Received: December 9, 2008
Accepted: May 15, 2009
Abstract published online: June 1, 2009
Full paper published online: August 31, 2009

J Venom Anim Toxins incl Trop Dis.
V.15, n.3, p.374-390, 2009.
Review article.
ISSN 1678-9199.

IMPACT OF MALNUTRITION ON IMMUNITY AND INFECTION

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ABSTRACT: Malnutrition may be a consequence of energy deficit or micronutrient deficiency. It is considered the most relevant risk factor for illness and death, particularly in developing countries. In this review we described the magnitude of this problem, as well as its direct effect on the immune system and how it results in higher susceptibility to infections. A special emphasis was given to experimental models used to investigate the relationship between undernutrition and immunity. Malnutrition is obviously a challenge that must be addressed to health authorities and the scientific community.

KEY WORDS: malnutrition, infection, immunity, experimental models.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Malnutrition can be a consequence of energy deficit (protein-energy malnutrition – PEM) or a micronutrient deficiency. In any case, it is still a major burden in developing countries and is considered the most relevant risk factor for illness and death, affecting particularly hundreds of millions of pregnant women and young children (1). This direct relationship between malnutrition and death is mainly due to the resulting immunodeficiency and, consequently, greater susceptibility to infectious agents.

It is estimated that 852 million people were undernourished between 2000 and 2002, with most (815 million) living in developing countries, particularly in southern Asia and sub-Saharan Africa (2-4).

Malnutrition by itself can cause death; however, epidemiological data reveal that it greatly increases susceptibility to and severity of infections, and is a major cause of illness and death concomitant with numerous diseases (5-7). It is the direct cause of about 300,000 deaths per year and indirectly responsible for about half of all deaths in young children (8). A direct correlation between a higher degree of malnutrition and higher risk of death is supported by the observation that severely malnourished children experience substantially higher mortality risk (9, 10).

Many factors affect the degree and distribution of PEM and micronutrient deficiency around the world, with poverty being at the top of the list. Other factors, in which the relationship between cause and consequence is hard to establish, are also deeply involved in malnutrition including: socioeconomic and political instability; impaired educational development; unsanitary conditions; poor food practices; breast-feeding habits and the shortage or ineffectiveness of nutrition programs (1, 11).

The causal relationship of malnutrition with immune suppression and infection is also aggravated by the profound effect of many infections on nutrition itself. For example, gastrointestinal parasites can lead to diarrhea, anemia and nutrient deprivation (12, 13). It is also well established that Aids, tuberculosis and other chronic infections cause cachexia and anemia (12, 14).

PEM is defined, in children, by measures that fall more than two standard deviations below the normal weight relative to age (underweight), the normal height relative to age (stunting) and weight for height (wasting). Among children aged less than 5 years in developing countries, about 31% are underweight, 38% have stunted growth and 9% show wasting (1). Severe malnutrition occurs almost exclusively in children

and manifests as marasmus (severe wasting) marasmic kwashiorkor (severe wasting with edema) and kwashiorkor (malnutrition with edema) (15). Marasmus is diagnosed when subcutaneous fat and muscle are lost because of endogenous mobilization of all available energy and nutrients. Clinical aspects include a triangular face, primary or secondary amenorrhea, extended abdomen and anal or rectal prolapse (16). Kwashiorkor usually manifests as edema, changes in hair and skin color, anemia, hepatomegaly, lethargy, severe immunodeficiency and early death (17, 18).

Severe PEM is typically characterized by the occurrence of fat degeneration in diverse organs including the liver and heart. In the heart, it causes subclinical or even overt cardiac insufficiency that demands urgent correction. The loss of subcutaneous fat, which markedly reduces bodily capacity to regulate temperature and also to store water, can also occur, provoking dehydration, hypothermia and hypoglycemia (19-21). Moreover, PEM is associated with atrophy of the small intestine that triggers the loss of both absorption and digestion capacity (22, 23).

MALNUTRITION AFFECTS IMMUNITY

A condition that results from a genetic or developmental defect in the immune system is called a primary immunodeficiency. Secondary or acquired immunodeficiency is the loss of immune function that results from a variety of extrinsic factors. The most well known secondary immunodeficiency is caused by the human immunodeficiency virus (HIV) infection; however, the most prevalent cause of immunodeficiency worldwide is severe malnutrition, which affects as much as 50% of the population in some impoverished communities (24). The consequent abnormalities of the immune system affect both the innate and adaptive immunity.

