Deficiencies in Selenium and/or Vitamin E Lower the Resistance of Mice to Heligmosomoides polygyrus Infections

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ABSTRACT Previous studies have shown that deficiencies in selenium (Se) and/or vitamin E (VE) can exacerbate the infectivity and pathogenesis of coxsackievirus B3 and influenza. Both Se and VE play a role in immune function and antioxidant defense. To determine whether these deficiencies would affect the normal course of infection with a metazoan parasite, mice were made deficient in Se and/or VE and inoculated with the gastrointestinal nematode parasite Heligmosomoides polygyrus. Both primary and secondary infections were assessed. Although the course of a primary infection with H. polygyrus was unaffected by diet, diets deficient in Se, VE, and both Se and VE (Se/VE double-deficiency) all caused delayed adult worm expulsion and increased fecundity during a secondary infection; suggesting an impaired intestinal response. H. polygyrus-induced IL-4 levels were diet-independent; but Se/VE double-deficiency blocked the H. polygyrus-induced IL-4 receptor-associated decrease in sodium-dependent glucose absorption in the jejunum that contributes to worm expulsion. In contrast, Se/VE double-deficiency had no effect on the infection-induced, IL-4R-associated increase in epithelial cell permeability that accompanies the infection. These results suggest that both Se and VE are required for specific IL-4-related changes in intestinal physiology that promote host protection against H. polygyrus. J. Nutr. 135: 830–836, 2005.

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Intestinal parasites infect approximately a billion people worldwide, cause substantial morbidity in affected populations (1), interfere with the uptake and utilization of nutrients (2), and can increase susceptibility to other infectious organisms (3,4). Malnutrition is also a worldwide problem that coexists with a high incidence of intestinal parasite infection and can lead to increased susceptibility to other infectious agents (5). Malnourished individuals are likely to be deficient in multiple nutrients including selenium (Se)2 and vitamin E (VE). Deficiencies in Se or VE can increase the virulence of coxsackievirus B3 and combined deficiencies increase susceptibility in genetically resistant mice (6–8). The intensity of infection with influenza (9) and the severity of infection with the protozoan parasite Trypanosoma cruzi are also enhanced by Se deficiency (10).

Both Se and VE are important in host antioxidant defense and immune function. Vitamin E is a lipid-soluble antioxidant present in cellular membranes that quenches free radicals and prevents lipid peroxidation (11,12), and it is found in especially high concentrations in immune cells (13). Vitamin E deficiency has been associated with increased oxidative stress (14,15) and impaired immune function including both humoral and cell-mediated immunity, phagocyte function, and lymphocyte proliferation (16). Additional studies have shown that age-related declines in immune function can be restored by VE supplementation (17,18).

A substantial body of research has also defined an important role for Se in both antioxidant defense and immune function. Selenium is important for the control of oxidative stress and, therefore, the redox state of the cell, due to its incorporation as selenocysteine into glutathione peroxidase (19) and thioredoxin reductase (20). Selenium is important for cytotoxic T lymphocyte and natural killer cell activity (21,22) and for protection against endotoxin-induced oxidative stress (23). Selenium supplementation suppresses TNF-α-induced HIV replication in culture (24) and Se deficiency can alter chemokine and cytokine expression in viral infections (25).

We were interested in the characterization of Se- and VE-dependent immunity to a metazoan parasite where host
resistance is expressed in the intestine because of the combined prevalence of geohelminth infection and malnutrition worldwide. Heligmosomoides polygyrus is a gastrointestinal nematode that naturally infects mice and provides a well-defined animal model to study immunity to helminth infection (26). Gastrointestinal nematode infections, in general, elicit a CD4+ Th2-dependent type 2 cytokine response that is characterized by elevated IL-4, IL-5, IL-9, and IL-13, eosinophilia, goblet and mucosal cell hyperplasia, and production of non-complement fixing reaginic antibodies including IgE and IgG1. Susceptible strains of mice, such as BALB/c, develop a primary infection that can be cured by anthelmintic drugs (27). Resistance to reinfection is IL-4 dependent and results in expulsion from the intestine around d 10 after inoculation. Clearance of a primary infection is enhanced by injection of exogenous recombinant IL-4 (28), whereas resistance to a secondary challenge infection is reduced by neutralizing IL-4 or blocking the IL-4 receptor (R) (27). IL-4R-mediated responses to adult H. polygyrus in the intestine include increased mucosal epithelial cell permeability, decreased sodium-dependent glucose absorption, and increased smooth-muscle cell contractility (29). In toto, the combined effects of IL-4 create a hostile environment for worms that ultimately leads to worm expulsion.

