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GASTROINTESTINAL NEMATODES, NUTRITION AND IMMUNITY: Breaking the Negative Spiral

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■ **Abstract** Nutritionists have long understood that intestinal nematode parasites have deleterious effects on host nutritional status, but only recently has the importance of malnutrition as a predisposing factor to intestinal nematodes been recognized. Here we review experimental and field studies on the effects of protein, energy, zinc, vitamin A, and iron deficiencies on gastrointestinal (GI) nematodes of humans, livestock, and laboratory rodents, and draw certain conclusions about the state of our current understanding. In general, malnutrition promotes the establishment, survival, and fecundity of these parasites, but the magnitude of the effect depends on factors such as host species, parasite species, particular infection protocol used, magnitude of the infection, severity of the nutritional deficiency, and presence of single or multiple infections and single or multiple nutritional deficiencies. We highlight the Th2 arm of the immune system as a component of primary importance in the association between malnutrition and GI nematode infections. We summarize what is known about underlying mechanisms that may account for the observed patterns. Finally, we suggest future research directions.

CONTENTS

GASTROINTESTINAL NEMATODES AND MALNUTRITION

Their Place in Our World

Gastrointestinal nematodes are chronic pervasive infections that contribute to widespread morbidity and mortality worldwide. Estimated to infect over one quarter of the world's population, the four most common human gastrointestinal (GI) nematodes, *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americana*, and *Ancylostoma duodenale*, are responsible for an annual mortality of 18,000 people and for over 4 million years of life lost per annum due to premature death or disability (164). In livestock, related parasites are such an important cause of morbidity and mortality that producers use millions of dollars worth of anthelmintic drugs each year despite the full knowledge that such practices lead rapidly to drug resistance in the parasites (115). Family pets are tested regularly for intestinal nematodes and wild animal populations are host to a variety of similar parasites. All GI nematodes discussed in this review have a direct life cycle involving a single host in which adult worms mature and reproduce in the GI tract, and with one exception (*Trichinella spiralis*), all release eggs or larvae into the environment through the host feces.

Reasons to Postulate Causal Associations Between Malnutrition and Infection

The association between undernutrition and GI nematode infection has been recognized for many decades by veterinarians and health care workers who have observed that malnutrition and intestinal parasitism share a similar geographical distribution, with the same individuals experiencing both disease states simultaneously (104). The co-existence between undernutrition and nematode infections has been explained by invoking two causal pathways: Infection leads to malnutrition and alternatively malnutrition increases susceptibility to infection (122, 133, 136, 157). However, because both pathways occur concurrently it is often difficult to resolve whether the malnutrition preceded or resulted from the parasitic infection. Intestinal nematodes may lead to malnutrition because they

cause anorexia and a variety of pathophysiological responses in the GI tract (vomiting, diarrhea, malabsorption) that directly affect the ability of the host to gain nutriture from the diet; these effects can account for observations that more heavily infected individuals suffer from more severe malnutrition (132). On the other hand, one can explain the concurrent presence of high infection rates and severe malnutrition in the same individuals by citing the now classical literature that describes how malnutrition impairs immunity and that impaired immunity, in turn, increases susceptibility to infection (24, 123).

We envisage the interactions between malnutrition and infection as a negative spiral whereby malnutrition promotes infection, and infection leads to malnutrition. This construct of a negative spiral raises important questions when managing these two interacting conditions. Can and should treatment of intestinal nematodes be used as an indirect means of improving nutritional status? Can nutrient supplements be used as a means of indirectly reducing infection? Should drug treatment be given before, concurrent with, or after nutritional interventions? An understanding of the dominant pathway may be critical when proposing intervention strategies, especially in an epidemiological setting where multiple factors influence both nutritional status and infection and may modify the interaction between the two. The strength (and even the direction) of the association between infection and malnutrition depends on the parasite species (54, 152), the age and sex of the individual (102, 104), concurrent infections (119), and on factors that themselves are associated both with malnutrition and infection such as family income and level of education (118). Over time, the importance of measuring potential environmental and social confounders has influenced design of epidemiological studies on nematodes and undernutrition, but still most studies ignore multiple nutritional deficiencies and concurrent helminth, protozoa, viral, and bacterial infections.

In this review, we explore our understanding of the interactions between nutritional deficiencies and intestinal nematode infections, the nutritional factors that influence host immunity to GI nematodes, the implications for control, and areas in need of further research. Because of the number of excellent reviews on the effect of GI nematode infections on nutritional status (87, 132, 136, 157), more focus is directed toward the effects of malnutrition on GI nematodes.