From a practical point of view, it is impossible to separate innate and specific arms of immunity because they work in an intrinsically connected manner in the body. However, for the sake of clarity, we will first refer to some mechanisms considered innate, i.e., the ones used against any type of pathogen. For example, availability of complement components and phagocyte function are compromised during malnutrition, which will directly affect pathogen elimination. This happens since the complement system by itself can destroy bacteria or viruses or because complement receptors present on the phagocyte surface mediate capture of pathogens. Significantly lower levels of complement, especially C3 that is the main opsonic

component, were described by Sakamoto *et al*. (25). In addition, phagocyte ability to ingest and kill pathogens was also reduced (26).

Antigen-presenting cells (APC) play a cardinal role during the induction, regulation and maintenance of innate and acquired immune responses (27). A series of studies demonstrated that the biological function of different cell types (B lymphocytes, macrophages and Kupffer cells) is clearly decreased during nutritional deficiencies (28-31).

The most relevant immunological alterations found in humans or in experimental malnutrition models that affect mechanisms associated with adaptative immunity will be briefly described below. Severe protein malnutrition in newborns and infants is clearly associated with atrophy in the so called primary lymphoid organs, i.e., bone marrow and thymus. Consequences are devastating because these organs are generators of B and T cell repertoires. Furthermore, malnutrition clearly affects hematopoiesis, determining anemia, leucopenia and severe reduction in bone marrow. Production of IL-6 and TNF-α by bone marrow cells is also significantly lower in malnourished animals (32). The capacity of malnourished hematopoietic stroma to support the growth of hematopoietic stem cells (CD34+) *in vitro* is also decreased (33). This is a very relevant finding because CD34+ cells are able to generate multiple lymphohematopoietic lineages as myeloid, erythroid and lymphoid (B and T) (34).

Severe protein malnutrition, mainly in newborns and small children, also provokes thymus atrophy that, in turn, reduces thymus cell number and also severely affects the development of peripheral lymphoid organs (35). The immediate consequence of this atrophy is leucopenia, decreased CD4/CD8 ratio and increased number of immature T cells in the periphery. Recently, Cortés-Barberena *et al*. (36) observed that significant lower numbers of CD3+ lymphocytes were present in the spleen of moderately and severely malnourished rats. They also detected a considerable impairment of T cell activation characterized by decreased expression of CD25 and CD71 in these cells.

These disturbances in the thymus have been more intensely investigated in malnutrition experimental models. For example, it has been demonstrated that the patent atrophy is due to decrease in T cell proliferation and increased depletion by apoptosis, affecting mainly immature TCD4+ and TCD8+ cells. This has been, at least partially, attributed to lower leptin levels during starvation or malnutrition (37,

35). Morphological changes in thymic epithelial cells associated with decreased thymic hormone production have also been described during malnutrition. This feature is apparently connected with a hormonal imbalance, involving a decrease in leptin and a consequent increase in glucocorticoid hormone levels in serum.

Immune responses from the epithelial barrier are also deeply affected by malnutrition. These alterations are mainly characterized by changes in the architecture of gut mucosa including flattened hypotrophia microvilli, reduced lymphocyte counts in Peyer's patches and reduced immunoglobulin A secretion (38, 39).

In our experience, BALB/c mice submitted to dietary restriction (80% of the amount of food consumed by a control group) during 40 days showed similar alterations. Together with a significant loss of body and spleen weights (Figure 1), these animals also presented modifications in the lymphoid organs (Figure 2). By comparison with the normal thymus presented in Figure 2a, a severe atrophy was observed in this organ in malnourished animals. Furthermore, the distinction between cortical and medullar areas was lost in the dietary restricted group (Figure 2b). Striking changes were also observed in mucous membrane associated with small intestine. Besides being smaller and irregular, intestinal villi also lost their brush borders. These alterations can be observed in Figure 2d, compared to the normal correspondent structures shown in Figure 2c.

