Dietary Selenium Modulates Activation and Differentiation of CD4⁺ T Cells in Mice through a Mechanism Involving Cellular Free Thiols¹–³

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Abstract

The immune-enhancing effects of selenium (Se) supplementation make it a promising complementary and alternative medicine modality for boosting immunity, although mechanisms by which Se influences immunity are unclear. Mice fed low (0.08 mg/kg), medium (0.25 mg/kg), or high (1.0 mg/kg) Se diets for 8 wk were challenged with peptide/adjuvant. Antigen-specific CD4⁺ T cell responses were increased in the high Se group compared with the low and medium Se groups. T cell receptor signaling in ex vivo CD4⁺ T cells increased with increasing dietary Se, with all 3 groups differing from one another in terms of calcium mobilization, oxidative burst, translocation of nuclear factor of activated T cells, and proliferation. The high Se diet increased expression of interleukin (IL)-2 and the high affinity chain of the IL-2 receptor compared with the low and medium Se diets. The high Se diet skewed the T helper (Th)1/Th2 balance toward a Th1 phenotype, leading to higher interferon-γ and CD40 ligand levels compared with the low and medium Se diets. Prior to CD4⁺ T cell activation, levels of reactive oxygen species did not differ among the groups, but the low Se diet decreased free thiols compared with the medium and high Se diets. Addition of exogenous free thiols eliminated differences in CD4⁺ T cell activation among the dietary groups. Overall, these data suggest that dietary Se levels modulate free thiols levels and specific signaling events during CD4⁺ T cell activation, which influence their proliferation and differentiation. J. Nutr. 140: 1155–1161, 2010.

Introduction

Selenium (Se) is a nutritional trace mineral essential for various aspects of human health (1). The biological effects of Se are mainly exerted through its incorporation into selenoproteins as the amino acid, selenocysteine. Twenty-five selenoproteins have been identified in humans, all but one of which exist as selenocysteine-containing proteins in mice and rats (2). Several members of the selenoprotein family exhibit antioxidant or redox functions, including the glutathione peroxidases (GPx1⁷). Through 4) and thioredoxin reductases (Trxrd1, 2, and 3) (3). Several of these and other selenoproteins have been shown to be expressed in nearly all tissues and cell types, including those involved in innate and adaptive immune responses (4–6). Thus, it is not surprising that levels of Se intake have been shown to affect immune responses (7).

Data from experimental animal studies as well as limited human studies have demonstrated that Se deficiency results in less robust immune responses to vaccinations and infections compared with Se-adequate controls (8–10). The influence of host Se status on infectious agents such as viruses, bacteria, parasites, and fungi depends on the microorganism involved (7,11). Our laboratory recently used a mouse model of allergic airway inflammation to investigate how levels of dietary Se affected the development of this Th1 helper (Th2)-driven immune response (12). Interestingly, Se intake was not related to the development of allergic airway inflammation in a simple dose-response manner. In particular, low levels of dietary Se resulted in low levels of allergic responses, which is consistent with results by others showing that several different types of immune responses are decreased in individuals with low Se status (13). However, supplementing diets with Se at levels higher than Se-adequate diets resulted in lower Th2-driven responses. Comparing these results to those involving viruses (14–17) and...
intracellular pathogens (18,19) suggests that Se supplementation may affect Th1 and Th2 responses differently. The notion that Se polarizes immunity during the activation of naive T cells may help explain studies showing that Se exerts differential influences on various types of immune responses (7,20).

Redox tone has been shown to play a key role in modulating the activation of T cells into effectors (21–23), and selenoproteins regulate redox tone. Despite the crucial role that CD4+ T cells play in initiating immune responses, there is a lack of information available regarding the direct effects of Se levels on these cells. A recent study demonstrated that a complete lack of selenoproteins in T cells led to decreased pools of mature T cells and defective T cell activation (24). Complete depletion of selenoproteins is physiologically improbable even under conditions of very low Se intake and questions remain regarding how less overt changes in Se status affect T cell biology. We examined the effects of Se intake on the activation of CD4+ T cells in terms of proliferation and differentiation.

