The effect of selenium supplementation on DTH skin responses in healthy North American Men

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Abstract

The trace element selenium (Se) is essential for immune system development and function in animals. However, the exact functions of Se in the human immune system and the achievable health benefits from Se supplementation remain unclear. To test whether an increased intake of dietary Se affects immune function, we conducted a randomized, controlled trial of Se supplementation in healthy free-living men. Forty-two men were administered 300 μg of Se a day as high-Se Baker’s yeast, or low-Se yeast for 48 weeks. Serum immunoglobulins, differential complete blood counts and lymphocyte sub-populations were measured every 6 weeks. Tests of delayed-type hypersensitivity (DTH) skin responses to mumps, candida, trychophyton, tuberculin-purified protein, and tetanus were performed at baseline and at the end of 48 weeks of treatment. Supplementation increased blood Se concentration by 50%. Surprisingly, consumption of the low-Se yeast induced anergy in DTH skin responses and increased counts of natural killer (NK) cells and T lymphocytes expressing both subunits of the high affinity interleukin-2 receptor (IL2R). DTH skin responses and IL2R+ cells did not change in the high-Se group, suggesting Se supplementation blocked induction of DTH anergy. There were no differences between groups in quality of life indicators, number of days sick, other leukocyte phenotypes, serum immunoglobulins, or complement factors. These results suggest that Se plays a role in immunotolerization, a cell-mediated process involved in many aspects of immune function.

Keywords: Selenium supplementation; Immune function; Immunotolerance; Natural killer cell; T-cell

Introduction

Selenium (Se) is an essential trace element required in microgram amounts by humans and by all animals in which it has been tested. Signs of selenium deficiency include liver necrosis in rats, pancreatic atrophy in chickens, nutritional muscular dystrophy in sheep, and skeletal myopathy in patients receiving total parenteral nutrition without supplemental Se [1]. The essential functions of Se in mammals are mediated by a group of 25 selenoproteins in which Se is in the form of selenocysteine (Sec), the Se-containing homolog of cysteine. Enzymatic activities have been assigned to 12 of these selenoproteins: four are forms of glutathione peroxidase, three are iodothyronine deiodinases, three are thioredoxin reductases, and there is one methionine sulfoxide reductase B and one selenophosphate synthetase. Most of these selenoprotein genes are expressed in white blood cells [2–4]. Selenium is the only element specified in the genetic code (“TGA”) and Sec has become recognized as the 21st protein amino acid [5].
Se has multiple functions in the immune system and deficiency has pleiotropic effects on humoral and cellular immunity [6]. Se is essential for development of the bursa and supports development of the thymus in chicks [7]. Se is reported to prevent myocardial lesions from Coxsackie virus B3 infection, increase killing of and protection from *Candida albicans*, and increase antibody titers in mice [8–10]. Se increased splenocyte blastogenesis and protection against *C. albicans* and *S. aureus* in rats [11,12]. Se raised serum IgM and lymphocyte proliferation *in vitro* in lambs and increased chemotaxis in goat neutrophils [13–15]. Se improved humoral and colostral antibody titers, and protection from *E. coli*-induced mastitis in cattle, and increased IgG and hemagglutination titers in ponies [16–19]. Despite the number and variety of Se’s effects on animal immune systems, little is understood about the underlying mechanisms.

Relatively few studies have investigated the role of Se in human immune function. Supplementation of Se-replete US subjects with 200 μg/d Se as sodium selenite for 8 weeks increased tumor cell lytic activity of natural killer (NK) cells and cytotoxic lymphocytes [20,21]. Se supplementation improved polio vaccination response and poliovirus handling in UK subjects with marginal Se status [22], and supplementation of elderly subjects with Se-enriched yeast increased *in vitro* lymphocyte proliferation in response to pokeweed mitogen [23]. Se deficiency was associated with increased mortality in HIV-positive intravenous drug users, HIV-positive children, and pregnant HIV-positive Tanzanian women [24–26].

In a prior study, we observed that healthy men fed high-Se foods for 99 days had 2–1/2 fold higher antibody titers in response to a second vaccination with a recall antigen compared to men fed low-Se foods [27]. That study suggested Se affected immunological memory, a T-cell-mediated process. In the present study, we supplemented healthy men with 300 μg/d Se as high-Se yeast for 48 weeks to test if Se supplementation alters immune status or cell-mediated immunity.

