Stress, Nutrition, and the Immune Response

Jeffrey S. Kennedy

Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, Massachusetts, USA

This chapter highlights the effects of stress on host immunity to disease and reviews the new insights gained over the past few years on the potential therapeutic role of nutrition in altering stress-related disease processes. Recent data suggest that stress may have an effect on both innate and adaptive (specific) immune defenses (1,2). Furthermore, the relation between malnutrition and immune defense indicates a role for nutrition, as many cellular functions are dependent on adequate host nutrition. It is generally accepted that every nutritional deficiency has an effect on the immune system. This has led to the belief that nutritional supplementation can prevent or reduce the impact of disease by augmenting immunity.

The effects of stress and nutrition on innate immune responses has been reviewed elsewhere (3,4). This chapter will focus on the effects of stress on adaptive (specific) immunity. Recent studies demonstrating the effects of stress on immune function have provided important new insights into how individuals vary in their response to different stress loads (allosteric loads). This information is providing a means by which individuals can reduce their risk of stress-associated disease. Interventions such as exercise, nutrition, behavioral modification, or combinations of these may eventually be shown to improve health and prevent disease.

The relation between nutrition and health is especially apparent in the setting of malnutrition. Deficiency of most macro- and micronutrients affects the immune response. This interaction between nutrition and the immune response is bidirectional, with nutrition affecting immune cell response to pathogens, and infectious agents producing detrimental effects on host nutritional status (Fig. 1). There is, however, difficulty in defining optimal nutritional intake when the aim is to augment immune function or prevent disease. Although most investigators agree that suboptimal nutrition increases susceptibility to infectious diseases, we have less understanding of the impact of nutrition in preventing disease. Maintaining the competency of the im-
mune system during times of stress may serve to decrease the variety of pathogen-related diseases, from infections to cancers and autoimmune diseases.

A landmark review by Scrimshaw et al. (5) underscores the effects of malnutrition on the immune response. In the 30 years since this work, numerous reviews have been written on the effects of protein-energy malnutrition on the immune response (6–8). Nutritional deficiencies can affect delayed-type hypersensitivity (DTH), antibody response to pathogens or vaccines, mucosal (immune) integrity, and lymphocyte cytolytic activity against invading pathogens. Generalized malnutrition is a spectrum of deficiencies that can result not only from starvation but also from surgical or medical illness and perhaps strenuous physical or emotional stress. The ensuing acquired immune dysfunction increases host susceptibility to respiratory, intestinal, and systemic infections and assuredly leads to increased morbidity and mortality that is not invariably reversed by nutritional repletion. The interrelation between nutritional status and illness raises questions regarding the role of neuroendocrine responses on the immune system. The influence of the disease process on appetite, intestinal absorption, and end organ utilization must also be considered when attempting to use nutrition as a method of treatment.

Intact adaptive (specific) immunity is essential for host survival. Protein-energy malnutrition suppresses resistance to infections typically encountered in populations in developing countries (9–11). This increased susceptibility to infection appears to be secondary to alterations in T-cell function (12,13). Recovery of immune function following deficiency can be accomplished with nutritional interventions, but it is often delayed when compared with nutritional recovery (14,15). What remains to be determined is whether nutritional supplementation of well-nourished individuals can ameliorate or prevent disease. Clearly, demonstrating a role for nutritional supplements in preventing disease will require studies that delineate, at the molecular level, the mechanisms by which nutrition alters immune cell function and immunity to disease.
IMMUNE FUNCTION: THE ADAPTIVE IMMUNE RESPONSE

The immune system orchestrates the defense against harmful foreign invaders while attempting to spare autologous cells and tissues from injury. First, a protein structure had to be developed, such as an antibody or receptor, which would initiate specific destruction of foreign material. Second, the response to an overwhelming invading force had to be rapid, yet ensure the survival of the host while eliciting for future encounters a memory of the pathogen. Finally, a system would have to evolve that ensured complete destruction of the invading organism, yet protected autologous cells and organs from collateral destruction—a mechanism to distinguish self from nonself.

The human host has developed three distinct but overlapping systems to engage its immune response against pathogens:

1. Antibodies, produced by B lymphocytes and plasma cells, to recognize foreign molecules, called antigens
2. Immune cells specific in recognizing and reacting with antigens on either autologous or foreign cells, called T cells
3. Cytokines and acute phase proteins, designed to maintain immune cell responses and mediate site-directed response to invasion of the host

The specific (or adaptive) immune response is the general term delineating the response of the immune system toward specific antigens. This process involves receptors on T cells that are restricted to recognizing peptide antigens on the surface of cells only when associated with the major histocompatibility complex (MHC), a process referred to as presentation. Recognition of protein antigens requires the involvement of antigen-specific helper T cells. This process is fundamental to the initiation of the adaptive (specific) immune response.

The basic elements of the adaptive immune system are cells that process and present antigens. This results in the development of humoral or cytolytic effector responses. Macrophages, and dendritic and Langerhans' cells, although not immunologically specific, are capable of phagocytosis and of processing and presenting antigens to T cells. These cells are important in the secretion of inflammatory cytokines such as interleukins (IL)-1, 2, 6, 10, 12 and tumor necrosis factor-α (TNF-α). These cytokine mediators are important in the induction of fever and innate immune defense molecules such as complement, acute phase proteins, and bactericidal enzymes. Macrophages are, therefore, critical to the initiation of specific immune responses by presenting antigen to T cells and eliciting innate defense mechanisms.

Lymphocytes are immunologically specific cells subdivided into two classes: B and T lymphocytes. B lymphocytes synthesize immunoglobulins and express surface immunoglobulin receptors that recognize soluble intact antigens. T lymphocytes consist of regulatory helper and suppressor cells, and effector T cells, such as cytolytic T cells (CTL) that search and destroy cells bearing specific antigen. The unique specificity of T cells lies in the T-cell receptor which recognizes foreign peptide antigens in association with autologous MHC molecules. The major classes of T cells, as
marked by their surface receptors, location, and effector responses are depicted in Table 1.