Materials and Methods

Mice and diets. C57BL/6 mice purchased from Jackson Laboratory were fed standard diets containing 0.2–0.25 mg/kg Se. At the time of weaning (3–4 wk of age), males were fed Open Source Diets purchased from Research Diets containing either 0.08 mg/kg (cat. no. D19101), 0.25 mg/kg (cat. no. D10001), or 1.0 mg/kg (cat. no. D05030403) Se. These diets were formulated with purified ingredients (Supplemental Table 1) and contained 20.3% protein, 66% carbohydrate, and 5% fat. The protein source was casein, which was also the main source of Se in the low Se diet. For medium and high Se diets, sodium selenite was added to achieve final Se levels. Each lot was independently tested to confirm the Se concentration by inductively coupled plasma-MS (Bodycote) with lot-to-lot variation at or below the detection limit of inductively coupled plasma-MS testing (0.02 mg/kg). Mice were fed the special diets for 8 wk prior to isolation of CD4+ T cells from spleens. To ensure that the different Se diets were not altering their overall health, mice were monitored for indicators of general health, including body weight (measured 8 wk after weaning), coat appearance, and general grooming. All animal experimental protocols were approved by the University of Hawaii Institutional Animal Care and Use Committee.

Antibodies and reagents. Antibodies used for flow cytometry included allophycocyanin (APC)-anti-CD4 and fluorescein isothiocyanate (FITC)-anti-CD4, FITC-anti-CD3, phycoerythrin (PE)-anti-CD40L and APC-anti-CD40L, PE-anti-CD44, PE/cyanine 5 (Cy5)-anti-CD16/32, PE/Cy5-anti-CD19, and anti-CD25 (eBioscience). Stimulation of CD4+ T cells was carried out with anti-CD4 (L3T4) (eBioscience) and anti-CD28 (37.51) (BioLegend). Propidium iodide (Ebioscience) was used for live cell determination. Lymphocytic choriomeningitis (LCMV) gp66–77 (D37KGVYQFKSV) peptide was purchased from Peptide 2.0 and APC-conjugated I-A(b) tetramer preconjugated with LCMV gp66–77 was obtained by the NIH Tetrramer Facility. All antibodies and reagents listed above were used at final concentrations recommended by manufacturers. N-acetyl-cysteine (NAC) and 2-mercaptoethanol were both purchased from Sigma.

In vivo antigen challenge. To induce CD4+ T cell immune responses, an established protocol was followed (25) with modifications. Briefly, 50 μg LCMV gp66–77 peptide in 200 μL of a vaccine adjuvant comprised of cationic liposomes and noncoding plasmid DNA (CLDC) was injected intraperitoneally at d 0 and 12. Five days later, spleens were removed and 2 × 10^6 cells were stained with APC-conjugated tetramer for 2.5 h at 37°C. For the last 30 min of this incubation, a cocktail of surface-staining antibodies was added, including FITC-anti-CD4 plus PE-anti-CD44 to detect activated, antigen-specific T cells, and PE-Cy5-anti-CD16/32, PE-Cy5-anti-CD19, and propidium iodide detected in FL-3 to eliminate nonspecifically bound tetramer (dump channel). Four-color flow cytometry was performed on a FACScaliber to enumerate antigen-specific T cells.

Purification of ex vivo CD4+ T cells. Spleens were removed from mice in a sterile hood and cell suspensions were prepared as previously described (25). A negative-selection CD4+ T cell isolation kit (Miltenyi) was used to purify untouched CD4+ T cells per the manufacturer’s instructions and cells were resuspended in RPMI with 10% mouse sera pooled from mice in the same dietary Se group. Purity of cells was determined by evaluation of CD3+CD4+ via flow cytometry on a FACScaliber (BD Biosciences) and consistently ≥95%.

