasma cells to the lymphocytes circulating

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in the blood or present in the aggregated lymphoid tissues of lymph nodes, the spleen, thymus and lymph follicles elsewhere in the body. In fact, circulating lymphocytes were considered to be dormant resting cells, all of which looked alike and possessed no known or even suspected function. There was no concept of immunoregulation, the notion that the immune system is a finely tuned and complex multicell response machine that is closely regulated by signals generated during the immune response from various cells within and outside the immune system.

Most of the available information was derived from animal studies, in which animals were deprived of certain nutrients in the diet and subjected to challenges with living or dead microorganisms of one sort or another. The outcome measure was antibody activity, usually measured 4 wk postchallenge, which means all of the early events and their kinetics were overlooked. Unfortunately, the animal models represented, at best, only moderately faithful simulations of the human situation. This was because animals were fed a defined diet deficient in the specific nutrients of interest, with everything else in sufficient amounts, because the diet was provided by a defined schedule or even ad libitum, because the animals were maintained on a strict cycle of light and dark, and because they were generally protected from external infectious diseases. In each of these features the animal models differed totally from the human situation. Although we now know there are marked similarities in the organization and function of the immune system between animals and humans, there are also significant differences, especially between humans and chickens, which were often used for studies of antibody immunity in these earlier studies.

From the perspective of human nutrition, it was considered that protein-energy malnutrition (PEM), or protein-calorie malnutrition as it was then called, was attributable principally to dietary deficiency and therefore it could be prevented or treated by dietary measures alone. There was also little or no contact between immunologists or infectious diseases specialists who studied host susceptibility to infection and those interested in nutrition. Without doubt, the best immunology of the day was not being applied to nutritional diseases.

The renaissance: 1959–1968

In 1959, an exceptional review paper by Scrimshaw, Taylor and Gordon appeared in the *American Journal of the Medical Sciences*, documenting the extensive and cyclical interactions between malnutrition and infection (2). The authors made the case that malnutrition resulted in increased susceptibility to infection, and that infection caused deterioration of nutritional status, ushering in a cycle of malnutrition–infection that would ultimately lead to kwashiorkor (the clinical manifestations of which were usually triggered by an infection as well) and, if untreated, to death.

The interactions were described as generally synergistic, occasionally antagonistic, as amplified by Scrimshaw in this symposium. Synergistic interactions, the most common, were those in which the combined effects of malnutrition and infection were more profound than the sum of the individual effects of either one alone. Antagonistic interactions were occasionally documented in which the presence of malnutrition lessened the impact of an infectious disease. One example, described in mechanistic terms a number of years later, is hepatic schistosomiasis, in which the formation of hepatic granulomas is diminished by the effects of PEM on the cell-mediated immune limb of the immune response. Because the liver pathology in schistosomiasis is attributed to the host's own granulomatous response to the deposition of worm eggs in the liver, diminished granuloma formation results in a

lessening of the pathological response in the liver or an apparent antagonistic response.

The cycle of malnutrition–infection–more nutritional deterioration–more infection was a powerful pathway. The evidence suggested that simply intervening in this cycle by improving nutritional intake in the presence of repeated exposure to infection (as occurs in malnourished children in developing countries) was not sufficient to fully reverse the cycle, and that greater attention to the reduction of infection was necessary if clinical responses were to be improved and mortality rates diminished. This was because infection itself caused a loss of critical body stores of protein, energy, minerals and vitamins. It was a common occurrence to observe children who were improving during the initial phase of nutritional treatment for acute kwashiorkor suddenly deteriorate when they developed an infection such as varicella, measles or bacterial pneumonia, often with a fatal termination, or to see children fail to respond to nutritional therapy until an inapparent ongoing infection, such as in the urinary tract, was detected and treated. Experiences such as these suggested that a dual attack on nutrition and infection was needed for an optimal response. An analogy was made to the attempt to fill a leaky bucket, that is, pouring in nutrients at the same time that infection resulted in ongoing nutritional losses. Notwithstanding, it was understood that nutritional rehabilitation was critical and work proceeded to carefully and quantitatively define the nutrient needs through the metabolic balance studies in children admitted to a metabolic ward at INCAP. These studies were classic and they have produced data that have never been seriously challenged but only somewhat refined up to the present.

This period of renaissance ended, as it began, with an even longer, more fully documented and more sophisticated review of the field by Scrimshaw, Taylor and Gordon (1). Published as a World Health Organization monograph, the book discussed the nature of the interactions between nutritional status and infection, and implicated the immune system as the critical intermediary. However, consistent with the relatively rudimentary knowledge of the times, the lymphoid system was hardly mentioned, and, although the early underpinnings were being explored in murine models of tuberculosis, cell-mediated immunity was a concept still to be developed.