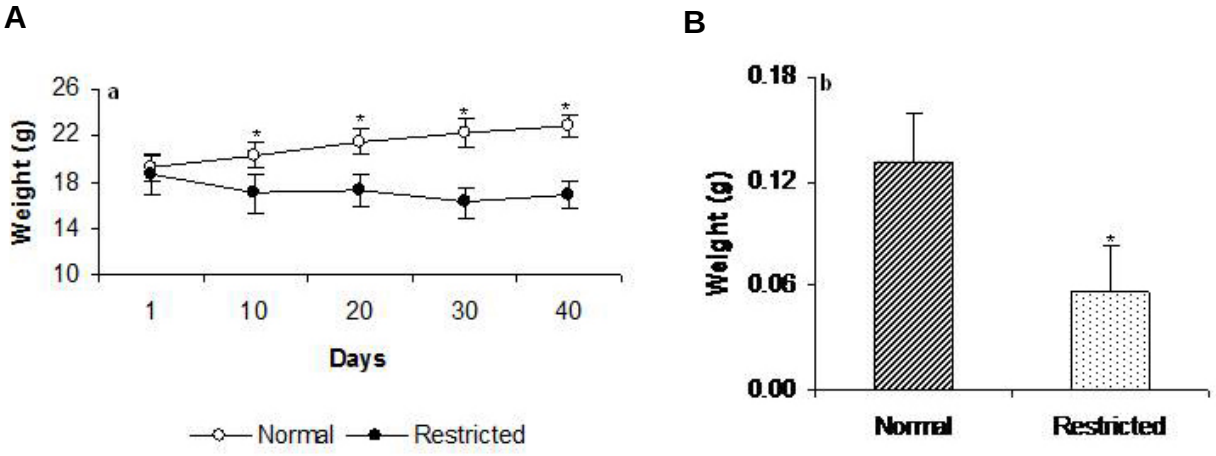

Figure 1. Effect of dietary restriction on body (**A**) and spleen (**B**) weights. Body weight was measured every day, each group included eight mice. Spleen weight was measured only on the 40th day after the beginning of dietary restriction; the normal group included four mice and the restricted one included three.
*Mean value was significantly different from the normal group ($p < 0.05$).

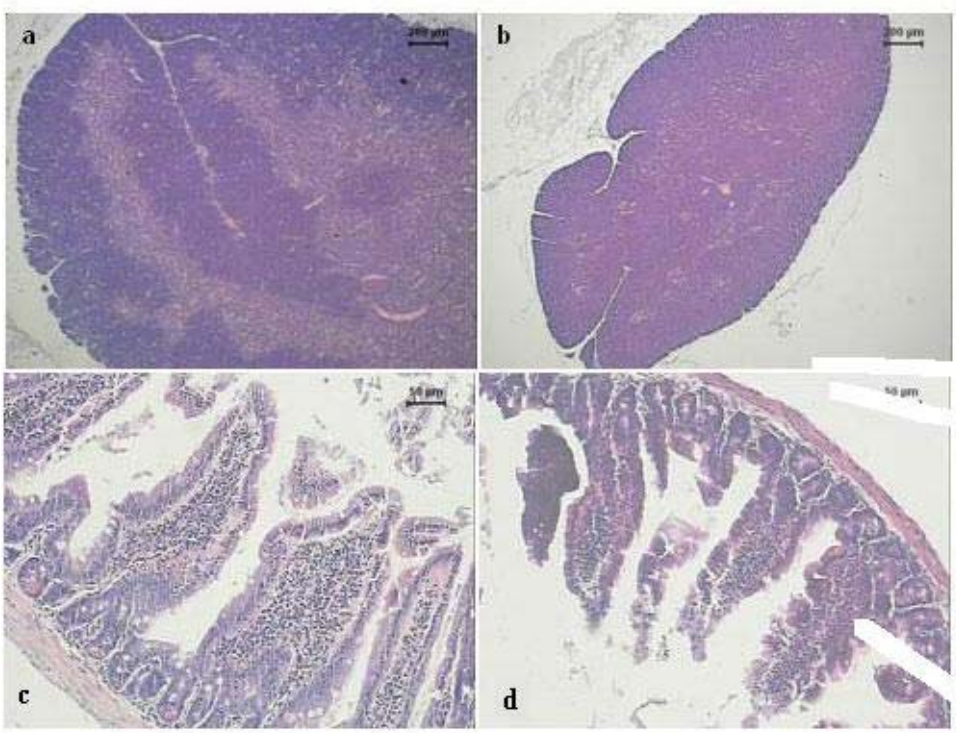

Figure 2. Effect of dietary restriction on the thymus (**a** and **b**) and small intestine (**c** and **d**) architecture. BALB/c mice were fed a normal diet (left column) or 80% of normal diet (right column). Sections were obtained 40 days after and stained with hematoxylin and eosin.