![FIGURE 1](https://academic.oup.com/jn/article-abstract/140/6/1155/4600362/fig1)

**FIGURE 1** Selenoenzyme activity in erythrocytes (A), plasma (B), and CD4+ T cells (C,D), and in vivo CD4+ T cell responses (E) in mice fed low, medium, or high Se diets for 8 wk. Values are means ± SE, n = 3 (A–D) and 10 (E). For all graphs, means without a common letter differ, P < 0.05.
Calcium mobilization and proliferation assays. Established techniques were used to assay for Ca^{2+} mobilization (26,27) with slight modifications. In brief, purified CD4^+ T cells (5 \cdot 10^5 cells/well) were loaded with 2 \mu M fluo-3 AM (Invitrogen) in 0.5% pluronic acid for 30 min followed by 2 washes in RPMI with 10% fetal bovine serum. Cells (10^10/L) were recovered at 37°C for 30 min and in some cases media contained 50 \mu M 2-mercaptoethanol or 10 \mu M NAC, commonly used sources of exogenous free thiols (28). The cells were then analyzed with a FACScaliber for fluorescence 1–2 min to determine baseline Ca^{2+} levels. After this initial period, the cells were stimulated using soluble hamster anti-CD3 (10 \mu g/L) and mouse anti-hamster-CD28 (5 \mu g/L) precoated coverslips in 500 \mu L complete RPMI media and incubated at 37°C for 10 min. Cells were fixed, permeabilized, and stained for NFAT (anti-NFAT followed by 488-secondary antibody) and nuclei (Hoeschst) using an NFAT Activation kit (Thermo Scientific). Cells were analyzed by fluorescence microscopy for colocalization of green and blue fluorescence using ImageJ software (NIH).

Real-time PCR and flow cytometry. Total RNA was extracted and real-time PCR carried out as previously described (29). Oligonucleotides used for real-time PCR included mIL2 (fwd: 5'-gct gtt gat gga cct aca gga-3'; rev: 5'-ttc tct tgg gcc tgc tt-3') and mCD23 (fwd: 5'-cca aca cag tct atg cac caa-3'; rev: 5'-aga tct tgg aat cct gat c-3'). Also, proprietary primers were purchased from SuperArray Biosciences for interleukin (IL)-4 (cat. no. PPM03013E) and interferon (INF)γ (cat. no. PPM03121A). Flow cytometric analyses for surface markers were performed as previously described (12).

Statistical analyses. All statistical tests for comparison of means were performed using GraphPad Prism version 4.0. A 1-way ANOVA was used to determine the effects of diet on outcomes for T cell receptor (TCR)-stimulated cells with Tukey post test used to compare means of dietary groups. For the experiment involving exogenous free thiols and proliferation, a 2-way ANOVA was used to test the main effects of diet and exogenous free thiols on proliferation. In addition, interactions between diet and exogenous free thiols on proliferation were analyzed. Bonferroni’s post hoc test was used to identify the means that differed. Differences were considered significant at P < 0.05. GraphPad Prism was also used for linear and nonlinear regression analyses for curve-fitting.

Results

High dietary Se increases CD4+ T cell responses. As expected with the chosen Se levels, the dietary groups did not differ in general health, lymphoid tissue development, or T cell populations in lymphoid and nonlymphoid tissues (data not shown). The diets had the desired effects in establishing low, medium, and high Se status in the mice as determined by GPx activity in erythrocytes and plasma (Fig. 1A,B). CD4+ T cells were purified from spleens and GPx and Txrd enzymatic activities were measured. GPx activity was significantly lower in cells from the low Se group compared with cells from the medium and high Se groups. These lower activities were associated with reduced Trxrd enzymatic activities and reduced proliferative responses to TCR stimulation in the low Se group. High Se status was associated with increased proliferative responses to TCR stimulation compared with medium Se status (Fig. 2B). The proliferative responses to TCR stimulation were not impacted by exogenous free thiols, but the proliferative response to TCR stimulation was significantly reduced in the low Se group compared with the other experimental groups (Fig. 2A). The proliferative responses to TCR stimulation were not impacted by exogenous free thiols, but the proliferative response to TCR stimulation was significantly reduced in the low Se group compared with the other experimental groups (Fig. 2A).