The specific immune response can be broken down into two broad types: humoral and cellular immunity. The humoral immune response has been described in detail elsewhere (16). Cellular immunity is essential for survival. Genetic or acquired defects in T-cell function result in increased susceptibility to numerous infections, often to otherwise nonpathogenic organisms (Table 2). Organisms such as *Mycobacterium tuberculosis* (or *M. leprae*), *Listeria*, *Leishmania*, and *Bartonella* have the capacity to replicate within the macrophage. Individuals with impaired cellular im-

### Table 1. Low-molecular-weight mediators of the acute phase response

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandins</td>
<td>Vasodilatation</td>
</tr>
<tr>
<td>Thromboxane A₄</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>Leukotriene B₅</td>
<td>Chemotaxis, initiates phagocytosis</td>
</tr>
<tr>
<td>Leukotrienes C₄, D₄, E₄</td>
<td>Contraction of smooth muscle</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Mediates activation of pain nerve fibers</td>
</tr>
<tr>
<td>Histamine</td>
<td>Chemotaxis, allergic response</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
</tr>
<tr>
<td>Platelet activating factor</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Markers and distribution of human T and NK cells

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular weight (kd)</th>
<th>Function</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>γ 25-28, δ 21, ε 20, ζ 16, π 21</td>
<td>Signal transduction in T-cell gene activation</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD4</td>
<td>60</td>
<td>Coreceptor with the TCR complex for MHC antigen recognition</td>
<td>MHC class II</td>
</tr>
<tr>
<td>CD8</td>
<td>α 34, β 34</td>
<td>Maturation and positive selection of MHC class I restricted T cells</td>
<td>MHC class I</td>
</tr>
<tr>
<td>CD28</td>
<td>44</td>
<td>Costimulatory molecule signaling independent of TCR</td>
<td>CD86, CD80</td>
</tr>
<tr>
<td>TCR</td>
<td>αβ 45/40</td>
<td>αβ: T-cell antigen-specific receptor for MHC-peptide complexes on APC</td>
<td>αβ-MHC-peptide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δγ: antimicrobial and cytolytic activity</td>
<td>γδ-single molecule, ligand</td>
</tr>
<tr>
<td>CD16 (FcγRIIIa)</td>
<td>50-65</td>
<td>Associates with TCRγ and FcεRI, NK cells, and activated monocytes</td>
<td>IgG immune complexes</td>
</tr>
<tr>
<td>CD56 (NCAM)</td>
<td>175-185</td>
<td>Cytotoxicity to NCAM</td>
<td>Unknown</td>
</tr>
<tr>
<td>NKB1</td>
<td>70</td>
<td>Inhibits cytotoxicity</td>
<td>HLA-Bw4 alleles</td>
</tr>
<tr>
<td>NK1.1</td>
<td>80/38</td>
<td>Murine marker, human equivalent unknown but presumed present activation of cytotoxicity</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

TCR, T-cell antigen receptor; MHC, major histocompatibility complex; APC, antigen-presenting cell; NK, natural killer; NCAM, natural cell adhesion molecule.
munity, as seen in malnutrition, often display accelerated and more lethal consequences when infected by these organisms (17–19).

The clinical integrity of cellular immunity continues to be assessed by the ability of individuals to develop inflammatory skin reactions to recall antigens. The first such test developed involved the ability of individuals previously exposed to *M. tuberculosis* to develop inflammatory skin wheals on re-exposure to intradermally applied tubercle bacillus-derived protein. This test is characterized by local induration, rubor, and swelling, and in rare cases, necrosis, 24 to 48 hours after administration. This subacute reaction, known as the *delayed-type hypersensitivity reaction*, has been subsequently developed for a variety of common bacterial and fungal antigens.

Immunopathologically, the reaction is characterized by perivascular infiltrates of mononuclear cells, is immunologically specific, and is transferred passively by T cells (but only to recipients with matched MHC) but not by plasma. The response is directly linked to macrophage production of IL-2, IL-6, IL-12, and γ-interferon (IFN-γ) by antigen-specific cells. Inhibition of the cytokine response can result in abrogation of the DTH response and is typically seen in disease, malnutrition, and during stress (20,21).

Delayed type hypersensitivity reactions are usually triggered by antigen-mediated stimulation of Th1 effector subset of CD4⁺ helper T cells (22). These cells produce the cytokines IL-2, IFN-γ, and TNF-α. In a DTH reaction, an antigen stimulates the secretion of these cytokines from Th1 cells that have developed in response to previous exposure to that antigen. The effector response of various cells and cytokines involved in the development of the DTH reaction is depicted in Figure 2. IL-2 promotes expansions of antigen-specific T-cell clones, whereas the other principal cytokines of DTH, IFN-γ and IL-12, initiate an amplification of the response. Other cytokines, which are produced by the Th2 subset of CD4⁺ helper T cells, function to regulate the DTH reaction. Interleukin-10, an inhibitor of macrophage activation, and IL-4, an antagonist of IFN-γ action, are the principal inhibitory cytokines designed to prevent unchecked tissue injury in DTH reactions.

![FIG. 2. Cytokine-driven, T-helper cell differentiation.](image-url)
The immune response to protein antigens is influenced by the differential effect of various antigens on the types of T cells that are induced. Naive CD4+ T cells can be induced to differentiate into either or both effector subsets known as Th1 or Th2 cells. These cell populations produce specific mixtures of cytokines that can be used clinically to mark the type of effector response. The exact mechanisms by which naive T cells differentiate into these effector populations are not completely understood, but the types of cytokine produced locally during antigen exposure appear to be major determinants of differentiation. The pattern of cytokine production as well as signals generated through the T-cell receptor complex may be influenced by disease states (23).

Signals generated through T-cell receptor complex are not sufficient for full activation of T cells. Additional antigen nonspecific co-stimulatory signals through receptors such as CD28 and CTLA-4 are necessary for augmenting IL-2 production and the proliferation and differentiation of effector cell function. Throughout this decade considerable progress has been made in understanding the mechanisms of signal transduction by the T-cell receptor. Various events initiated by T-cell receptor signaling may be important in modulating immune function by hormones, neural input, stress reactions, and nutrition.

Tyrosine phosphorylation is a crucial part of the T-cell signal transduction pathway (Fig. 3). Multiple protein tyrosine-containing motifs, located in the chains of the T-cell receptor complex, are involved in T-cell activation. Two families of protein tyrosine kinases utilize these tyrosine motifs as both substrates and sites for binding, followed by complex formation and activation of downstream signaling events. The src-kinase LCK or the syk-kinase ZAP-70 play important roles in T-cell receptor signaling, as mutation of either kinase aborts T-cell activation (24,25). These kinases associate with the intracellular chains of T-cell receptor complex (ZAP-70 with the ζ chain and LCK with the ε chain of the complex), initiating a series of downstream events that ultimately direct the T cell to enhance proliferation and IL-2 production (Fig. 4).

The crucial question of how stress can alter response of T cell to MHC presented antigens remains to be explored. Clearly, disruption of DTH and vaccine response

![FIG. 3. T-cell signaling and immune response.](image-url)
during stress may imply altered T-cell signaling through protein tyrosine kinases. The recent use of altered peptide ligands that engage the same T-cell receptor but induce qualitatively different responses in T cells may help explore the effect of stress on T-cell receptor signaling (26). In addition, antigen-presenting lymphocytes can at times act as antagonists of the T-cell response by altering CD3 phosphorylation through SHP-1 (27). This may be regulated by mitogen and stress-activated protein kinases (MAPK and SAPK) and serine phosphorylation of Lck (28). This altered pattern of ζ-phosphorylation is also associated with a failure to activate ZAP-70 and LCK. These events have been shown to result in loss of IL-2 gene transcription and induction of T-cell anergy in T-cell clones (29). Induction of anergy and lack of lymphocyte proliferation are associated with malnutrition and disease states. It is interesting to speculate about the possible mechanisms underlying this anergic response and the signaling pathways within the T cell and how nutrition may play a role in correcting dysfunctional T-cell responses.