EVIDENCE THAT NUTRIENT DEFICIENCIES PROMOTE NEMATODE SURVIVAL

Macronutrient Deficiencies: Protein-Energy Malnutrition

Combined protein-energy malnutrition (PEM) remains the most widely studied nutrient deficiency associated with infectious disease and acquired immunosuppression. There is extensive literature on the consequences of PEM on immunity (69, 100), but a much smaller body of literature on protein, energy, or combined PEM and their association with immunosuppression during GI nematode infections

(16, 100). Furthermore, even though energy, and not protein, is now considered the most prevalent nutritional deficiency worldwide (82), most studies have not distinguished between the two despite a growing body of evidence suggesting that protein and energy deficits modify host defense mechanisms differently.

Epidemiological Studies in Human Populations Attempts to use epidemiological approaches to unravel cause/effect relationships between PEM and GI nematode infection explore either the causal pathway that malnutrition leads to high infection rates or the other directional pathway that presumes that high infection rates lead to malnutrition. Neither approach presents uniform findings. A study of children in rural Nigeria found that protein intakes were inversely proportional to the number of co-existing intestinal parasitic infections (116), whereas an Indonesian study that stratified children by intensity of *Trichuris* or *Ascaris* showed stunting to be positively associated with *Trichuris*, but not *Ascaris*, burden (54). The alternative approach, assessing risk of infection across variable degrees of malnutrition, has shown severely malnourished Ghanian children to have a tenfold greater prevalence of *Strongyloides stercoralis* than moderately malnourished children (100) and Zairean children with kwashiorkor to have higher *Trichuris* infection than normal, stunted, or wasted children (152). However, the outcomes with *Ascaris* differed. Two reports (54, 100) showed that *Ascaris* infection was independent of nutritional status and in Zaire (152), kwashiorkor was negatively, not positively, associated with infection intensity. One of the few observations on energy per se (90) reported a strong relationship in two-year-old children between infectious disease and a lifetime of low energy but not protein intake.

Cause-effect relationships between malnutrition and GI nematodes may be investigated using controlled interventions. Two common experimental interventions—drugs and diet—have been attempted, but each intervention presumes one unidirectional causal pathway. Evidence exists for and against both interventions modifying GI nematodes during PEM. Anthelmintic intervention studies often measure short-term growth (57) under the premise that removal of GI nematode infections will facilitate catch-up growth, perhaps because of improved protein digestibility (20, 64). Although drug treatment alone does not always result in catch-up growth, perhaps because of low infection levels (44), marginal malnutrition (53), or small sample sizes (20, 57), many studies have reported consistent improvements in growth following drug treatment of *Ascaris*, *Trichuris*, and hookworm (3, 54, 55, 91, 134, 135, 137–139, 149). Unfortunately, very few dietary intervention studies that target PEM monitor impact on intestinal nematodes. Despite nutritional repletion following 29 weeks of high-protein diets resulting in improved serum albumin, hookworm egg production did not change relative to baseline levels in 12 malnourished adults with heavy hookworm infestations, leading the authors to conclude that hookworms were unaffected by improvements in host protein status (151). Later, Gupta et al (52) reported that nutritional status was unimproved by supplementary feeding of preschool children in India unless they also received anthelmintic treatment. This latter investigation is

one of very few human studies using both protein supplements and anthelmintics simultaneously.

Field and Experimental Studies in Livestock Much research on GI nematode-PEM interactions is described within the literature on parasitic infections in livestock, where producers and researchers have long recognized that GI nematodes impair productivity of cattle, sheep, and pigs as a result of parasite-induced reduction in feed intake, poor digestibility and absorption of feed, and pathophysiological defects in epithelial processes such as intestinal leakage of plasma proteins (13, 26, 157). Considerable attention has been directed toward demonstrating the deleterious impact of intestinal parasites on nitrogen utilization and livestock performance, but the reciprocal relationship, that well-nourished animals resist intestinal parasitism better than those less adequately fed, is gaining increasing prominence as investigators recognize that prolonged parasitism in protein-deficient animals can be reversed by protein supplementation (26, 113, 156, 157). Studies in lambs have demonstrated that high-protein diets enhance the development of immunity against *Haemonchus contortus* (1), *Trichostrongylus colubriformis* (66), and *Ostertagia circumcincta* (27). The long-term ability of sheep to control their GI nematode infections under natural grazing conditions has been shown to be directly proportional to the protein content during the post-weaning diet (33). Recently, Knox & Steel (77) reported improved resilience to infection in sheep given urea supplements. In fact, when given the choice of diets, infected sheep selected the diet with the higher protein concentration (79, 80). Protein, rather than energy, was identified as the major factor limiting the ability of *T. colubriformis*–infected lambs to utilize feed (17) because of a speculated parasite-induced loss of endogenous protein. Only one study has directly examined the consequences of energy restriction; energy-restricted sheep infected with nematodes have increased mortality (51). Taken together, these studies indicate that infection with GI nematodes in livestock increases the protein needs of the host or reduces the effectiveness of the immune response. However, the contribution of energy restriction requires further investigation.