Reactive oxygen species, glutathione, and free thiol measurement. For reactive oxygen species (ROS) measurement, purified CD4+ T cells (10^10 cells/L) in complete RPMI media were incubated with anti-CD3 (10 mg/mL) on ice for 30 min. At 15 min prior to flow cytometric analysis, cells were loaded with 2 \mu M 5-(and 6-) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (Invitrogen) in pre-warmed (37°C) PBS containing 5 mg/mL cross-linking anti-hamster antibody. Reactions were terminated by adding 4.5 mL ice-cold fluorescence-activated cell sorting (FACS) buffer (PBS with 2% fetal bovine serum). Cells were pelleted and washed once with 3 mL ice-cold FACS buffer, resuspended in FACS buffer, and analyzed on a FACScaliber. For free thiol measurement, equivalent numbers of unstimulated CD4+ T cells from each dietary group were lysed with Mammalian Cell Lysis buffer (Sigma) with no reducing agent added, and lysates were analyzed using a DetectX Thiol Detection kit (Arbor Assays) and a Victor II fluorimeter (Perkin Elmer). Glutathione (GSH) levels were spectrophotometrically measured using an OxisResearch Total GSH kit. The readouts for total thiol and GSH were normalized to total protein as measured by the Bradford assay.
medium and high Se groups (Fig. 1C). Trxrd activity was significantly higher in cells from the high Se group compared with cells from the low and medium Se groups (Fig. 1D). CD4+ T cell responses to in vivo antigenic challenge were greater in the high Se group compared with the low and medium Se groups (Fig. 1E).

Dietary Se increases Ca2+ mobilization, oxidative burst, and NFAT translocation. We next examined the effects of Se on CD4+ T cell activation by focusing on an early cell signaling event that occurs within seconds of TCR stimulation: Ca2+ mobilization. TCR-induced Ca2+ mobilization increased with increasing dietary Se, with all 3 groups differing from one another (Fig. 2A). There were no differences in Ca2+ mobilization when cells were treated with the Ca2+ ionophore, ionomycin, suggesting TCR signaling was specifically affected by Se levels and Se was not having a general effect on plasma membrane Ca2+ flux (data not shown).

A time-course experiment was performed to measure TCR-stimulated oxidative burst. ROS levels increased with increasing dietary Se, with all groups differing significantly from one another at 45 min post-TCR stimulation (Fig. 2B). Interestingly, there were no differences in ROS prior to TCR stimulation despite results showing that increasing dietary Se increased antioxidant selenoenzyme activity in CD4+ T cells (Fig. 1). A crucial signaling event downstream of Ca2+ mobilization, NFAT, was evaluated. NFAT translocation increased with increasing dietary Se, with all groups differing significantly from one another (Fig. 2C). Interestingly, TCR-induced phosphorylation of extracellular signal-regulated kinase was not affected by dietary Se levels (data not shown).

Se levels influence IL-2 transcription, IL-2 receptor expression, and proliferation. A time-course experiment was performed to evaluate IL-2 mRNA levels in CD4+ T cells after activation through TCR engagement (Fig. 3A). IL-2 mRNA was higher in the cells from the medium and high Se groups compared with cells from the low Se group from 3–8 h poststimulation. IL-2 mRNA was higher in cells from the high Se group compared with cells from the low or medium Se groups at 12 h poststimulation.