Another area of interest to nutritional immunologists will be the characterization of the process that controls early T-cell development. In both infants and elderly populations, the activation of naive or T-cell progenitors is altered (30). In B cells, a pre-B-cell receptor complex regulates developmental progression and allelic exclu-
sion (16). Recent work has begun to shed more light on the T-cell receptor complex and on the signal transduction pathway and ligand for this receptor (31). Regulation of early T-cell development may be an important area by which nutrition can play a role in disease prevention.

One final area in need of further investigation is the role of T-cell co-stimulatory pathways in the initiation of T-cell responses to pathogens during stress. Much in vivo and in vitro data have implicated CD28 as a major co-stimulatory receptor on T cells and B7, its ligand, on antigen-presenting cells. The importance of the CD28–B7 interaction in T-cell responses has been confirmed by CD28-deficient mice. T-cell–dependent antibody responses were essentially absent in these mice, as were T-cell responses to some but not all viruses. Subsequent studies have revealed that T-cell proliferation in the presence of antigen-presenting cells was markedly reduced (32). Stress has been shown to affect proliferative response, attenuate IL-2 production, and alter antigen response. Co-stimulatory receptors may also be affected by mediators of the stress response and, therefore, amenable to modulation by nutrition.

CTLA-4 was originally identified as a cDNA expressed in T cells after activation (33). The gene coding for CTLA-4 is highly homologous and is genetically linked to CD28. CTLA-4 binds to B7 with an affinity much higher than CD28, and a soluble form of CTLA-4 has been shown to block T-cell responses in vivo and in vitro. The function of CTLA-4 remains controversial. Early reports indicated that CTLA-4 might synergize with CD28 to enhance co-stimulation and thereby function to sustain IL-2 production and T-cell proliferation. A more recent report showed that cross linking of CTLA-4 together with T-cell receptor and CD28 resulted in a profound inhibition of IL-2 production and proliferation (34).

Present understanding of the proximal events of signal transduction mediated by CD28 or CTLA-4 is limited. The cytoplasmic domain of CD28 contains a motif, YMNM, that has been shown in other signaling receptor systems to be a site for binding the SH2 domain of phosphoinositide 3-kinase (PI3-kinase). CTLA-4 likewise contains a PI3-kinase domain (YVKM motif) and an association of CTLA-4 with PI3-kinase has been reported (35). PI-3 kinase has recently been shown to be modulated by nutrition (36) and appears to play a role in altered immune function in elderly (37) and aging mouse models (38).

The level of current knowledge regarding the downstream signaling events of CD28 co-stimulation is limited. Augmentation of IL-2 appears to be one important consequence of co-stimulation; however, controversy exists over whether enhanced transcription or mRNA stabilization is the mechanism. At present, it appears that both may play a role. Recent reports of a role for stress-activated protein kinases (SAPK or JNK) in the downstream regulation of induction of IL-2 transcription by T-cell receptor suggest a role of neuroendocrine mediators in modulating T-cell activation during stress.

One intriguing question for the future is whether these biochemical differences can account for the changes in DTH and vaccine response (MHC recognition) seen during stress and malnutrition. Do these changes account for the positive and negative
selection in the thymus and can they be reversed by nutrition and other therapeutic approaches?

**STRESS, NEUROENDOCRINE RESPONSE, AND IMMUNITY**

The relation of stress to immune function has been the subject of much debate and research since the early work of Cannon and Selye. Cannon's summation on the sympathetic nervous system introduced the concept of homeostasis and the intricate balance between the nervous system and health. Selye's notion is that stress is a nonspecific noxious stimulus that elicits an adaptation response encompassing both nervous system elements as well as alterations in the adrenohypophyseal axis (39). The paradox lies in the fact that physiologic systems activated by stress not only protect but may also damage the host. Selye's description of thymolymphatic atrophy in response to stress led to a new understanding of neurohumoral control of the immune response (40).

Stressful experiences, although stimulated by many factors, can best be viewed as acute—in essence *fight or flight*—or chronic, resulting from the cumulative load of day-to-day stress. The latter can be caused by a variety of factors—malnutrition, psychological stress, physical stress, and disease—each influenced by many other factors, as depicted in Figure 5. Genetic factors do not account for all the variability seen in response or sensitivity to stress, as evident from the lack of concordance in identical twin studies (41). Genetic factors also do not explain the differences seen across socioeconomic levels. However, certain factors do appear to have a primary influence on an individual's response to stress. The first relates to how an individual perceives a stressful situation, which is determined not only by genetic factors but also by the person's ability to adjust or habituate to repeated stress and current state of

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**FIG. 5.** Host defense and susceptibility to disease.
health; second, and most crucial, is whether the individual perceives a situation as a threat, either psychological or physical (42).

Physiologic and hormonal responses activated by stress can both protect and damage host end organs (43). This paradox confounds our ability to understand the role of stress on disease susceptibility, virulence, and pathophysiologic response. The stress response can be induced by a wide variety of environmental events: cold, sleep deprivation, starvation, and physical injury. The endocrine effects of stress have broad physiologic and metabolic consequences as well as inducing immunologic changes. During acute stress, immune function can be attenuated by the release of glucocorticoids and damage as a result of stress-induced immune activation may be prevented by negative neuroendocrine feedback on immune cells.

The evidence for a relation between stress and disease is compelling. Animal as well as human studies have shown a relation between repeated stress and the development of cancer (44), rheumatoid arthritis (45), and heart disease (46). The available evidence also points to a role of stress in initiating alterations in both specific and nonspecific antigen immunity (47). Numerous studies continue to point toward a role of stress in adversely affecting both arms of adaptive immunity—humoral and cell-mediated (48).

The immune system responds to pathogens by eliciting cellular as well as noncellular immune responses, often referred to as the acute phase response. Activation of both the hypothalamic-pituitary axis and the autonomic nervous system during stress has been shown to attenuate the acute phase response and cell-mediated immunity (49). Acute stress results in redistribution of lymphocytes and macrophages throughout the body and margination to certain organ systems such as the skin, lymph nodes, and bone marrow. This margination requires blood vessel endothelial contact, important in priming the immune system for the assault against intruders. However, it may also predispose certain individuals toward the development of stress-related disease. As examples, DTH during acute stress is enhanced because of increased trafficking of lymphocytes and monocytes to the site of the acute challenge (50). However, individuals experiencing chronic stress have decreased DTH reactions (51). Individuals afflicted with asthma can exacerbate and enhance the frequency of attacks during periods of emotional stress (52). This latter effect may relate to an increased acute phase response on neutrophil function (53).