The reformation: 1970–1980

In this decade greater interest in the mechanisms underlying the malnutrition–infection cycle was facilitated by the increasing sophistication and availability of improved tools to assess immune function in humans. This work established the significant adverse impact of cyclical malnutrition–infection interactions on the complement system, mucosal immunity and cell-mediated immune responses. Application of methods to distinguish between the T- and B-cell systems demonstrated that B-cells remained functionally intact if the proper help from mature T-cells could be provided. Part of the defect in antibody immunity in malnourished persons was shown to be attributed to the profound effect of these deficits on the maturation of T-cells, resulting in a reduction in fully functional mature T-cells and an excess of poorly functional immature T-cells.

Consumption of complement during infection and the inability to keep up with the needs by synthesis of new complement proteins were shown to result in a significant functional complement deficiency. Because the initial events in phagocytosis and microbial killing are complement dependent, this deficiency resulted in a significant impairment in leukocyte

microbicidal capacity, especially for gram-negative organisms, in the early stages of infection when complement was critical. In vitro studies demonstrated that addition of complement-rich sources from normal plasma restored the microbicidal capacity of polymorphonuclear leukocytes derived from PEM patients, compared to PEM cells incubated in autologous serum, indicating that humoral factors were of greater importance than cellular function in limiting microbicidal activity of neutrophils.

Insights gained from the observation of individuals with hereditary defects of specific limbs of the immune system revealed that these defects caused specific rather than generalized susceptibility for classes of infectious agents, and within classes, specific agents. Because all limbs of the immune system were affected in one way or another in PEM, the almost universal heightened susceptibility of these patients could be understood. These descriptive immunological studies shed considerable light on the nature of the host defect, but did not provide an explanation for the mechanisms underlying them.

A critical new insight by William Beisel and his colleagues at the Walter Reed Army Institute of Research, another contributor to this symposium, first suggested a role for leukocyte-derived mediators in initiating the catabolic changes and loss of nutrient stores characteristic of the infected host. These studies employed partially purified mixtures of the growth medium in which leukocytes were incubated and stimulated, which Beisel's group dubbed leukocyte endogenous mediator (or LEM). This product appeared to reproduce many of the critical metabolic changes occurring during the acute-phase response in infection. Had they appreciated that they were dealing with a mixture of mediators and had changed the word "mediator" to the plural "mediators," they would have been entirely on target from the beginning of their studies.

Reconstruction: 1980–1990

During this decade, the endogenous pyrogen derived from activated leukocytes and responsible for the febrile response during infection was purified, sequenced and the gene identified. With this information, this protein was renamed interleukin 1 (IL-1), the first of a number of peptide mediators with different functions found in LEM to be clearly characterized. Identification of other interleukins soon followed, including tumor necrosis factor alpha (TNF- α , previously described and never renamed), IL-6 and a number of others. These critical mediators of cell function and host response are now known as cytokines. When it was appreciated that many of these same cytokines were involved in the activation of the immune response, it became clear that the immune and metabolic responses to infection were intimately entwined, with common pathways of activation and regulation, suggesting that both responses had survival value, and that attempts to manipulate the metabolic response to diminish the deterioration of nutritional status during infection might have potential downsides.

These discoveries began to capture the interest of immunologists to study the effects of nutrition on immune function, and the initiation of greater collaborations between immunologists and nutritionists. For example, the decades-old observation that the thymus gland involuted during childhood malnutrition led immunologists to appreciate the role of the thymus gland in the differentiation of T-lymphocytes, and the relevance of thymic involution to the decrease in the number of mature, differentiated T-cells during malnutrition. The consequence of this was functional impairment of cell-mediated immunity and diminished antibody responses to protein anti-

gens dependent on T-cell help. Further analysis of the mechanisms by which the thymus gland triggers differentiation of T-cells suggests that secreted thymic peptides are involved, as well as still uncharacterized signals provided by interaction of immature lymphocytes with the thymic epithelium.

This period also saw an increase in the number of studies conducted in humans, as the methodology continued to improve and new methods to obtain and purify relevant cell types from human peripheral blood and other tissues were developed, as well as new and better animal models. Some of this was ascribed to the full realization that malnutrition of a degree sufficient to impair immune function was not just confined to children in developing countries without access to nutritionally complete diets, but occurred in up to half of the adult patients hospitalized on medical or surgical services in the United States. In fact, the same metabolic events induced by infection were found to be caused by trauma or surgery. Malnutrition was also common in the elderly, who often consumed inadequate diets because of a number of social and medical factors including disease and drug-induced anorexia. Nutritional rehabilitation during hospitalization and greater attention to nutrition and diet in general medical and surgical care became higher priorities.