Levels of mRNA for CD25, the high-affinity chain of the IL-2 receptor (IL-2R), were examined at 12 h poststimulation. CD25 mRNA was higher in cells from the high Se group compared with cells from the low and medium Se groups (Fig. 3B). Surface expression of CD25 on TCR-stimulated CD4+ T cells was higher in cells from the high Se group compared with cells from the low and medium Se groups (Fig. 3C). Interestingly, TCR-induced proliferation increased with increasing dietary Se, with all groups differing significantly from one another (Fig. 3D). Levels of CD25 mRNA, CD25 surface expression, and proliferation did not differ in unstimulated cells from the 3 dietary Se groups (data not shown).

Se levels affect CD4+ T cell differentiation. Increasing Se intake affects various types of immune responses differently and the differentiation of CD4+ T cells plays a central role in shaping immune responses (7). Thus, we evaluated TCR-stimulated CD4+ T cells for mRNA levels for the Th1 and Th2 cytokines, IFNγ and IL-4, respectively. IFNγ mRNA increased with increasing dietary Se, with all groups differing significantly from one another at 12 h post-TCR stimulation (Fig. 4A). In contrast, IL-4 mRNA was higher in cells from the low Se group at 3–8 h post-TCR stimulation compared with cells from the medium and high Se groups (Fig. 4B). Surface expression of CD40L, a molecule important for differentiation of Th1 effectors, was measured on TCR-stimulated CD4+ T cells. CD40L expression was higher on cells from the high Se group.
compared with cells from the low and medium Se groups (Fig. 4B). CD40L surface expression did not differ on unstimulated cells from the 3 dietary Se groups (data not shown).

**Se influences T cell activation through modulation of free thiol levels.** To determine cellular redox tone, free thiol groups (-SH groups arising under more reduced conditions, i.e. lower oxidative stress) were measured in freshly isolated CD4+ T cells. Free thiol levels were lower in cells from the low Se group compared with cells from the medium and high Se groups, which did not differ significantly from one another (Fig. 5A). Because GSH is a major intracellular thiol, GSH levels were measured in CD4+ T cells. GSH levels were lower in cells from the low Se group compared with cells from the medium and high Se groups, which did not differ from one another (Fig. 5B). To determine whether dietary Se affected T cell activation through a mechanism involving free thiols, we repeated the experiments from above with and without the addition of exogenous free thiols in the form of NAC. The addition of NAC eliminated the effects of dietary Se on proliferation (Fig. 5C). NAC did not affect proliferation in unstimulated CD4+ T cells (data not shown). The addition of exogenous free thiols also eliminated the effects of dietary Se on Ca2+ flux (Supplemental Fig. 1).

**Discussion**

The data presented in this study demonstrate that TCR signal strength in CD4+ T cells is increased by Se intake, affecting their proliferation and differentiation. For several different measurements of TCR-induced activation, low dietary Se resulted in weak T cell activation. While several other studies have used tortilla-based diets containing <0.02 mg/kg Se to severely deplete selenoprotein activity in rodents (19,31,32), the low Se diet (0.08 mg/kg) used in the present study was marginally lower than the nutritional requirement for rodents (0.1 mg/kg) (33). This level was chosen to reflect moderately low Se intake in a diet consumed by humans, with selenite added to increase Se intake to medium and high Se levels (34,35). Although CD4+ T cells are not the only type of immune cell detrimentally affected by low Se levels (11,36,37), they play a crucial role in driving a variety of different immune responses. The dramatic effects of low Se intake on CD4+ T cells in terms of weak TCR signals and reduced proliferation highlight the importance of acquiring sufficient levels of dietary Se for properly functioning Th cells.

In addition to increasing TCR signal strength, our data suggest that Se intake influences differentiation of CD4+ T cells into Th1 compared with Th2 effector cells. Increasing dietary Se may shift intracellular redox status toward a more reduced state and induce a bias toward Th1 effector cells. This would benefit antiviral immune or antitumor responses that depend on robust Th1 immunity (12). The notion that Se increases Th1 responses is consistent with studies showing higher dietary Se levels led to an increasing ability to generate CTL and to destroy tumor cells in mice (38). In contrast, Se supplementation and decreased oxidative stress may decrease Th2 responses such as those that drive allergic asthma and this notion is consistent with our previously published data (12). Particularly interesting was the effect of increasing Se intake on the expression of CD40L, an accessory molecule that plays a key role in driving the differentiation process (39–41). The effect of redox status on expression of CD40L or other accessory molecules involved in Th1:Th2 polarization is an understudied area that warrants further investigation.