These immune-enhancing effects of acute stress last from hours to several days and depend on intact and effective adrenal response. The hypothalamic-adrenal axis functions as a check and balance of the immune response. This allows for heightened surveillance by the immune system and development of immunologic memory to pathogens without self-destructive mechanisms overwhelming the host. It has been suggested that this system, in genetically susceptible individuals, can lead to the development of autoimmune or allergic responses during stress. Chronic or repeated stress has been shown to suppress cellular immunity and lead to inhibited DTH (54), increased severity of viral upper respiratory infections (55), and decreased viral antibody titers (56).

This idea of neuroendocrine control of immune activation has strong support from
three models: prolonged physical exertion (exercise-immune model), experimental allergic encephalomyelitis in susceptible strains of rats, and studies in humans and nonhuman primates showing an increased incidence of atherosclerosis and hypertension in situations of chronic high stress (57–59).

Marked individual variation exists in the hypothalamic-pituitary axis response to stress and subsequent effects on systemic immune function (60). The mediator of this systemic response may, in part, be IL-6. The pleiotropic function of IL-6 on immune cells and its involvement in the regulation of endocrine, metabolic, and acute phase response suggests that the role of IL-6 during stress is complex and in need of further study. Recent data from my laboratory suggest a role for IL-6 in the chronic stress response after the usual acute hypothalamic-pituitary axis response of acute stress has subsided. A subset of individuals showed marked changes in T-cell subsets and function, suggesting that extended stress also alters T-cell responses to antigens that could play a role in the development of autoimmune diseases (Table 3).

Integrated homeostatic mechanisms between the nervous and endocrine systems have recently been explained by new information showing that both have important modulating effects on immune function. Regulatory peptides and their receptors, previously thought to be limited to the brain or the immune system, are now known to be expressed in both. This finding helps to explain how stress, particularly the behavioral response to stress, could modify effective immune response to disease.

Toward this end of defining the effect of stress on immune function, research in exercise immunology is guided by the acceptance that strenuous exercise is a model in which to study the effects of stress on the immune system. This model uses exercise as a means of temporarily suppressing the immune system. Disease amelioration and prevention has been the major focus of studies relating exercise and neuroen-

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene defect</th>
<th>Function</th>
<th>Infection risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>CD3γ</td>
<td>TCR signaling</td>
<td>Intracellular and atypical pathogens</td>
</tr>
<tr>
<td>Moderate T-cell immunodeficiency</td>
<td>CD3ε</td>
<td>TCR signaling</td>
<td>Intracellular and atypical infections</td>
</tr>
<tr>
<td>X-linked SCID</td>
<td>IL-2Rγ</td>
<td>Lack of circulating T cells</td>
<td>All pathogens</td>
</tr>
<tr>
<td>X-linked hyper-IgM SCID</td>
<td>CD40 ligand</td>
<td>Failure of Ig switch</td>
<td>Lethal in first year of life</td>
</tr>
<tr>
<td>SCID</td>
<td>Zap-70</td>
<td>TCR signaling</td>
<td>No CD8+ cells; all pathogens</td>
</tr>
<tr>
<td>X-linked agammaglobulinemia</td>
<td>Btk</td>
<td>BCR signaling; lack</td>
<td>Infections at 5 months with declining maternal</td>
</tr>
<tr>
<td>Common variable immunodeficiency</td>
<td>Unknown</td>
<td>B- and T-cell deficits</td>
<td>Antigen disease, gastric</td>
</tr>
</tbody>
</table>

SCID, severe combined immunodeficiency; TCR, T-cell antigen receptor; BCR, B-cell antigen receptor.
doctrine or immune function. The role of exercise in cancer prevention, as well as limiting the effects of such diseases as coronary heart disease, osteoporosis, and diabetes, tends to suggest that exercise can have a positive impact on health. In addition, exercise-induced muscle injury represents a model of activating the acute phase response of injury or repair and adds the potential of determining whether nutritional supplements can alter overall immunocompetence and repair mechanisms. Current data are inconclusive to the value of this model in studying the role of nutrition in disease prevention.

Physical exercise has been promoted as a panacea for a host of illnesses (61). However, the complexities and redundancies of the immune system and the unique mechanisms of virulence of specific pathogens confound the role of exercise in altering immune function and human disease processes. Epidemiologic studies point to a reduction in morbidity and mortality from cardiovascular disease, cancer, the human immunodeficiency virus (HIV), osteoporosis, and autoimmune diseases (62–67). Morbidity, and more often mortality, used as outcome measures, make the assumption that they measure the intactness of an integrated host immune defense response. Studies in exercise immunology to date often show abnormalities of lymphocyte sub-populations in peripheral blood or changes in proliferative response to mitogens such as phytohemagglutinin or concavalin A. These assays, although descriptive, lack specificity and fail to define defects in immune function that might be causally linked to disease risk.

Like any other homeostatic system, the immune system is composed of redundant and overlapping mechanisms to ensure adequate response to environmental threats. These overlapping mechanisms are most clearly seen in congenital deficiencies. For example, one of every 500 humans is deficient at synthesizing IgA, which binds pathogens at sites of mucosal defense, yet no increased risk for infectious diseases has been found in these individuals. In contrast, congenital deficiencies of complement can lead to abnormalities in phagocytosis, and in the case of terminal complement deficiency, can lead to increased susceptibility to Neisseria infections. This unique quality of the immune response to use several mechanisms in responding to infectious challenges points to a danger in drawing conclusions about the overall resistance to infectious agents based on changes in one isolated mechanism. In addition, defining risk for disease based on immune cell deficiencies requires rigorous analysis of immune function at the cellular and molecular level. This may require future studies to focus, for example, on vaccine antibody response (host), T-cell signaling response (cellular), and MHC genetic polymorphisms (molecular) to adequately define the role of exercise or nutrition in disease risk.

Physical exercise, as with all stressors, can have potential adverse consequences. Severe prolonged exercise and overtraining syndrome leads to immunosuppression, resulting in an increased risk of upper respiratory tract infections (68) (Table 3) and immune cell changes consistent with other forms of stress, such as aging and severe psychological stress (69) (Fig. 6). Aging is associated with increased risk of cancer, autoimmune diseases, and infectious diseases. Are the immunologic changes seen with extreme physical stress comparable to the risks of disease inherent in aging?
TABLE 4. Changes in immune cell populations after a 21-day SFAS training and the risk of upper respiratory tract infection

<table>
<thead>
<tr>
<th>T-cell populations (cells/μl blood)</th>
<th>CD4+ (helper)</th>
<th>CD8+ (killer)</th>
<th>IL-4+CD4+ Th2 cells</th>
<th>γIFN+CD4+ Th1 cells</th>
<th>Infection risk over baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 (baseline)</td>
<td>990</td>
<td>545</td>
<td>0.02</td>
<td>83</td>
<td>n.a.</td>
</tr>
<tr>
<td>Day 19 (stressed)</td>
<td>780</td>
<td>400</td>
<td>0.35</td>
<td>61</td>
<td>2.3</td>
</tr>
</tbody>
</table>

IL, interleukin; IFN, interferon; n.a., not applicable.