MATERIALS AND METHODS

Mice. Three-week-old weanling female BALB/c mice were purchased from the Small Animals Division of the National Cancer Institute. Mice were housed in a LabProducts microisocorator rack, 4–5/cage, and were allowed ad libitum access to specialized diets and chlorinated and deionized water. Mice (n = 4–12) received one of five isocaloric torula yeast-based diets (prepared by Harlan Teklad) that were adequate in all nutrients except those specified and are modifications of previously described diets (6). Two control diets were utilized, an adequate diet with 4% lard + 1% corn oil as the major fat source (Se+, E+, L), or an adequate diet with 4% menhaden oil (MO) + 1% corn oil in place of lard as the fat source (Se+, E+, MO). The control diet contained 0.2 μg/kg of sodium selenite and 50 mg/kg of d-a-tocopherol acetate. The MO-containing diet served as the control diet in experiments where VE-deficient diets were utilized. Menhaden oil increases the requirement for VE and therefore hastens the onset of VE deficiency (30). Three additional diets were used: 1) an Se-deficient, lard-containing diet (Se−, E+, L); 2) a VE-deficient, menhaden-oil-containing diet (Se+, E−, MO); and 3) an Se- and VE-deficient, menhaden-oil-containing diet (Se−, E−, MO). Mice were fed the diet for 3 or 5 wk prior to inoculation with H. polygyrus and continued with the respective diets for the remainder of the experiment. Vitamin E deficiency was confirmed by liver VE analysis (31). Selenium deficiency was confirmed by determining liver glutathione peroxidase (GPx) activity, an Se-dependent enzyme sensitive to dietary Se levels (32).

Parasite. Infective, ensheathed, third-stage H. polygyrus larvae (L3) (specimens on file at the U.S. National Parasite Collection, U.S. National Helminthological Collection, Collection 81930) were propagated and stored at 4°C until used (33). Mice were inoculated by oral gavage with 200 H. polygyrus L3 using a 20-gauge ball-tip feeding tube. To study the effect of diet on infection with H. polygyrus, mice were followed after mice were fed their respective diets for 3 (for doubly deficient mice) or 5 wk prior to the primary inoculation and 14 d after the start of the infection. Other mice were treated similarly and fed their respective diets during the anthelmintic drug-cure and resting phases prior to a secondary inoculation with H. polygyrus to study the effect of diet on a challenge infection. Mice used for studies examining the effect of diet on primary infection were fed the diet for the same length of time as mice receiving both a primary and a secondary challenge. Thus, all mice had been fed their respective diets for ~3 mo at the time of assay. Body weights were monitored both prior to and after H. polygyrus infection.

In vivo cytokine capture assay. Circulating cytokine levels of IL-4 and IFN-γ were measured using the in vivo cytokine capture assay (IVCCA) (34). IL-4 production was measured by injecting mice intravenously with 10 μg of biotin-BVD4–1D11 (anti-IL-4), and mice were bled 18 h later. Quantitative levels of IL-4-biotin-anti-IL-4 mAb complexes were measured by ELISA using microtiter plates coated with BVD6–24G2.3, a mAb to a second IL-4 epitope, and horseradish peroxidase-streptavidin. IFN-γ production was similarly measured by injecting mice with 10 μg of biotin-R4–6A2, an anti-IFN-γ mAb, and serum levels of IFN-γ-biotin-anti-IFN-γ mAb complexes measured with microtiter plates coated with AN-18. The IVCCA increases the sensitivity of detection of IL-4 and IFN-γ ~100-fold and does not interfere with cytokine-dependent processes.