In addition, there was a realization that the acute-phase response induced by infection was a closely regulated and highly complex set of events, and that further refinement in our understanding of the mechanisms and mediators involved might lead us to targeted interventions after all. This exciting period of discovery is further discussed by Michael Powanda in this symposium. We are still at the early stages of this line of research and there are no therapeutic "magic bullets" yet available.

The modern era: 1990–2000

During this period the role of micronutrient deficiency as a conditioning factor in host response to infection became widely recognized, as multiple large field studies of vitamin A supplementation in different populations around the world demonstrated a marked decrease in childhood mortality attributed to all causes in children compared to those who were not supplemented. Although it has been difficult to show that the reduction in deaths associated with supplementation of infants and young children was specifically attributable to an effect on susceptibility or the ability to respond to individual infectious diseases, with the exception of measles, there is no other plausible explanation for the large effects noted in these studies. This discrepancy between the overall effect and the lack of an explanatory mechanism has triggered considerable discussion and some degree of skepticism. There are, however, plausible explanations that remain undocumented. For example, vitamin A deficiency is known to result in keratinization of the respiratory epithelium, leading to a decrease in mucus production and diminished capacity of the respiratory epithelium to clear bacterial pathogens. These events have not been studied in vivo. In addition, vitamin A and other retinoids regulate the expression of the genes for multiple proteins involved in host defense; in fact, aside from its role in visual function, the major effect of vitamin A is through the regulation of genes. Although the specific pathways involved in the enhanced mortality associated with vitamin A-deficiency states remains elusive, major programs to provide vitamin A supplements to those at risk in developing countries have been initiated in many developing countries.

Deficiency of other minerals, including iron and zinc, are well documented to impair immune function in experimental

animals, and to the extent studied, in humans as well. One postulated mechanism is that both of these metals are essential for the function of a number of metalloenzymes required for nucleic acid synthesis and cell replication. This is a particularly critical barrier to an effective immune response to infectious diseases that is based on the rapid reproduction of antigen-specific responsive clones of stimulated lymphocytes and the capacity of bone marrow to churn out increasing numbers of neutrophils and monocytes. Without the ability to make new DNA and RNA for cell division the host response sputters, and this is clearly documented in a variety of *in vitro* studies. It has been more difficult to demonstrate these effects *in vivo* in humans, however, and the clinical importance of zinc and iron deficiency remains in doubt. Interestingly, iron excess also appears to impair immune function, in this instance because of iron-catalyzed toxic oxidative reactions that physically damage immunocompetent cells.

Interesting studies on Keshan disease, the cardiomyopathy ascribed to Coxsackie B virus infections in selenium-deficient individuals in Keshan province in China, demonstrated that the antioxidant deficiency acted to select for a more virulent form of the causative virus. As is the case for many RNA viruses, including HIV, the relatively common infidelity of RNA replication leads to the production of an array of slightly different genotypes, commonly known as quasi-species. Alterations in certain specific nucleotide positions also correlate with increases or decreases in viral virulence, as tested in a mouse cardiomyopathy model. Infection of normal mice with fully virulent, but not avirulent, forms of the virus results in disease and death. Infection of selenium-deficient mice with avirulent virus also causes disease, and when the progeny virus is reinfected into normal mice, severe disease results. Analysis of this virus before and after this serial passage in animals demonstrates the selection of virulent virus associated with nucleotide changes in the relevant virulence positions in the genome (3). Although the precise nature of this selection for virulence in selenium deficiency remains to be determined, these studies have established another mechanism of micronutrient influence on infection.

During this decade, further studies of the catabolic wasting syndrome in AIDS have suggested that the underlying mechanism involves an imbalance in the levels of pro- and antiinflammatory cytokines produced by mononuclear cells. A number of studies have shown high circulating levels of proinflammatory cytokines such as IL-1, IL-6 and TNF- α in patients with the AIDS wasting syndrome. Equally high levels of these cytokines were also reported in a group of African women, known to have long-standing (>5 y) HIV infection who, nonetheless, remained relatively asymptomatic, with limited weight loss or decrease in lean body mass. When the cytokine antagonists of IL-1 and TNF- α were assessed, however, they were found to be in a sufficient molar excess to block the proinflammatory functions of the agonist cytokines (4). Why these particular subjects were able to balance the increase in proinflammatory cytokines with antiinflammatory cytokines is not known at present, but of interest not only for this situation but also to the possible use of antiinflammatory cytokines in other clinical settings.