Laboratory Studies in Rodents In both humans and livestock, infection is a continuous process where the host is constantly exposed to parasite eggs or larvae. Certainly in human studies, it is very difficult, if not impossible, to distinguish between susceptibility to incoming infections and clearance of existing infections. In laboratory models, researchers are able to separate these two processes by controlled infection protocols. Most GI nematodes used in laboratory rodents elicit a protective immune response that eliminates the parasites within 2–3 weeks of the first infection in well-nourished hosts. Is this protective response lost during protein deficiency? In rats fed a low-protein diet (5–10% versus 30%), the initial establishment and short-term survival of *Nippostrongylus brasiliensis* was unaffected, but the protein-deficient rats had increased worm survival (15, 30). Similarly, in both *Trichinella spiralis*-infected mice (47) and in *Trichuris muris*-infected mice

(92, 93), 4% protein deficiency delayed expulsion of adult worms. However, studies using the mouse–*Heligmosomoides polygyrus* model are less conclusive. Worm survival was unaffected when mice were fed a 2% casein diet, despite reduced body weight gain and overt hypoalbuminemia (18), whereas worm survival was prolonged when mice were fed both marginal (7%) and low (3%) protein diets (16) despite absence of hypoalbuminemia. The discrepancy among findings may be explained in part by differences in diet composition, but it is more likely a consequence of differences in protective immunity. Primary infections with *N. brasiliensis*, *T. spiralis*, and *T. muris* induce strong protective immunity whereas a primary *H. polygyrus* infection normally invokes a weak host immune response, due to the immunosuppressive action of adult worms (35, 97), that promotes long-lasting chronic infections.

Repeated infection ("challenge") protocols with *H. polygyrus* normally stimulate strong protective immunity upon re-infection, and all studies using these infection protocols support the hypothesis that restriction of dietary protein enhances GI nematode survival. Several studies using a challenge infection protocol have shown that a 2–3% protein diet increased worm survival, suggesting that protein deficiency impaired the capacity for acquired host resistance in previously immunized mice (16, 61, 71, 130). In repeated infection protocols, *H. polygyrus*–infected mice fed 2–3% protein accumulated worms in proportion to the intensity of exposure, whereas worm accumulation in mice fed 8–16% protein actually declined at higher infection doses (71, 129). Under semi-natural infection conditions in which a group of susceptible animals was continuously exposed to *H. polygyrus*, a 2% protein diet increased the rate at which uninfected mice acquired the parasites and also increased the survival of adult worms (129). Effects on parasite reproduction have been less consistent. Worm fecundity was unaffected by the 2–3% protein diets in some studies (16, 130) but was elevated in the malnourished mice in other studies (71, 129). Thus, severe protein deficiency prolongs survival of GI nematodes in rodents, and in all cases where elimination of the parasite is normally immunologically mediated, the data strongly suggest that the mechanism underlying the prolonged parasite survival is perturbed host immunity.

A few studies have directly examined the effects of energy restriction independent of protein deficiency in laboratory models and the results strongly suggest that the effects of energy restriction during GI nematodes infections are independent of protein restriction. Lunn et al (88) demonstrated prolonged *N. brasiliensis* survival in energy-restricted rats that was accompanied with hypoalbuminemia, which the authors attributed to protein leakage into the intestine through parasite-induced lesions. Recent work in our laboratory successfully produced a mild to moderate energy deficit that was not accompanied by any signs of protein deficiency. We showed that a 20% or 25% reduction in energy intake, independent of any alteration in protein status, exerted dramatic effects on survival of *H. polygyrus* in mice (78). What was particularly striking about this observation was that, in this host-parasite model, a smaller energy deficit than protein restriction produced comparable changes in parasite numbers; furthermore, there were contrasting

differences in host immunity between the energy and protein restrictions demonstrating independent effects of dietary deprivations (61, 78).

Micronutrient Deficiencies

As with macronutrients, studies on the association between micronutrient deficiencies and intestinal nematode infections reveal the complexity of the interrelationship. Although beneficial effects of potassium, molybdenum, and cobalt, but not selenium and copper, have been reported against GI nematodes in livestock (157), most research on trace elements and GI nematode infection in humans and in laboratory models focuses on deficiencies of zinc, vitamin A, or iron, which are among the most pervasive nutritional deficiencies in humans worldwide.

Zinc The first study to suggest a relationship between zinc deficiency and increased nematode infections was a cross-sectional survey of Jamaican children (21) that showed a weak but significant negative correlation between plasma zinc levels and numbers of *Trichuris*. A later randomized, double-blind clinical trial of Guatemalan children reported contrasting results with no effect of zinc supplementation on re-infection with GI nematodes following drug treatment (49). This absence of effect was attributed to the lack of pre-existing zinc deficiency in these Guatemalan children. In addition to effects on parasite numbers, an intriguing association between refeeding of severely malnourished infants and parasite reproduction emerged when Bundy & Golden (21) noted a transient increase in *Ascaris* egg production during refeeding that was attributed to a decrease in plasma zinc. This relationship highlights a possible unexpected consequence of refeeding programs and we suggest that this temporary decline in plasma zinc may lead to further immunosuppression releasing the parasite from immunologically induced constraints in egg production (126).