Functional assays. Gastrointestinal function was assessed by measuring tissue resistance and sodium-dependent glucose absorption (29). One-centimeter segments of mucosa (4/mouse) were stripped of muscle and mounted in Ussing chambers that exposed 0.126 cm² of tissue to 10 ml of Krebs’ buffer. Agar-salt bridges and electrodes were used to measure the potential difference. Every 50 s the tissues were short-circuited at 1 V and the short-current circuit was monitored continuously. In addition, every 50 s, the clamp voltage was adjusted to 1 V for 10 ms to allow calculation of tissue resistance using Ohm’s law. A basal measure of tissue resistance, which reflects tissue permeability, was made initially. Changes in short-circuit current (isc) in response to the addition of glucose to the mucosal side of the jejunal segment were measured next.

Data analysis. Worm burden and egg production data were analyzed using a t test, one-way ANOVA with post hoc Dunnetts’s test, or the Mann-Whitney nonparametric test when the data could not meet the normalcy and equal variance requirements of the t test or ANOVA. Differences with P < 0.05 were considered significant. Comparisons were made between the appropriate control and deficient groups; i.e., groups fed the same fat (lard or menhaden oil) were compared to one another. A two-way ANOVA with a post hoc Student-Newman-Keuls analysis was used for comparing the in vivo production of cytokines in response to diet or H. polygyrus infection.

RESULTS

Effect of diet on body weight and liver biochemistry. The diets affected weight gain in VE and doubly deficient mice, which weighed 5–10% less than mice in the corresponding control group after being fed the diets for ~3 mo; only double-deficient mice suffered weight loss after secondary infection (data not shown).

The experimental diets resulted in VE deficiency as indi-
cated by hepatic VE concentrations [1.7 ± 0.1 μg/g (3.9 ± 0.2 × 10^{-9} \text{mol/g}) E+, MO diet vs. 0.07 ± 0.02 μg/g (1.6 ± 0.5 × 10^{-10} \text{mol/g}) E-, MO diets]. Selenium deficiency was confirmed by liver GPx activity, which was 473.3 ± 16.5 and 586.3 ± 17.2 μU/g of tissue in mice fed the Se-adequate lard- and MO-containing diets, respectively, while in mice fed the Se-deficient lard- and MO-containing diets, it was 6.3 ± 1.2 and 7.3 ± 0.8 μU/g of tissue, respectively. Thus, Se and VE levels were reduced by >95%.

**Effect of host Se, VE, or combined Se and VE deficiencies on H. polygyrus worm burden and egg production.** Initial studies examined the effect of diet on the primary response to infection with *H. polygyrus*. There was no effect of Se (P = 0.298), VE, or combined Se and VE deficiency (P = 0.813) on adult worm burden on the response to a primary *H. polygyrus* infection 14 d after inoculation (data not shown). Similarly, there was no effect of Se (P = 0.971), VE, or combined Se and VE deficiency (P = 0.260) on fecal egg production on the response to a primary *H. polygyrus* infection 14 d after inoculation (data not shown).

Mice with an *H. polygyrus* infection that is cleared by an antihelminthic drug express an adaptive immune memory response to a secondary challenge infection. To maximize any potential dietary effects on the host antioxidant defense and immune response to infection with *H. polygyrus*, a second study focused on the combined effect of Se and VE deficiencies on host resistance to a secondary challenge infection with *H. polygyrus*. As expected, mice fed the control diet had nearly cleared the infection by d 23 with both adult worm burden and fecal egg production at low levels (Fig. 1A and B). Only 3 of 8 mice fed the control diet had measurable egg production. In contrast, mice fed the combined Se- and VE-deficient diet had elevated adult worm numbers (P < 0.001) and higher egg production (P = 0.004), indicating that the adult worms were not perturbed by the local immune response. This was confirmed when adult worms were cultured overnight and the eggs/worm ratio measured (Fig. 1C). Egg production by worms from mice fed the doubly deficient diet was greater than the production by worms from mice fed the control diet (P < 0.001). Thus, adult worms from a secondary challenge infection with *H. polygyrus* persist in Se- and VE-deficient mice and have greater fecundity than worms from mice fed a control diet.