The millennium era: 2000 and beyond

What does the new millennium portend for these areas of research? Opening on a positive note, a textbook dedicated to nutrition and immunology was published (5), demonstrating not only the general interest in the field, but also

the involvement of increasing numbers of serious immunologists as research partners. It is certainly not too much to expect that there will be an improvement in existing methods and the development of better methods to assess nutritional status as well as immune function. For example, there is already considerable knowledge waiting to be applied in the analysis of cell surface markers to identify specific lymphocytes and monocyte/macrophage populations and their activation state, so that the dynamics and kinetics of the specific immune response can be followed and assessed in real time (6). As a part of this, assessment of RNA and protein synthesis can help track the activation of genes during immune responses and production of the relevant proteins that mediate the ultimate responses of the host. Interest in regulatory mechanisms, and the role of newly described cytokines in the control of nutritional status and immune activation will continue to yield new insights (7). Explorations are much easier now with the new microchip array technology, which allows the rapid and simultaneous analysis of multiple pathways, at both the transcriptional and translational levels (8,9). Although there are some possibilities to use excreted samples such as saliva or urine for analysis, standardization is a problem and invasive sampling of cells and body fluids remains the standard. Nonetheless, it is conceivable that new photolabeling and imaging techniques could make possible real-time noninvasive sampling methods.

The biological revolution initiated by the Human Genome Project and the development of rapid sequencing methods have created the possibility of identifying minor sequence variations in specific genes that predict expression patterns of the proteins they encode, and thus the nature of the response at the protein and phenotypic levels (10). Single nucleotide variations (or SNPs), now readily detected, can indicate functional differences in genes between individuals. There is a major ongoing collaborative international effort to map and identify the truly important variances. Because gene sequence reveals protein sequence, the level of functional action, a new science, proteomics, has emerged (11). This will ultimately lead to new products that block, enhance, delay activation or deviate the locus of action of individual response proteins (including receptor and regulatory membrane proteins). This may permit the selective activation or inhibition of specific pathways that mediate disease or host responses to exogenous challenges, such as infections. In the context of nutrition, it may be possible to determine the specific mechanisms by which individual nutrients affect the immune system, and thus to target activation and regulation of immune pathways.

Because the acute-phase response is so crucial to host defense, the application of these methods of molecular analysis and the identification of ways to affect the regulatory pathways is of the highest immediate priority. All that is necessary is that there are investigators trained and ready to move down this route, and that sources of research funding become available. The path ahead is clear; what is not clear, however, is the time frame for making progress. We need only to find a few scientists with the dedication of Nevin Scrimshaw to begin this journey.

LITERATURE CITED

1. Scrimshaw, N. S., Taylor, C. E. & Gordon, J. E. (1968) Interactions of nutrition and infection. Monogr. Ser. World Health Organ. 57: 3-329.
2. Scrimshaw, N. S., Taylor, C. E. & Gordon, J. E. (1959) Interactions of nutrition and infection. Am. J. Med. Sci. 237: 367-372.

3. Beck, M. A. (2001) Antioxidants and viral infections: host immune response and viral pathogenicity. *J. Am. Coll. Nutr.* 20(suppl.): 284S–288S.
4. Thea, D. M., Porat, R., Khondi, N., Matela, B., St. Louis, M. E., Kaplan, G., Dinarello, C. A. & Keusch, G. T. (1996) Relationship of cytokine and cytokine antagonist plasma levels to disease progression in African women with HIV-1 infection. *Ann. Int. Med.* 124: 757–762.
5. Gershwin, M. E., German, J. B. & Keen, C. L. (2000) *Nutrition and Immunology*. Humana Press, Clifton, NJ.
6. Field, C. J., Johnson, I. R. & Schey, P. D. (2002) Nutrients and their role in host resistance to infection. *J. Leukoc. Biol.* 71: 16–32.
7. Faggioni, R., Feingold, K. R. & Grunfeld, C. (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. *FASEB J.* 15: 2565–2571.
8. Heller, M. J. (2002) DNA microarray technology: devices, systems, and applications. *Annu. Rev. Biomed. Eng.* 4: 129–153.
9. Winssinger, N., Ficarro, S., Schultz, P. G. & Harris, J. L. (2002) Profiling protein function with small molecule microarrays. *Proc. Natl. Acad. Sci. USA* 99: 11139–11144.
10. Collins, F. S. (2001) Contemplating the end of the beginning. *Genome Res.* 11: 641–643.
11. Daniel, H. (2002) Genomics and proteomics: importance for the future of nutrition research. *Br. J. Nutr.* 87(suppl. 2): S305–S311.