Antigen presentation to T cells is a fundamental event that precedes the development of both humoral and cellular immunity. Three different cell types (B lymphocytes, macrophages and dendritic cells) have been described as the main APC. Among them, only dendritic cells (DC) have been more thoroughly studied during malnutrition. There is a general consensus that different aspects of DC as number, cytokine production and ability to trigger proliferation of antigen-specific memory T lymphocytes are significantly affected by undernutrition (40). Very recently, it was demonstrated that adoptively transferred DC were also able to restore primary cell-mediated inflammatory competence to acutely malnourished weanling mice (41).

There is a general agreement that deficits of protein, energy or even both, produce a profound depression in acquired cell-mediated immune competence, whereas humoral competence is less predictably affected. This was supported by the observation that Th2-type immunoglobulins (IgG1 and IgE) were selectively elevated in weanling mice submitted to acute malnutrition, while levels of Th1-type immunoglobulins (IgG2a and IgG3) were unaffected (42). Similar and complementary

information was obtained in a vitamin A deficiency mice model. The shortage of this vitamin at the time of initial antigen exposure significantly enhanced the development of IL-10 producing Th2 or T regulatory cells while diminishing the expression of Th1 memory cells (43). More recently, Sakai *et al*. (44) corroborated these findings, demonstrating that protein deficiency impairs the induction of antigen-specific T cell proliferation, but not the B cell response in DNA immunized mice.

One of the main mechanisms that prevents or decreases immunity during undernutrition appears to be related to the activation of T lymphocytes. It is well established that the voltage-dependent K (V) potassium channels are vital for the activation of T cells. Fernández *et al.* (45) reported a significant decrease in the K^+ current and positive activation of the membrane potential by 20 mV in T lymphocytes derived from rats with severe malnutrition. It has also been demonstrated that moderately and severely malnourished rats present lower absolute and relative numbers of CD3+ and CD4+ lymphocyte subpopulations. This was also associated with a major decrease in the expression of CD25 and CD27 that are molecules absolutely required for T cell activation and proliferation (36).

The impaired activation of T cells has been clearly associated with deficits of cytokine production which are the main molecular mediators of immunity. This was evident in malnourished children that showed reduced production of type 1 cytokines (IL-2 and IFN-γ) (46).

MALNUTRITION INCREASES RISK OF INFECTION

The strong relationship between malnutrition and infection was originally described by Scrimshaw *et al*. (47). From this framework, much investigation was done in this area and there is a total agreement among authors that mortality is significantly more elevated in undernourished child compared to healthy ones. The study by Man *et al*. (48), which included a large population of hospitalized Gambian children, clearly illustrated the relationship between undernourishment, characterized by lower weight relative to age, and higher mortality indexes associated with many infectious diseases.

One-third of the world's population is infected with *M. tuberculosis*, the main agent that provokes death among infectious diseases (49, 50). This infection is particularly influenced by undernutrition and is a major cause of morbidity and mortality in developing countries where PCM is also prevalent (51). Furthermore, malnutrition as

an important risk for tuberculosis has also being reinforced by findings in experimental models (52). Similarly, undernutrition may also affect the clinical outcome of tuberculosis (53). A recent meta-analysis suggested that low serum vitamin D levels are associated with higher risk of active tuberculosis (54). It is important to emphasize that tuberculosis is a typical condition whose evolution, characterized by a chronic inflammatory process, accentuates undernutrition and causes a typical cachexia. This has been partially attributed to IgG1 antibodies, that up-regulate TNF-α and IL-6 (proinflammatory cytokines), but not to IL-10 (anti-inflammatory cytokines) (55). Emergence of highly virulent drug-resistant strains of *M. tuberculosis* has been largely attributed to a combination of poorly implemented drug regimen and coinfection with HIV. It has been suggested by Prentice *et al*. (56) that malnutrition may contribute to the appearance of resistant *M. tuberculosis* strains (57).

Even though effective vaccines are licensed for measles, it continues to cause death and severe disease in children worldwide. Complications from this viral infection can occur in almost every organ or system including pneumonia, croup and encephalitis. Among other factors, malnutrition and vitamin A deficiency increase complication rates (58). There is experimental evidence that vitamin A supplementation in children is associated with a reduction from 23 to 30% in mortality risk and attenuation in disease severity (59). For this reason, the World Health Organization recommends administration of an oral dose of vitamin A to children with measles that live in vitamin A deficiency areas (60).