What is the functional impairment of aging that explains this generalized risk of multiple types of illness, and does this impairment exist with prolonged physical stress? Are these processes reversible? Do exercise and nutrition have a role in reducing the risk? By understanding these relations we will be better able to identify at-risk individuals as well as learn how to prevent disease.

The results from animal studies are complicated and the isolated effects of physical stress are difficult to interpret. Models using swim to exhaustion may reflect more survival stress than actual mortality observed following infection or diminished resistance to infection. Timing of exercise (rats are nocturnal) can influence the results of studies because sleep deprivation is known to suppress immune function (69). Use of electric shocks to prod the animal to exercise is in itself a proven immunosuppressant. Finally rodents, if given access to their running wheels at night, will typically run several miles a night, which is much more significant exercise than the experimental challenge used during most studies. Additionally, the effect of the exercise on pathogen virulence and susceptibility is less consistent. The swim model of animal exercise has been shown to increase the replication of viruses such as coxsackie A9 (70), B3 (71), and influenza (72), but not bacterial or protozoan infections (72,73). This form of exercise increased mortality secondary to coxsackie B3, influenza, and trypanosomiasis (although perhaps deaths may have been related more to drowning than to the infectious agent). Many of these
studies suffer from the efforts to maximize infection, in that the route of infection (intraperitoneal and intracerebral injection of organisms) may have evaded the typical host immune response and defense mechanisms, leaving the question of whether exercise would have played a role if the normal portals of entry had been used.

Studies conducted in humans emphasize the difficulty in assessing a possible role of exercise in altering immune defense. Two studies (74,75), one of 6 months and the other of 1 year in duration, were used to assess the incidence of upper respiratory infection in young adults active in athletic sports. No correlation was found in either study on risk of upper respiratory infection and exercise activity, although in the former study (74), a negative correlation was found in women. In a study of sedentary obese women, those assigned to a regimen of 15 weeks of supervised walks reported a significantly reduced duration of symptoms when suffering upper respiratory infection. In both studies, a lack of control over the extent and type of pathogen exposure complicates the interpretation of the role played by exercise in reducing upper respiratory infection risk or symptoms. More recently, a large study of 394 subjects given nasal drops containing one of five respiratory viruses versus placebo saline showed that the incidence of infection correlated with measures of psychological stress, but was not related to exercise frequency, intensity, duration, or type (76). This study serves to illustrate that many other factors (e.g., psychological stress) must be controlled for during studies in humans that assess the effects of exercise on immune function.

Acute exercise results in several blood compartment changes in lymphocyte populations. T cells show a decline in both helper (CD3^+CD4^+CD8^-) and suppressor (CD3^+CD4^-CD8^+) cells as well as declines in B cells (CD19^+) and natural killer cells (CD16^+CD56^+). Natural killer cell activity has been shown to decline after exercise (77) and it has been hypothesized that this is the cause of the exercise-related risk of upper respiratory tract infection. It has also been shown that antibody production is inhibited and mucosal IgA levels are diminished (77). Whether these cellular changes are associated with altered immune function or risk of disease is not known. Most have only made conjectures from combining the results of several studies.

Several regulatory peptides, first described in immunocompetent cells, are known to have profound effects on both the immune cell and the central nervous system. These include the interleukins and growth factor peptides. Exercise has been shown to induce the acute phase response with the induction of three families of cytokines (IL-1, IL-6, and TNF). Conflicting data are found on the role of IL-1 in the exercise-induced acute phase response. Currently, many studies have reported a lack of IL-1 in the plasma following strenuous exercise (78,79). IL-6, a member of a family of cytokines that triggers gp-130 type receptors (important in the activation of stress kinases), has been shown to be enhanced by strenuous exercise (80). This family of cytokines includes other immunologically important cytokines such as LIF and IL-2, which will be discussed later.

Bruunsgaard et al. (80) showed that eccentric exercise (known to induce severe muscle injury) increased plasma IL-6 (fivefold after 2 hours), whereas concentric
exercise did not. This study suggests that cytokine production after exercise is connected with activation of the immune response to injury rather than injury itself. In a recent study, it was shown that 21 and 60 days of continuous intense physical training results in a ten to twenty times increase in IL-6, IL-1Ra, and TNFsRp55 (unpublished results). The increase in IL-6 was sustained throughout the duration of the exercise. In addition, these cytokine elevations were not reflective of changes in circulating monocyte or lymphocyte populations, and they were consistent with western blot analysis of lymphocytes that indicated activation of kinases associated with gp130 receptor ligation. The elevation of IL-6 was not associated with significant increases in serum creatine phosphokinase, as seen in eccentric exercise models. It is unlikely, therefore, that muscle injury played a significant role in inducing IL-6 release from immune cells during this training. Other mediators, perhaps in response to stress, may play a more significant role during long-term strenuous exercise.

Immune responses alter neural and endocrine function, although the activity of the latter clearly modifies host immune function. Links are established between neuroendocrine function and response of the immune system to infection and inflammation. The relation between neuroendocrine function and immune responses may explain how emotions, stress, and aging modify individual capacity to fight infection or influence the course of chronic diseases such as cancer and autoimmune disorders. Data from our studies in prolonged stress models show that IL-6 may have a role in the regulation of energy balance. During 60 days of continuous physical and psychological stress, induction of acute phase response and IL-6 was associated with a dramatic decline in plasma leptin levels and diminished T-cell responsiveness following ligation of T-cell receptor (unpublished data). Lymphocytes harvested from these individuals when incubated with leptin showed a return to normal T-cell receptor signaling when compared with controls. A T-cell receptor signaling abnormality seen during stress is consistent with findings from multiple studies indicating that stress alters DTH skin responsiveness and IL-2 production of isolated lymphocytes (81).

Our model used a multistress environment simulating the extreme stress seen during combat, natural disasters, and extended bereavement. This model, involving young men training in US Army Special Operations, enabled us to study the effects of sleep deprivation, mental stress, and intense physical exertion on immune function and risk for infectious diseases. Initial investigations showed that individuals experiencing this stress developed anergic skin DTH at incidences seen in elderly people, people with HIV, and critically ill patients in the hospital (Fig. 7).