Data are now accumulating in rodent models to show a graded response depending on the magnitude of the dietary zinc restriction. In primary *H. polygyrus* infections where immunity is not normally stimulated, moderate (3 mg/kg) (16) and severe (0.75 mg/kg) (126) dietary zinc restriction prolonged *H. polygyrus* survival whereas a marginal deficiency (5 mg/kg) did not (95). This pattern correlated well with biochemical and physiological indicators of tissue, but not plasma, zinc deficiency, and with host immune effectors. Investigators have also examined several infection models where immunity is fully functional and have confirmed a relationship between zinc deficiency and increased worm burdens. Fenwick et al (41) reported delayed expulsion and higher burdens of *T. spiralis* in rats fed a moderately deficient diet (3 mg/kg) compared with control animals, whereas intake of 3 mg zinc/kg diet had no effect on the number or size of *N. brasiliensis* in rats but increased total egg output (38). When rats were infected with *Strongyloides ratti*, the 3 mg/kg dietary zinc delayed but did not abolish the ability to eliminate intestinal worms by day 28 pi (40). Similarly, a diet of 3 mg/kg zinc during challenge infection with *H. polygyrus* (16) had no effect on *H. polygyrus* worm burdens

whereas a more severe deficiency (0.75 mg/kg) profoundly impaired the immune response and facilitated *H. polygyrus* survival (126, 127). Interestingly, data from repletion studies (40, 41) using both *S. ratti* and *T. spiralis* hint that repletion actually improves ability to control infection over that of the well-nourished control animals, supporting the therapeutic use of zinc.

A complication associated with laboratory models of zinc deficiency is the reduced food intake that accompanies zinc-deficiency. Whereas concurrent energy restriction appeared to have no effect on *N. brasiliensis*, *T. spiralis*, or *S. ratti* infections, many of the effects attributed to zinc deficiency in *H. polygyrus* were a consequence, at least in part, of the concurrent energy restriction (125–127. The greater number of worms and higher per capita egg production in zinc-deficient mice after challenge infection were also detected in pair-fed control mice, indicating effects of both zinc and energy restriction (126, 127). Parasites developed more rapidly in energy-restricted mice during a primary infection and even more so in zinc-deficient mice, perhaps reflecting zinc- and energy-dependent changes in worm migrational habits, local inflammatory response to the larval stage, and/or GI physiology such as gut transit time or intestinal contractility. Zinc deficiency and energy restriction produced opposite effects on position of worms during a primary infection: In zinc-deficient mice, adults moved posteriorad whereas in energy-restricted mice, fourth stage larvae were more anteriorad (126). The parasites themselves were not zinc deficient (126), suggesting that the negative impacts of zinc deficiency on worm survival, development, and migration were likely mediated through perturbations on the host immune response. During both primary and challenge infections, *H. polygyrus* were more sensitive to dietary protein deficiency (3%) than to zinc restriction (3 mg/kg) (16). Furthermore, each nutrient deficit was independent of the other, suggesting that each had a different physiological effect on the host immune system.

Vitamin A Several studies suggest an association between vitamin A and *Ascaris* infection, with the strong implication that *Ascaris* leads to malabsorption of vitamin A (136, 147, 148). However, this area remains controversial. Ahmed et al (4) were unable to demonstrate impaired vitamin A absorption in *Ascaris*-infected children, yet Curtale et al (31) reported that *Ascaris* was a significant risk factor for xerophthalmia in Nepal. Vitamin A supplementation had a stronger effect on vitamin A status than treatment of *Ascaris* in Indonesian children (146), yet vitamin A status improved following treatment for *Ascaris* in other studies (63, 89, 108). Very few studies have examined the effects of vitamin A deficiency on host resistance to GI nematode infections in animal models. During primary infection with *T. spiralis*, vitamin A deficiency did not alter the number of larvae in the intestine or muscle, the worm expulsion rate, or the level of egg output despite induction of multiple systemic and gut-associated immunological defects in mice (23). Both numbers and egg production of *H. polygyrus* were elevated in vitamin A–deficient mice (45).