The effect of single deficiencies in Se or VE on a secondary challenge infection with *H. polygyrus* was also examined. Adult worm burden and egg production was compared early (d 11) and late (d 30) after inoculation with *H. polygyrus* to determine whether clearance was less efficient in mice deficient in Se, VE, or both after a secondary challenge infection with *H. polygyrus*. Effect of a combined Se/VE deficiency on a secondary challenge infection with *H. polygyrus*. *Mice (n = 4–8) fed an adequate (Se+, E+, MO) or a Se/VE doubly deficient (Se-, E-, MO) diet were infected with* *H. polygyrus* and assayed for the number of adult worms (A) and fecal eggs (total eggs detected in the cecum and colon) (B). Egg production by adults worms cultured overnight in vitro was expressed as the number of eggs produced per worm (C). Values are means ± SEM, n = 4–8. *Different from Se+VE+, P < 0.05.

**FIGURE 1** Number of adult worms (panel A), fecal eggs (total eggs detected in the cecum and colon; panel B), and egg production by adult worms cultured overnight in vitro (panel C) in mice fed diets deficient in Se, VE, or both after a secondary challenge infection with *H. polygyrus*. Effect of a combined Se/VE deficiency on a secondary challenge infection with *H. polygyrus*. *Mice (n = 4–8) fed an adequate (Se+, E+, MO) or a Se/VE doubly deficient (Se-, E-, MO) diet were infected with* *H. polygyrus* and assayed for the number of adult worms (A) and fecal eggs (total eggs detected in the cecum and colon) (B). Egg production by adults worms cultured overnight in vitro was expressed as the number of eggs produced per worm (C). Values are means ± SEM, n = 4–8. *Different from Se+VE+, P < 0.05.

More marked effects of the nutrient-deficient diets were observed 30 d after inoculation. Both Se- and VE-deficient mice had higher adult worm burdens (Fig. 2A; P < 0.001) and fecal egg counts (Fig. 2B; P < 0.001). Egg production was higher from cultured adult worms (Fig. 2C; P = 0.030) obtained from all (11/11) Se-deficient mice compared to adult worms from only 3 of 7 mice fed the control diet. Fecundity tended (P = 0.065) to be higher in VE-deficient mice. Infection was detected in only half (4/8) Se+, E+, MO mice, but in all (8/8) VE-deficient mice. Thus, both Se and VE deficiency increased the persistence and fecundity of *H. polygyrus* adults in mice and the adult worms are more robust when cultured in vitro.

**Effects of nutrient deficiencies on GI function in response to infection.** The effect of an Se and VE double deficiency on epithelial cell permeability and sodium-dependent glucose absorption was measured. Epithelial cell permeability was similar in uninfected control and in Se and VE doubly deficient mice and increased equally in *H. polygyrus*-infected mice fed either diet (data not shown). *H. polygyrus* infection induced the
expected decrease in sodium-dependent glucose absorption in control mice but not in mice fed the Se−, E−, MO doubly deficient diet, whereas the doubly deficient diet alone had no effect on sodium-dependent glucose absorption in uninfected mice (Fig. 3).