Specific immunity is critical to host defense against disease. Medicine today is faced with an array of new technologies geared toward enhancing specific immune response against cancer, autoimmune disease, and pathogens. The role of nutritional supplementation in augmenting host defense mechanisms is only beginning to be explored. The future holds the promise of identifying individuals at risk, which will allow further study of the effects of nutritional interventions on the prevention of disease.
NUTRITION AND STRESS

The study of the effects of dietary nutrients on immune cells and immunity to disease is complicated by the multiple interactions that various types of nutrients have on cellular physiology and metabolism. Dietary proteins, lipids, and complex carbohydrate-based nutrients may act as antigens when absorbed, stimulating mucosal immune responses independent of any innate effects on immune cell function. In addition, the effect of any single nutrient depends not only on its relative concentration in tissues but also on its interaction with other critical nutrients involved in the response measured. For an immunologist, it is, at first glance, difficult to imagine that vitamins or minerals could have effects on immune function except when deficient. However, several nutrients seem to have pharmacologic value. In other words, when supplied at levels far in excess of requirements for normal physiologic processes, they exert an effect that appears to alter the relative risk of disease or disease progression. Finally, in many studies of the effects of nutrients on immune function, a nutrient imbalance exists. The duration of this imbalance, as well as factors such as stress, disease, and the age of the host, may influence the measured immune response and cloud the interpretation of the effect of nutrient supplementation.

In 1971, Smythe et al. documented the impact of nutrition (protein-energy malnutrition) on cell-mediated immunity (11). This work substantiated a longstanding belief that the cell-mediated immune system bore the brunt of poor nutritional status. This work was supported by later investigations showing that the types of infection seen in malnutrition were consistent with altered cell-mediated immunity (82).

Three specific examples of the interaction of nutrition and the immune cell response serve to illustrate the complexity and difficulties scientists will encounter as they try to elucidate how nutrition can prevent or ameliorate disease. It is widely known that the response to immunization as measured by specific antibodies to an immunogen is often normal in malnutrition, yet cellular immune responses, particularly T-cell function, are impaired. This altered T-cell response correlates with an increased risk of infectious disease morbidity and mortality in malnourished individuals, hospital inpatients, infants, and elderly people. For example, in a child with a

<table>
<thead>
<tr>
<th>Group</th>
<th>% Anergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (non-stress)</td>
<td>2%</td>
</tr>
<tr>
<td>Children (0.5–2 yrs)</td>
<td>38%</td>
</tr>
<tr>
<td>Children (&gt; 2 yrs)</td>
<td>7%</td>
</tr>
<tr>
<td>Adults (&lt; 40 yrs)</td>
<td>5–8%</td>
</tr>
<tr>
<td>Elderly (&gt; 65 yrs)</td>
<td>15–25%</td>
</tr>
<tr>
<td>Continuous Stress</td>
<td></td>
</tr>
<tr>
<td>21 days</td>
<td>25%</td>
</tr>
<tr>
<td>60 days</td>
<td>45%</td>
</tr>
<tr>
<td>HIV (CD4 = 100–500)</td>
<td>30–60%</td>
</tr>
</tbody>
</table>

FIG. 7. Frequency of anergic skin delayed type hypersensitivity response.
recent history of measles, malnutrition increases the risk of death from diarrheal disease (83). This effect appears not to be related to antibody titers against measles virus but rather to be the result of cell-mediated immune suppression induced by the viral infection compounded by malnutrition (84–87).

Measles virus infection can result in near complete suppression of cell-mediated immunity (84). Lymphocytes possess surface molecules called common differentiation antigens (CD), which serve as receptors and signal-transducing proteins to initiate the immune system’s battle with invading pathogens, as well as to protect the host from cancer and autoimmune diseases. Measles utilizes one such receptor, CD46, a component of the complement system, as a means of binding to lymphocytes and suppressing the immune response. CD46 is upregulated on the surface of lymphocytes during acute inflammation, and the measles virus has adapted to this opportunity, using CD46 as a receptor to enter the cell and evade destruction. Once inside, the virus initiates a series of cytokine effector responses designed to limit host destruction of cells infected with the measles virus.

Normally, the host response to intracellular viruses such as measles involves T-cell production of γ-interferon and IL-2, which elicits a type 1 (Th1) T-cell immune response (88) (Fig. 8). Interestingly, malnutrition also impairs this type of T-cell response (89). The impairment by measles virus of this response is so complete in susceptible hosts (e.g., infants) that other pathogens, in particular enteric bacteria (dysentery), can opportunistically invade a host and cause death.

As a second example, studies by Beck and others (71,90) have extended the concept that nutritional state can alter susceptibility to disease; in this case, virulence and

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**FIG. 8.** Host response to measles virus.

![Diagram of Host Response to Measles Virus](image)
pathogenicity of coxsackie B virus for inducing myocarditis during selenium deficiency is increased.

This work serves to illustrate the multidimensional relation between nutrition and immune health. Keshan disease (91) is an endemic cardiomyopathy typically seen in northern China. In endemic areas, individuals have low plasma concentrations of the trace element selenium and repletion of selenium in the diet significantly reduces the prevalence of cardiomyopathy (92). This disease presents an epidemiologic pattern consistent with an infectious agent as a risk factor in endemic selenium deficiency for the development of cardiomyopathy. Initial studies in selenium-deficient mice demonstrated increased heart damage when mice were inoculated with a strain of coxsackie virus (CVB3/20) isolated from individuals with Keshan disease (93). Subsequently, a series of elaborate studies in selenium-deficient mice showed that an animal model with a risk profile similar to that seen in human Keshan disease could be developed (94,95).

Investigators hypothesized that the selenium-deficient environment altered viral genotype, which resulted in increased virulence of the CVB3 virus. In selenium-deficient animals, CVB3 introduced into the animals mutated in six of seven genomic nucleotide positions characteristic of the more virulent strain, CVB3/20. The CVB3/20 virus is the pathogenic strain causing myocarditis (96,97). This hybrid formation occurs in vivo, under conditions of selenium deficiency, suggesting that selenium may play a role in altering virulence of a virus (98). The alteration of the viral genome was not unique to selenium, but was also seen under conditions of vitamin E deficiency (99). This later finding suggests, in contradiction to Beck's hypothesis, that altered nutritional status may only be a marker of impaired immune surveillance. Altered immune response may be responsible for the emergence of more pathogenic strains of viruses through natural selection rather than through nutrient deficiency altering viral replication and genetic mutation. Although the exact role of selenium deficiency on CVB3 virulence is unknown, these studies nonetheless point to an important role for nutrition in modulating host immune response and risk of disease.

The role that diet can play in modulating the immune response to infections, as well as in chronic disease states, can be viewed from the role these cytokines play in disease processes. To understand more clearly the role that cytokines, and in particular IL-1, play in disease, one must first understand that the production of cytokines is often only the first step in a cascade of physiologic and cellular responses to inflammation. In septic shock, for instance, the mechanism of action of IL-1 appears to relate to its ability to stimulate the production of small effector molecules such as platelet-activating factor, bradykinins, prostaglandins, and nitric oxide. In rheumatoid arthritis, IL-1 is produced in the synovium of patients, and in animal models intra-articular injection of IL-1 results in the breakdown of cartilage, infiltration of leukocytes, and periarticular bone remodeling. In arteriosclerosis, IL-1 stimulates the proliferation of smooth muscle cells and may play a role in plaque formation by stimulating IL-1 by low density lipoproteins (100). In addition, o-3 fatty acids, known to alter the course of atherosclerosis, have been shown to decrease the production of IL-1 by monocytes in humans fed diets supplemented with n-3 fatty acids (101,102). The
impact nutrition can have in altering the inflammatory response could influence the morbidity and mortality from many acute and chronic inflammatory diseases. To date, research on the role of nutrition in altering disease or the injury process has been limited. The need for further investigations is quite apparent.