Iron Iron deficiency and anemia have been frequently observed in patients with hookworm infection in a dose-dependent relationship such that individuals with higher levels of infection have greater risk for lower hemoglobin status (5, 58, 102, 140, 141). Hookworms derive their nutrients from host blood and tissue and as a result can induce significant iron losses in the intestine (140). Human studies have shown that high levels of iron intake do not lower prevalence of infection (83, 151) and have led to the conclusion that hookworm infection causes iron deficiency, rather than iron deficiency predisposing to infection. This generalization appears not to be true under controlled experimental conditions. Iron deficiency increased larval establishment and adult survival of *N. brasiliensis* in rats (15). Repletion of iron did not fully restore hemoglobin values but was effective in accelerating parasite expulsion (15). During challenge infection with *N. brasiliensis*, Duncombe et al (37) observed higher worm burdens in rats fed a iron-free diet compared with well-fed controls, suggesting that acquired resistance to secondary nematode infections is dependent on adequate iron nutriture. More research is required on effects of iron deficiency on other GI nematodes, particularly those causing chronic infections, before one can make definitive comments of the role of dietary iron in development and control of intestinal parasitism.

Conclusion PEM as well as poor vitamin A, zinc, and iron intakes predispose to GI nematode infections, which in turn exacerbate the nutritional deficiencies and further prolong nematode survival in human, livestock, and rodent models. Interestingly, the degree of deficiency required to increase worm burdens differs for each nutrient, and for protein and energy, changes in worm burdens occurred without overt signs of compromised nutritional status. This suggests that the changes in host defense mechanisms occurred before biochemical signs of clinical deficiency for protein and energy, whereas for vitamin A and zinc, severe dietary deficiencies that were accompanied by declines in serum and/or tissue concentrations were required before effects on host defenses were noted.

IMMUNOLOGICAL MECHANISMS UNDERLYING NUTRITION-INFECTION INTERACTIONS

Th1 versus Th2 and Nematode Immunity

Immune responses to infectious organisms involve two categories of T helper (Th) cells: type I Th (Th1) cells are responsible for cell-mediated immunity against bacterial, protozoal, viral infections, and intracellular parasites whereas type 2 Th (Th2) cells mediate antibody-dependent immunity against extracellular parasites including GI nematodes. Each response phenotype produces a dominant pattern of cytokine and immune effectors. Moreover, each phenotypic profile (Th1 versus Th2) is antagonistic to the differentiation and activity of effectors belonging to the reciprocal phenotype (98). Primed Th2 cells secrete interleukin (IL)-4, IL-5,

IL-9, and IL-10, which promote the proliferation and activation of Th2-associated effectors such as IgE or IgG1 secreting plasma cells, eosinophils, and mucosal mast cells (MMC) (28) whereas Th1 cells produce IL-2 and IFN-γ and interact with APCs to synthesize IL-12. Th1-associated cytokines are important in macrophage activity and isotype selection for IgG2a, IgG2b, and IgG3, which mediate responses against bacterial and viral infections (86). The distinction between these two arms is not complete, however. Both Th cell types secrete IL-3, tumor necrosis factor-α, and granulocyte-macrophage colony stimulating factor (98). Furthermore, each cytokine has pleiotropic effects on multiple types of lymphoid cells, and there is much duplication of function among the different cytokines.

Experimental studies show that functional immunity to GI nematodes involves systemic Th2-type cytokines and effectors and, with rare exception (84), that IL-4 is a requirement for the Th2 cell response. In humans infected with helminth parasites, serum IgE and IgG1 levels are directly proportional to parasite-induced IL-4 production and inversely related to IFN-γ synthesis (74) where IgE-mediated eosinophil and mast cell activities are associated with effective resolution of helminth infections (6). The role of IL-4 has been clearly demonstrated in laboratory studies with *H. polygyrus* where anti-IL-4 antibodies and anti-IL-4 receptor antibodies block immunological control (154, 155). Consistent with these data, mice infected with *N. brasiliensis* and deficient in the signal transducer and transcriptional activator of IL-4, secreted lower amounts of Th2-dependent antibodies (145), but not Th1-dependent antibodies (128).

Evidence that Nutrient Deficits Impair Systemic Th1 and Th2 Immunity

In immunology, a working theory is that the reciprocal cross-regulation of Th1 and Th2 cytokines and their effectors produce a dominant immunological phenotype, which for GI nematodes is represented by the Th2 phenotype. It has been proposed that nutritional deficiencies may prevent the expression of the dominant Th2 phenotypes and that energy deficits (78), vitamin A deficits (23), and protein deficits (61) result in the overexpression of the Th1 cytokine IFN-γ and the down-regulation of essential Th2 cytokines. The absence of Th2 cytokines and their effectors results in prolonged survival of GI nematodes. Recently, we have shown that down-regulation of IL-4 and other Th2 cytokines and effectors occurred during zinc deficiency (121), protein deficiency (61), and energy restriction (78) and was associated in all cases with prolonged survival of *H. polygyrus*. However, energy restriction lowered both Th1 and Th2 cytokines (78), whereas protein malnutrition decreased Th2 and increased Th1 cytokine profiles (61). Vitamin A deficiency also failed to support the hypothesis of an up-regulation of a Th1 during *H. polygyrus* infection (45); however, during *T. spiralis* more IFN-γ was secreted by spenocytes in the vitamin A–deficient mice (23). Taken together, these results demonstrate important mechanistic differences between nutrients in their ability to modify the reciprocal relationship between Th1 and Th2 phenotypes and

would further suggest that immunopathology could result from defects in specific pathways, not simply a result of dysregulation in Th1 versus Th2 phenotypes.