**Effect of diet on IL-4 and IFN-γ production in response to H. polygyrus infection.** Consistent with a skewing of the type 2 cytokine response following inoculation with *H. polygyrus*, circulating levels of IL-4 were higher in all infected groups regardless of dietary regimen compared to uninfected mice (Fig. 4A; *P* < 0.001). In contrast, IFN-γ levels did not increase in response to *H. polygyrus* infection and the IFN-γ levels were lower in infected mice compared to uninfected mice (Fig. 4B; *P* < 0.05).
levels actually decreased in the Se−, E−, MO, and Se−, E−, MO doubly deficient groups, which were elevated in uninfected mice fed these diets (Fig. 4B; P < 0.05). IL-4 production was slightly increased in uninfected mice fed the Se−, E−, MO doubly deficient diet compared to those fed the Se+, E+, MO diet (Fig. 4A; P < 0.05) and IL-4 production increased less in H. polygyrus-infected mice fed the Se−, E−, MO doubly deficient diet compared to those fed all other diets (Fig. 4A; P < 0.05).

DISCUSSION

Diet-induced deficiencies in Se and/or VE inhibit resistance to H. polygyrus in the intestine of mice fed a secondary challenge infection. Mice doubly deficient in Se and VE had a significantly impaired clearance of a secondary infection compared to mice fed a complete diet at 23 d postinoculation (Fig. 1). Single deficiencies in Se or VE also resulted in impaired ability to clear infection (Fig. 2). Worm burdens and egg production did not differ at 11 postinfection regardless of diet; however, mice fed control diets exhibited minimal or no infection by d 30 after inoculation while mice deficient in either Se or VE had a significantly higher level of infection (Fig. 2). This suggests that the inhibition was due to a change in the intestinal response to adult worms after they emerge from the submucosal tissue of the jejunum (35). The adult worms present at d 23 in the doubly deficient mice or at d 30 in the single deficient mice appeared more robust because their fecundity was significantly higher both in situ and in vitro. Fecundity is a measure of stress on the parasite caused by changes in their microenvironment that can be immune mediated.

Vitamin E deficiency has been associated with indices of increased oxidative stress and lipid peroxidation (14,15) and impaired immune function (16). Vitamin E also inhibits IL-4 gene expression in peripheral blood T-cells (36) and, along with Se, blocks activation of NF-κB and AP-1 (37–39). Inappropriate activation of NF-κB or other transcription factors could alter the normal response to infection. In fact, low levels of reactive oxygen species (ROS) can act as signaling molecules and can activate transcription factors including NF-κB (39,40). Decreased Se levels translate into decreased GPx activity (41) and increased intracellular hydrogen peroxide concentrations that can activate NF-κB (39,40); GPx is the primary enzyme responsible within the cell for breaking down hydrogen peroxide. Thus, a deficiency in either or both Se and VE may increase oxidative stress, alter signal transduction and transcription factor activation, and affect the ability of the host to properly respond to a gastrointestinal tract infection.

Selenium provides at least part of its antioxidant function by its incorporation into 4 GPx isozymes and thioredoxin reductase. GPx2 or GPx-GI is found in high concentrations in the intestinal tract, accounting for about 50% of the total Se-dependent GPx activity, with most of the remainder made up by cytoplasmic or GPx1 (42). The gastrointestinal tract is the only tissue known to have all 4 isozymes present, suggesting that this tissue may require greater control over levels of ROS than other tissues (43). Thus, GPx levels may be important for controlling oxidative stress in response to infection in the intestine and Se deficiency may compromise this system. Host expression of ROS is thought to contribute to a changing environment in the intestine during infection with H. polygyrus, and parasite-derived enzymes that affect oxidative stress in that microenvironment are considered important in the maintenance of balance of the host:parasite interface (44).

Because functional changes to the epithelial cells of the gastrointestinal tract are related to the “weep and sweep” response associated with clearance of H. polygyrus (29), we examined the effect of double deficiency on epithelial cell permeability and sodium-dependent glucose absorption, 2 functional parameters normally altered by infection with H. polygyrus. Mucosal permeability was unchanged by a double-deficient diet in uninfected mice and the parasite-induced reduction in permeability was also unaffected by diet (data not shown). Sodium-dependent glucose absorption, which is normally depressed in mice during a secondary infection with H. polygyrus, failed to respond to infection in mice on the double-deficient diet (Fig. 3). Thus, diets deficient in both Se and VE selectively interfere with one aspect of the intestinal epithelial cell response to infection that controls fluid accumulation in the lumen.