The inflammatory reactions that occur as a part of the host response to infections can occur both before the development of specific immunity and as a component or sequel of the effector phase of specific immune reactions. Inflammation serves a protective function by eliminating infectious agents, and may also cause tissue injury, resulting in local and systemic pathologic abnormalities. As a result, inflammatory reactions that accompany immune responses are often severe and have unique features dependent on the nature of the eliciting antigen and subsequent immune response.

In a recent finding by Lord and others, leptin, a hormone known to regulate body fat stores, was shown to have a profound impact on T-cell immune function. In a mouse model of starvation-induced immunosuppression, leptin administration partially reversed T-cell cytokine, proliferation, and function of the T-helper cell subpopulation (103). In addition, these investigators found that the response to leptin was particularly confined to naive T cells, which may help explain the similar responses seen in human starvation-induced immune suppression. My laboratory has shown a similar response in human subjects experiencing stress coupled with moderate energy deprivation (Kennedy JS, unpublished data; Fig. 9). In this setting, leptin levels correlated with a rise in IL-6 and suppression of T-cell–mediated immune responses. The addition of exogenous leptin to lymphocyte cultures of these individuals reversed the T-cell deficits. Although no leptin was given, the reversal by leptin ex vivo suggests that the decreased leptin levels seen during stress may alter T-cell

![Graph](image-url)  
**FIG. 9.** Effect of leptin on peripheral blood lymphocyte IL-2 production: stressed versus non-stressed humans.
immune function. These advances in understanding the biochemical and molecular mechanisms underlying the effect of malnutrition on immune function will provide the basis in the future with which to make more cogent recommendations regarding supplementation to prevent or treat disease.

The role of nutrition in preventing disease in childhood is becoming well established. A randomized, controlled trial of zinc supplementation in India showed a 21% reduction in the duration of diarrhea (104), improved birthweight following prenatal supplementation, and improved weight gain in infants (105). Vitamin A supplements substantially reduce mortality from severe measles (106), although when supplemented at the time of vaccination they may reduce seroconversion rates in children (107). Recent meta-analysis reviews suggest that the relation between vitamin A supplementation and viral infection (e.g., measles) is more complex (108,109).

The effects of nutrition on disease are reviewed elsewhere in this volume. As research in the field of immunology advances, new and more specific assessments of the impaired immune response during malnutrition will allow investigators to target novel nutritional therapies. I hope the information provided here serves to highlight the new immunologic advances in the area of T-cell signaling, and may influence the development of new approaches to study the effects of stress and malnutrition on T-cell function. I would also like to emphasize the need to develop and fund research projects that incorporate expertise from the fields of immunology, epidemiology, nutrition, and molecular biology. Under rigorous multidisciplinary studies we will learn whether nutrition can have pharmacologic-like effects on enhancing immune function and whether these effects can influence disease prevention, susceptibility, morbidity, and mortality.

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DISCUSSION

Dr. Griffin: I thought your hepatitis A data were very compelling. I would like to introduce the idea of two other vaccines. The first is an oral vaccine, which would stress the mucosal immune system. I have used, for example, oral cholera vaccine successfully to probe various immune aspects of HIV disease. The other is the possibility of using an intramuscular DNA vaccine. In rodent models, when DNA vaccines are injected into skeletal muscle, the immune response is considerably potentiated when the animal is exercised, and that might be useful in your model because you have these two components of exercise stress and physiologic stress.

Dr. Kennedy: I think those are excellent suggestions. We have become interested in some of the adenoviral vector vaccines as a possible means of looking at the immune response. The difficulty lies in the side effects. We picked hepatitis A because we thought it would have the least side effects of all the vaccines. The subjects we were studying volunteered during a time in their military careers when they were going through a very important course. To do anything that would influence their ability to be successful in that course would have ended our research program!

Dr. Woodward: I firmly believe that in malnutrition at least a shift toward naive-like T cells is real, which has also been observed in elderly subjects (1). I was interested in your leptin results because I believe it is the naive-type cell that responds best to leptin (2). Is that correct?
Dr. Kennedy: Yes, that is correct. We find, as have others, that major shifts occur in memory-naive cell populations. We are not sure what to make of that in terms of either the leptin or the vaccine response, but I agree that the naive cells are probably the ones that are responding most here.

Dr. Woodward: And that is a shift that may occur, not only in the blood, but elsewhere as well. It seems to me that the blood may reflect what is going on elsewhere.

Dr. Kennedy: Yes, but a compelling argument exists that the blood compartment is not where we should be looking, and it may indeed not be reflective of the general immune response. The difficulty with the peripheral blood compartment is getting enough cells. If specific populations of lymphocytes are to be isolated, pints of blood must be drawn, and it becomes a much more difficult approach. Some very interesting ways now use flow cytometry to look at signaling. We have begun to look at calcium signaling and to develop antibodies that will allow us to look at phosphorylation of very specific proteins by flow cytometry after activation. So, I think with flow cytometry we will be able to do some of those studies with fewer cells.

Dr. Farthing: I think you have produced some extremely interesting data, but there is some difficulty in that this was a mixed model: physical stress, psychological stress, and stress from lack of sleep; also, presumably some of these individuals had infections. So, I find it very hard to interpret exactly what was driving what which way in your data. It may be a model that is only relevant to special forces. I presume that they could eat as much as they wanted? Was there any question of limiting their dietary intake?

Dr. Kennedy: There may have been an element of intake limitation in the 60-day model. These individuals had a time limit on their meals and you could see differences between individuals. Some would wolf down 4000 to 5000 calories with a large spoon, but others would be much slower and may not have taken as much as they would have in the mess hall. We think there was probably an appetite suppressing effect. Clear differences appear between individuals in their perception of how hungry they are and what they need.

Dr. Farthing: Those very low leptin levels would be an appropriate response to variation in feed intake.

Dr. Kennedy: The leptin levels were low across the board. After 20 days, occasionally an individual might have a leptin value of 200, but everybody in this group of more than 100 individuals had values of 300 or less, so they all had very significant declines. The standard error bars on those last two time points are very narrow.

Dr. Farthing: During these studies, did you look at the CRH-ACTH-cortisol pathway, because it would seem to be a possible explanation for the depressed appetite?

Dr. Kennedy: We did look at cortisol levels and the blood was drawn at the same time, 5 a.m., at each time point in the studies. We did not find any correlation with simple single point cortisol levels, but we did not do a dexamethasone suppression test, which would be a better way of assessing that axis.