The Importance of Intestinal Immunity to Nematode Infections

The GI tract is one of the largest immunological organs in the body and serves as the first line of defense against GI nematodes. Cells in the gut-associated lymphoid tissue (GALT) respond to intestinal pathogens by processing antigens for recognition by lymphocytes, by initiating a cascade of specialized Th2 immune responses to parasite-specific antigens at intestinal and systemic sites, by regulating the trafficking of immune mediators from the periphery back to the infected gut, and by participating directly in cytotoxic activities that limit parasite establishment and survival (19, 39, 109, 111, 153, 159). The GALT is categorized into two anatomically and functionally distinct compartments: the afferent limb [Peyer's patches, isolated lymphatic follicles, and mesenteric lymph nodes (MLN)] where antigen is presented to naïve lymphocytes (14, 81), and the efferent limb (intraepithelium and lamina propria of intestinal microvilli) where antigen-specific effectors mediate their anthelmintic effects (9). MMC progenitors, basophils, and eosinophils are drawn by chemotaxis to the intraepithelium and lamina propria, as seen for example in *N. brasiliensis* infection (7, 25, 67, 94). There they bind to IgE and parasite antigens (7, 103, 131), which leads to degranulation and release of histamine and serine proteases (75, 161) that are associated with worm expulsion. These events occur rapidly after infection. For example, infection with *T. spiralis* results in transport of IgE into the intestinal lumen within 24 hours of infection (110, 143). Previous studies have documented the early synthesis, production and uptake of Th2 cytokines by GALT (2, 12, 43, 65, 99, 114, 120). A further indication of the unique immune responses in the mucosa is the large percentage of T cells with gamma delta receptors that provide a contact signal for isotype switching to IgE production in the presence of IL-4 (42, 46, 68, 162). These observations have led to the conclusion that mucosal cells residing in the gut are the most important source of local cytokines during early parasitic infection (111).

Evidence that Intestinal Immunity is Perturbed by Nutrient Deficits During Nematode Infections

Studies comparing responses of intestinal with peripheral lymphoid sites in the infected, malnourished host have been recently completed in our laboratories using the *H. polygyrus* model. We have demonstrated important differential responses in both the pattern and timing of host immunity in these two lymphoid compartments during protein (61), energy (78), and vitamin A deficiency (45). We found that protein malnutrition was more detrimental to gut-associated than to systemic IL-4 production: Deficient MLN cells secreted less IL-4 and more IFN-γ shortly after challenge whereas deficient spleen cells secreted more IFN-γ only at 2 weeks

post-challenge infection (pci). Adequate protein intake was necessary for mRNA expression and protein synthesis of IL-4 in GALT but did not affect IL-5 or IL-10 production. Thus based on our protein results, decreased IL-4 combined with increased IFN-γ contributed to reduced levels of IgE, MMC, and gut eosinophils and to prolonged parasite survival, supporting the hypothesis that protein malnutrition increased the survival of a nematode parasite by decreasing gut-associated Th2 cytokines and effectors and increasing INF-γ(61).

However, neither vitamin A nor energy deficits support this classical theory that postulates down-regulation of Th2 and simultaneous up-regulation of Th1 cytokines. Energy deficits suppressed both Th1 (IFN-γ) and Th2 profiles (IL-4, IL-5, IgE, IgG1, and eosinophils) in both the gut and splenic lymphoid tissues (78). From our study we concluded that a surge in IL-4 production is required by both the systemic and GALT immune systems early during first exposure to a nematode infection, and that energy restriction prevents this. Similarly, in vitamin A–deficient mice (45), both IL-4 and IFN-γ decreased in MLN, whereas the expected up-regulation of IFN-γ associated with the corresponding down-regulation of IL-4, which is observed in *T. spiralis*–infected vitamin A–deficient mice (23), was only observed in the systemic response to a challenge infection in vitamin A–deficient mice infected with *H. polygyrus*.

Conclusion Current evidence shows that the immune system during nematode infections in malnourished hosts (protein, energy, zinc, and vitamin A) is characterized by declines in several Th2 immune effectors: IgE (16, 45, 61, 78, 125), parasite-specific IgG1 (16, 23, 45, 78, 125), and eosinophils (16, 45, 61, 78, 125). Additional evidence has been provided that both zinc and energy alter the functioning of specific cellular components, in particular T-cell and APCs (127), pointing to specific cellular defects resulting from nutritional deficiencies. Importantly, no single nutrient deficiency suppressed all immune responses nor did all immune responses respond similarly to each nutrient. Our studies on single deficiencies of protein, energy, and vitamin A in the *H. polygyrus*–infected mouse model provide evidence that each nutrient has a distinct role in modifying Th1/Th2 profiles during GI nematode infections, and that responses differ significantly by gut versus systemic tissue.