Infection with H. polygyrus typically induces a skewed Th2-dependent type 2 cytokine response in mice characterized by elevated levels of IL-4, IL-5, IL-9, IL-10, and IL-13 and no appreciable change in levels of IFN-γ (45). The level of IL-4 production is important for worm clearance during a secondary challenge infection with H. polygyrus (26) because administration of exogenous IL-4 induces a STAT6-dependent reduction in sodium-linked glucose absorption and increases mucosal permeability similarly to that seen in mice infected with H. polygyrus (29). In addition, increases in IFN-γ can reduce IL-4 production and inhibit protection against gastrointestinal nematodes (46). Furthermore, blocking H. polygyrus-induced IL-4 activity by neutralizing antibody in vivo or by treatment of mice with anti-IL-4R mAb inhibits IL-4-dependent STAT6 signaling and several of the H. polygyrus-induced functional changes in intestinal physiology that are associated with worm expulsion (29). Thus, it is possible that dietary deficiencies in Se and/or VE could inhibit IL-4-dependent changes in the intestine in response to H. polygyrus by altering the production of IL-4. This appears not to be the case, however, because circulating IL-4 levels were elevated and similar among mice on the various diets following inoculation with H. polygyrus. Alternatively, circulating IL-4 levels may not accurately reflect cytokine production locally in the intestine where they act to induce worm expulsion. This possibility is supported by the observation that protein malnutrition affected gut-associated IL-4 production more than systemic production (47). However, the normal IL-4-induced STAT6-dependent reduction in epithelial cell resistance following a second H. polygyrus infection was similar in mice on complete and doubly deficient diets, suggesting that local IL-4 production was functionally adequate and not diet-dependent. These observations do not preclude a potential interference in downstream type 2 cytokine-induced signaling events that could be affected by the deficiencies.

Recent studies have shown that gastrointestinal nematode parasites induce a stereotypical IL-4-dependent and STAT6-associated alteration in intestinal epithelial and smooth muscle cell function that accompanies parasite clearance from the gut (48). Thus, infection with Nippostrongylus brasiliensis, Trichinella spiralis, and H. polygyrus similarly alter epithelial cell resistance, sodium-dependent glucose absorption, and secretory responses to serotonin and acetylcholine, 2 critical mediators in the submucosal reflux pathway in the intestine that control fluid accumulation in the lumen. These responses are complex and are dependent on STAT6 activation and both direct and indirect enteric nerve-mediated effects on epithelial cells (48). The increased contractility of the intes-
tinal smooth muscle associated with the "weep and sweep" response to infection with *H. polygyrus* is also dependent on IL-4-induced STAT6 activation (49). We have recently completed additional studies in mice infected with *H. polygyrus* that were maintained on diets deficient in either Se or VE. Worm-induced reduction in epithelial cell resistance occurred regardless of Se or VE status, whereas VE but not Se deficiency prevented *H. polygyrus*-induced decreases in sodium-dependent glucose absorption and hypercontractility of the smooth muscle; either deficiency alone reduced the efficiency of clearance of adult *H. polygyrus* from the intestine (48).

In conclusion, Se, VE, and Se and VE double deficiencies can alter the normal course of a secondary infection with *H. polygyrus* in mice. Worm expulsion is delayed and fecundity enhanced. Mice doubly deficient in Se and VE do not support the *H. polygyrus*-infected mice was negatively affected by Se and/or VE deficiency. Alternatively, Se and/or VE deficiency may alter additional mechanisms downstream of IL-4 that bind to the IL-4R that promote immune-mediated worm expulsion. Further evaluation of the contributions of Se and VE to worm expulsion should contribute to both an understanding of the role these nutrients have in immune function and how the immune system protects against metazoan parasites.

**LITERATURE CITED**