Dr. Gershwin: Did you look at heat shock proteins, or any other stress protein in the model?

Dr. Kennedy: We were very interested in looking at heat shock proteins and these are being analyzed but the data are not available yet. One of our interests is whether or not heat shock proteins are altering the nuclear translocation of phosphorylated transcriptional activators.

Dr. Griffin: Just returning to your point about plasma, or cells in plasma, and reflecting what is going on in the whole immune system, I do not think that is necessarily true. For example, in HIV disease, people have been measuring CD4 kinetics in plasma for a long time, and the finding, of course, is that what is going on in the lymph node is far more important than...
what is going on in the plasma. A technique is now available for measuring the turnover in vivo in humans of any member of the white cell series. This involves the use of 13-carbon glucose, which is metabolized to 13-carbon ribose, enters DNA, and then, providing a cell from plasma can be purified, you can then look at its kinetics very easily. With a technique such as that, you could see if the fall in CD4 positive cells was caused by increased removal or decreased production. You could do the same for any of the white cell series that you could sort out and extract DNA from.

**Dr. Kennedy:** Because of the nature of my research, I am hoping the plasma compartment does mimic the lymph node and spleen compartments! I am not totally convinced that such is the case, but I think that at the moment we are at a point where without some major technical advance it would be difficult to know for certain.

**Dr. Keusch:** Some of my colleagues have accused me of having a prejudice against animal models, so I would like to try to redeem myself. One very interesting model is the mouse put to exercise on a wheel or a water bath and injected with coxsackie B4 virus. The exercise has a dramatic effect on viral titers of those myotropic strains. So, it might be an interesting model with which to look at the effect of exercise on the immune system and how that might relate to viral proliferation and virus-mediated tissue damage.

**Dr. Kennedy:** I agree. A paper published about 4 months ago showed exertional rhabdomyolysis in coxsackie B4 virus infection during eccentric exercise (3). The question was raised whether selenium deficiency might alter the inflammatory response and potentiate the virulence factor of coxsackie B4.

**Dr. Woodward:** It seems to me that a common factor could be oxidant stress. Beck showed the same thing with vitamin E deficiency (4), and more recently with a glutathione peroxidase knock-out mouse (5). It strikes me that the common factor is antioxidants, or at least oxidant stress.

**Dr. Kennedy:** I agree. I do not think the story is going to be as simple as selenium per se, although the selenium may be a key modulating factor.

**Dr. Meydani:** Data in humans show that eccentric exercise increases oxidative stress, so it could be a common pathway.

**Dr. Abu-Zekry:** Did you do any studies, or do you know of some studies, relating stress to the development of malignancies or autoimmune diseases?

**Dr. Kennedy:** That certainly has crossed our minds because of the transformation we see from a TH1 to a TH2 response, and now the alteration in signaling pattern. We are currently interested in the ability of allelic variance to alter the signal transduction pattern, which has a lot to do with autoimmune disease, particularly multiple sclerosis. In a multiple sclerosis model, many of the same signaling pattern abnormalities are seen. Various workers have been focusing on the role of lymphocyte signaling in autoimmune disease states. It is a fairly big leap to suggest that our data show that certain individuals under stress may be more susceptible to autoimmune diseases, but it is an intriguing thought.

**Dr. Haschke:** Could you comment on whether the changes in immune function that you observed during the period of endurance training in military personnel are comparable to those seen in athletes, for example during endurance training before the Olympic games?

**Dr. Kennedy:** I am not really sure how it compares with the exercise immunology models that have been done. I would define those studies as models of acute exercise and its effect on immune cells, cytokines, and so on, whereas we deliberately sought a multistress environment. Our primary reason was that we did not want to do all this work and come up with nothing, because we did not stress the subjects enough! We picked our model to ensure that the subjects
were as stressed as was ethically possible for a human study. Thus, I am not sure how our model compares with exercise physiology. I would say that if a parallel exists, it is probably best made with what is known as overtraining fatigue of the immune system.

**Dr. Haschke:** Were the outcome variables that you measured comparable, or similar?

**Dr. Kennedy:** Some are similar, some are not. The exercise physiology literature is difficult to interpret, because many different studies have indicated different things. I think it is hard to control that environment *per se* and compare one study with another. I think our data are probably more comparable to the aging model in that the changes we see in lymphocyte subpopulations, as well as IL-2, reflect the results obtained by Meydani and others in models of aging.

**Dr. Ormisson:** I have the same question as Dr. Haschke. Is there any possibility of measuring stress in a very sick baby, say a premature neonate on a respirator surrounded by machines and equipment? What is the stress level under such circumstances?

**Dr. Kennedy:** I can only speak as an immunologist. It would be a major leap to extrapolate what I have shown to an infant, especially an infant under the age of 2 months, and even more so a premature infant. I think the immune system there reacts in a totally different way to environmental signals, so I am not sure how you would apply our data. However, the assays we did are compatible with work on premature infants in that you do not need a large amount of blood. With well-designed, very specific questions you can be successful with no more than 2 ml of blood.

**Dr. Fjeld:** I was interested in the disparity between the energy expenditure and the energy intake. I saw Jim Delaney's name mentioned, so I assume you measured energy expenditure by doubly labeled water.

I was wondering whether you looked at the energy density of the weight lost. I did a quick calculation and I see a huge difference in the apparent composition of the weight loss. Did you do that too, and how accurate do you think those data are?

**Dr. Kennedy:** I think the doubly labeled water data are very accurate. The body composition data are also fairly accurate. We did a subset of individuals by DEXA scan, and the remaining individuals were done by six site caliper.

The caliper body composition data correlated very highly with the DEXA scan data, although our body fat measurements were consistently approximately 1% to 2% higher in the caliper data than in the DEXA data. In all, I think the discrepancy was pretty accurate.

**Dr. Fjeld:** How did you measure the energy intake?

**Dr. Kennedy:** We did it mostly by food cards and visual estimation. A period occurs in both these training programs where these individuals go out into the field and it really becomes very, very difficult to do such studies. In those situations, visual estimation was about as accurate as we could get. The recall food survey cards that we had were fairly accurate. They correlated reasonably well with visual estimation, although visual estimation always gave a slightly higher value.

**Dr. Suskind:** The role of leptin in immunity seems a very important one. It would be interesting to see if leptin levels are a reflection of food intake under conditions of exercise stress.

**Dr. Kennedy:** I think the leptin levels probably initially reflected the energy deficit and body mass loss. In the first 20 to 30 days, the values were linear and correlated highly in both the 60-day model and the 21-day model. In the 21-day model, we never saw the leptin values plummet. It was only after day 20 or 30 that we see the levels plummet, and then I think there is a dissociation between body fat mass and leptin levels. I believe that is a stress phenomenon, not an energy-deficiency phenomenon.
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