MALNUTRITION, INFECTION, AND IMMUNITY—A CO-EVOLUTIONARY PARADIGM

The concept of a co-evolutionary trade-off between host protective immunity and parasitic infection is not new (11, 96). GI nematodes have evolved a finely tuned ability to recognize biochemical and physiological cues of the host in order to initiate their establishment (142). Host resistance to GI nematodes is a heritable trait tied directly to the expression of host immunity (11, 96, 160), with a phenotypic spectrum ranging from exaggerated susceptibility to rapid clearance of infection (50). Because helminth infections in general do not induce long-lasting

immunity, the most effective immune strategy may not be to completely clear an existing infection, but to support a chronic low-level infection that provides continual antigenic stimulation.

On an evolutionary time scale, the co-evolution between host and parasite most likely occurred in undernourished individuals. This co-evolutionary environment raises important questions. As nutritional conditions improved, did the host evolve to invest the additional resources into growth, reproduction, and tissue maintenance, or into mounting a more vigorous immune response? The answer may be seen by contrasting what occurs today in developed versus developing countries. Developed countries are characterized by abundant food, excellent sanitation, adequate health care, and pharmaceutical interventions, and dramatically reduced prevalence of GI nematodes, yet increasing incidence of food allergies. In contrast, developing countries are characterized by malnutrition, GI nematode infections, yet remarkably little allergy (6). Some evolutionary biologists have suggested that intestinal parasites have exerted a strong selective force for a specific Th2 immune phenotype in GALT (34, 144). The GALT is considered to be the most primitive immune organ in vertebrates, and there is considerable support for the idea that the pathological consequences of Th2 response (i.e. food allergy) are an evolutionary hangover from a time when hosts needed a strong Th2 response to protect against parasites (107). If these observations are indeed true, then we could argue that the increased incidence of allergy arises not only from reduced exposure to nematode infections but also from improved availability of nutrients.

It is unlikely that links between host-parasite co-evolution and nutritional status end with host immunity. Recent studies in the viral literature highlight a need for sensitivity to how nutritional status may affect the parasites directly. Beck & Levander (10) have found that not only is the virulence of a benign strain of the Coxsackievirus B3 higher in selenium-deficient hosts, but also that the virus retains its virulent state when passaged into a well-nourished host. This suggests the possibility that parasitic organisms quickly co-evolve within their present nutritional environment. Therefore, issues such as the possible effects of nutritional status on the biology of the parasite, genetic heterogeneity in the host population with regard to resistance and susceptibility to infection, and the role of improved nutritional status not only in reducing infection but perhaps in increasing immunopathologies, may need to be considered when planning intervention programs that target nutritional deficiencies, GI nematodes, or both.

BREAKING THE NEGATIVE SPIRAL

Three main types of interventions are envisioned to directly attack the nutrition-infection-immunity triad: improved nutritional status, prevention or treatment of infection, and improved immunocompetence. Each intervention can be achieved in many ways, and each should in theory have beneficial repercussions on the other conditions. However, in a system as complex as a human community, such "simple" solutions may not be a panacea.

Nutritional Interventions

Although we presume that hosts are better able to control their helminth infections when well-nourished, there is a surprising lack of field studies in human populations where the benefits of nutritional improvement on helminth infection have been examined. Even in clinical trials with vitamin A supplementation where the outcome on infectious diseases has been monitored, helminth infections have largely been ignored (8). In free-grazing sheep, supplementary feeding with sunflower meal was shown to be more beneficial, but also more expensive, than slow-release anthelmintics in reducing production losses due to GI nematodes (156). The cost was reduced when urea-molasses blocks were used as nutritional supplements; these enhance the ability of ruminants to withstand GI infection (76). In the laboratory, repletion after zinc-deficient diets were fed restored the ability of the host to control *T. spiralis* infections (40, 41) and refeeding protein-deficient rats with methionine improved their ability to control *N. brasiliensis* (29). These studies indicate that improved nutritional status will reduce infection or infection-induced pathology.