**FIGURE 4.** IFNγ mRNA (A), IL-4 mRNA (B), and surface expression of CD40L (C) for TCR-stimulated CD4+ T cells from mice fed low, medium, or high Se diets for 8 wk. Values are means ± SE, n = 3 (A,B), and 5 (C,D). For A and B, means at each time point without a common letter differ, P < 0.05. For B–D, means without a common letter differ, P < 0.05.

Dietary Se has long been considered a potent antioxidant and our data suggest that higher Se intake increases TCR signal strength through mechanisms that involve free thiol concentrations. Early signaling events like Ca2+ flux and NFAT translocation were increased by increased Se intake, but dietary Se did not affect phosphorylation of extracellular signal-regulated kinase levels, which is consistent with other data suggesting no influence of oxidative stress on this particular signaling event (26). Interestingly, higher Se status increased TCR-induced oxidative burst in activated CD4+ T cells, despite the increased selenoenzyme activity resulting from higher dietary Se in these cells. Higher enzymatic activity of GPx1, Trxrd1, and other antioxidant selenoproteins in CD4+ T cells from Se-supplemented individuals may serve to rapidly quench the augmented ROS and mitigate cellular damage that unchecked higher ROS may cause.
FIGURE 5 Free thiols (A) and GSH levels (B) and effects of exogenous free thiols on TCR-induced proliferation (C) for CD4+ T cells from mice fed low, medium, or high Se diets for 8 wk. Values are the mean + SE, n = 3 (A,B). Means without a common letter differ, P < 0.05. For C, values are the mean + SE, n = 11. P-values from the 2-way ANOVA were: diet, P < 0.001; NAC, P < 0.001; interaction, P < 0.001. Means without a common letter differ, P < 0.05.

Also, the differences in free thiols in CD4+ T cells from the 3 dietary Se groups were small compared with differences in proliferation. This suggests that small differences in free thiols prior to stimulation may become increasingly important for redox tone during the exponential expansion of these cells. Although Se and selenoproteins have been shown to have direct effects on cellular redox status (3), it remains unclear whether selenoproteins act directly on the cell signals during T cell activation or in a more indirect manner through regulation of cellular redox tone.

Extreme Se deficiency in humans is rare except in particular regions in China and Russia (42). Results of the NHANES III-1988–94 indicated that most Americans’ diets provide the recommended levels of Se. However, Se supplementation (200 \mu g/d) may improve immune responses to cold or flu viruses or may augment vaccine responses to a variety of infectious diseases. In addition, Se supplementation may improve health by reversing declining immunity associated with aging and the immunosuppression associated with cancer, its treatment, and with HIV/AIDS. The data in the present study demonstrated that CD4+ T cells from mice fed high Se diets exhibited enhanced TCR signaling and in vivo responses compared with cells from mice fed medium Se diets. However, the immune-enhancing effects of Se supplementation must be considered with its other effects, as highlighted from a recently discontinued study involving Se supplementation and cancer prevention, the Selenium and Vitamin E Cancer Prevention Trial (43). Understanding the mechanisms by which dietary Se affects the immune system, such as modulation of free thiols in Th cells, may lead to better utilization of Se supplementation as a means of enhancing Th1 immunity.

Acknowledgments

We thank Xiaosha Pang and Allyson Fukuyama for assistance with experiments. F.W.H., M.J.B., and P.R.H. designed research; F.W.H. and P.R.H. conducted research; S.D. designed and supplied specialized adjuvants; A.C.H. performed all animal husbandry; L.A.S. assisted with biostatistical analyses; F.W.H. and P.R.H. wrote the paper. P.R.H. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