Malnutrition and intestinal parasitism share a similar geographic distribution, with the same individuals experiencing both diseases simultaneously (61). The coexistence between undernutrition and nematode infection involves two causal pathways, malnutrition that augments susceptibility to infection and the infection itself that leads to a more accentuated undernutrition (62). Intestinal nematodes may provoke malnutrition because they cause anorexia and a variety of pathophysiological responses in the gastrointestinal tract such as vomiting, diarrhea and malabsorption. Together, these alterations deleteriously affect the host ability to get enough nutrients from the diet (63). Parasites that clearly affect the nutritional status are soil transmitted helminths, *Giardia duodenalis*, *Entamoeba histolytica*, coccidia and *Schistosoma* sp. (64).

There is also a general consensus that PEM is associated with greater malaria morbidity and mortality in humans (65). Supporting this observation, controlled clinical trials of either vitamin A or zinc supplementation indicated that these nutrients can substantially reduce clinical malaria outbreaks (66, 67). Opinions regarding the effect of certain micronutrients, as iron, for example, are still contradictory (68, 56).

Noma is an opportunistic infection promoted by extreme poverty that evolves rapidly from a gingival inflammation to mutilating orofacial gangrene. Even thought it can be observed worldwide, it is much more common in sub-Saharan Africa. It results from very complex interactions among malnutrition, infection and compromised immunity, and is very frequently preceded by malaria, measles and severe necrosating ulcerative gingivitis (69).

EXPERIMENTAL MODELS

Many studies carried out in human populations from developing countries indicated important deficiencies in macronutrients (proteins, carbohydrates and fat leading to protein-energy deficiencies), micronutrients (electrolytes, minerals and vitamins leading to specific micronutrients deficiencies) or both (70-72). These works were very relevant because they permitted the identification of the most severely affected regions and consequent intervention by humanitarian organizations and local governments. However, laboratory animals have been very useful in studying the effects of different levels of malnutrition, since non-nutritional factors that affect humans can be controlled in this type of evaluation. The use of animal models in malnutrition has brought a great deal of information to molecular mechanisms involved in the higher susceptibility to infections and also to immunodeficiency secondary to undernutrition (73, 13).

The most employed models are adult mice and rats (outbread or isogenic) feed with reduced amount of proteins, vitamins or micronutrients. The percentage of dietary restriction varies from 10 to 70%, according to different authors. As immunodeficiency associated with prepubescent malnutrition underlies a staggering burden of infection-related morbidity, acute weanling mice have also been explored to investigate the effects of malnutrition (41). More seldom, cats and dogs have also been used. More recently, transgenic and knockout mice have also been employed to better understand the mechanisms involved in higher susceptibility to infectious agents in malnourished mice (72, 74).

These numerous animal models allowed a growing understanding and characterization of the immunological disturbances triggered by undernutrition. Some examples of the most relevant findings in this research area are presented in Table 1.

Table 1. Experimental models employed to study the effects of malnutrition on immunity and susceptibility to infection

	Diet restriction	Effect	Reference
Human reconstituted SCID mice	Total vitamin A restriction; 7-day gestation period	Impaired antibody production after tetanus toxoid immunization	74
BALB/c mice	Three diets containing 6, 3 or 1% protein; 6 weeks	Excessive production of PGE2 and decreased levels of IL-10 and nitric oxide	75
Swiss-Webster mice	Total protein restriction; 1 week	Decreased PKC activity and Bcl-2 protein expression, higher macrophage apoptosis	76
SD rats	Total Zn restriction; 34 weeks	Thymus atrophy, oligospermia, testicular atrophy and loss of sperm cells and spermatocytes	77
BALB/c mice	Multinutrient restriction (protein, iron and zinc); 6 weeks	12% body weight loss, lower NF-κB activity, decreased production of TNF-α and NO by macrophages	78
BALB/c mice	Total vitamin A restriction 2-5 weeks	Increased Th2 and T regulatory cells decreased Th1	43
C57BL/6 mice	Lower casein diet (75%); 4 weeks	Impaired T cell response to DNA vaccination	44
Swiss mice	PEM 4% protein	20% body weigth loss, anemia, leucopenia and severe reduction in bone marrow, lower production of TNF-α, IL-1α, IL-6	32
C57BL/6 mice	Total dietary restriction (70%); 52 weeks	Decreased humoral response to hepatitis B virus	79

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