The laboratory studies and much of the speculations about livestock and human infections focus on the benefit to an individual of improved nutritional status and consequent improved resistance to infection. However, the host population or community consists of a broad spectrum of individuals who differ in their genetically determined propensity to resist infections and who may differ in their response to supplementation. Three paradigms exist. If the effects of malnutrition are subtle relative to genetically determined resistance to infection, then nutrition interventions may not change the overall prevalence of infection, but may be beneficial only to those heavily infected individuals who have high need of nutrients for tissue repair. If nutritional deficiency acts in a synergistic manner in the genetically susceptible individuals, then nutrition will be very important for them, but will have little impact on the genetically resistant individual who is protected from infection despite being malnourished. This would be expected, for example, in ruminant parasites where protein deficiency does not completely abolish the capacity for antibody or eosinophil responses in resistant strains (26, 62). If malnutrition inhibits the development of resistance then nutrition interventions will be helpful in restoring the capacity of the genetically resistant individuals to control infection but may also increase their risk of immunopathology. Nutritional interventions may also be beneficial if the dietary constituents themselves have anthelmintic properties. Primates (60), pre-historic man (22), and present day (59, 105) appear to selectively ingest plants with medicinal properties as a natural means to manage helminth infections. Studies in livestock demonstrate anthelmintic properties of indigenous plants (56, 101), and much attention is being paid to the value of allelochemicals against human helminth infections (163). Thus interventions that promote use of a variety of indigenous plants may have both nutritional and antiparasite benefits, assuming plant products are selected wisely.

Anthelmintics

Drug efficacy itself is also affected by nutritional status. The ability of benzimidazole drugs to eliminate *N. brasiliensis* is significantly impaired in rats fed a diet deficient in iron and protein alone (37) or in combination (36), compared with well-nourished rats, presumably because of the reduced uptake of drug by the parasite (106). A variety of mechanisms may account for this observation, including the fact that nutritional status may affect drug absorption, drug metabolism, and drug uptake by the parasite. Malnutrition can reduce the success of drug interventions and the reduced efficacy is likely to promote selection of drug-resistant parasites (106), further limiting chemotherapeutic control of GI nematode infections. We might conclude therefore that nutritional intervention should precede drug treatments to ensure maximal drug effectiveness during intervention programs. However, parasitic infections may in turn reduce the absorption of nutrients and therefore the efficacy of nutritional intervention may be improved if parasites are eliminated first. Studies are urgently needed to evaluate the relative benefits of drug treatments before, during, or after nutritional interventions so as to achieve the goal of maximized benefits of such integrated approaches.

Enhanced Immunocompetence

GI nematodes do not induce life-long immunity, perhaps because they fail to stimulate long-lived IgE^+ B cells responsible for memory, as reported for *N. brasiliensis*–infected rats (85). A consequence of the incomplete protective response is the need to carefully consider when a nematode vaccine should be administered so as to attain maximum protection from the vaccine. Most clinical problems associated with GI nematodes occur in young animals, in children, or during pregnancy—that is, at times when malnutrition may be a problem. It is well known from the microparasite vaccination experience that vaccination is ineffective in malnourished children (78, 117) and the same problem could arise when vaccines become available for use against GI nematodes. Other forms of immune enhancements have been under consideration including injections of plasmids containing genes that express key cytokines (72, 112) and administration of probiotics (32) to stimulate Th1 response in the gut. The implication of such interventions for GI nematodes infections must not be ignored.

CONCLUSION

In reviewing the body of literature relating malnutrition, nematode infection and immunity, a few key points have become very clear. Despite the very large number of studies, a central dogma has yet to emerge in this interdisciplinary area of research. We know many facts, often about very specific effects of deficits of a certain nutrient on a receptor molecule or on levels of a specific cytokine, and there are

large numbers of field studies that repeatedly show that malnutrition and infection occur together. Yet more and more it is becoming clear that generalizations cannot be made about the effects of different nutrients on the various components of the immune response and that lack of understanding of the basis of functional immunity against nematodes makes it very difficult to pinpoint the nutrient deficiencies that should be of most concern. There remains a very large gap between the science of the molecular biologists and that of the public health sector, although both share the common objective of improved understanding of nutrition-infection-immunity interactions with the goal of reducing morbidity and mortality in human and livestock populations. We need gradually to move to greater cooperation and integration of research questions so that those mechanisms that will be of critical importance in making public health decisions emerge. For example, as energy deficiency is a dominant nutritional problem in the world, and as modest deficits in energy appear to have dramatic influences on the host immune response to GI nematode infections, more research should be directed toward energy deficiency, infection, and immunity. Such work would be of tremendous benefit to the large-scale interventions that are now in progress, but that target infection and vitamin and mineral deficiencies (48, 150). In addition, a better understanding of the relationship between malnutrition and drug efficacy in the nematode-infected host is required. Focus must be centred on the intestine; it is the site of nutrient absorption, it is the home of the majority of parasitic organisms, and it is arguably the largest immunological organ in the body. Breaking out of the negative spiral requires cooperation between immunologists, parasitologists, and nutritionists, between molecular biologists and public health workers. It is the communication among these groups that will uncover those research questions, the answers to which will best lead us to appropriate management of these important health problems.

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