**Subjects and methods**

**Subjects**

The present study was part of a larger investigation into the metabolic effects of long-term, high-level Se supplementation in healthy men. Fifty-four healthy, non-smoking men, aged 18–45 years were randomly assigned to treatment with high-Se yeast or low-Se yeast in the main study. Forty-two subjects completed the 48 weeks supplementation period, including the DTH skin response testing. Only the results pertaining to immune function and cellular and humoral immune status are presented herein. Other aspects of the main study are reported elsewhere [28,29]. Potential volunteers were recruited through newspaper advertisements and public service announcements in the Davis and Sacramento, CA areas, given a physical examination by a nurse practitioner and determined to be in good health. Inclusion criteria were self-reported absence of disease and clinically normal blood count and blood chemistries. Exclusion criteria were tobacco smoking, positive blood test for HIV, hepatitis B, syphilis, or positive urine tests for drugs of abuse (barbiturates, benzodiazepines, cocaine metabolites, opiates, amphetamines, and cannabinoids), and Se supplements providing more than 50 μg/d. Subjects were paid for their participation. The study protocol was reviewed and approved by the Institutional Review Board of the University of California at Davis School of Medicine, and informed consent was obtained in writing from all subjects.

**Experimental protocol**

Potential subjects meeting the recruitment criteria were enrolled into a run-in period lasting 3–6 weeks, during which replicate baseline measurements were obtained and compliance was assessed. Non-compliant subjects were dismissed before randomization and are not reported further. Two subjects at a time were randomized to treatment from July 2000 to November 2002, with one subject from each pair randomly assigned to each treatment group by coin flip. Fifty-four men satisfactorily completed the run-in period and were randomized to receive low-Se yeast tablets or high-Se yeast tablets for 48 weeks. Neither the subjects nor the study staff were aware of subjects’ treatment assignments. Subjects took their first tablet the same day that all baseline measurements were completed. Subjects visited the Center for duplicate blood draws 2 d apart every 6 weeks during the 48 weeks supplementation period, and then returned again for duplicate blood draws at follow-up visits at 72 and 96 weeks. Visits were scheduled relative to each subject’s first day of supplementation. When visits were missed, the next visit was scheduled based on the first day of supplementation to restore the original schedule. Unused tablets were counted at each visit and were collected at the end of the treatment period to measure compliance. Subjects consumed 93 ± 5.3% of the pills assigned. Every subject kept a notebook in which they recorded pills missed, symptoms, side effects and days sick. At each visit, subjects were administered the Profile of Mood States and SF-36 Health Status questionnaires and were interviewed to determine the number of days each subject had been sick since his last visit.
Se supplements

Supplements were manufactured by Pharma Nord (Denmark) and consisted of high-Se Baker’s yeast (Saccharomyces cerevisiae, strain PN0056) grown aerobically using a Pharmacopoeia-controlled growth medium containing sodium selenite (SelenoPrecise™, 300 μg Se per tablet, 3.81 μmol Se, Pharma Nord, Denmark). Low-Se tablets were compounded identically, except using the same yeast grown without added Se (≤ 1.3 μg Se per tablet, 16.5 nmol Se). Tablets contained 0.5 g of spray-dried yeast in an inert binder and were coated with titanium dioxide for an identical appearance, smell and taste. Tablets were provided in 28-tablet bubble packs. Se contents were determined by the present investigators in a random sample of the tablets using the method described below.

Dietary intake assessment

The intake of nutrients from foods was estimated from 3-d diet records. Twice during the run-in period and then at 24 and 48 weeks, subjects kept a written record of all foods eaten for a 3-d period, always including at least one weekday (Monday–Friday) and at least one weekend day (Saturday and Sunday). Records were analyzed for nutrient contents with the Minnesota Nutrition Data System 5.0 (Nutrition Coordinating Center, Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, USA), using food composition data derived primarily from the USDA National Nutrient Database for Standard Reference to calculate dietary intakes of Se and other nutrients [30].

Delayed-type hypersensitivity (DTH) skin response test procedure

Delayed-type hypersensitivity skin response to five recall antigens was determined during the baseline period and again after 48 weeks (end of the treatment period) by injecting 0.1 mL of each antigen solution intradermally into the forearm. The antigens used were tuberculin purified-protein derivative (1 international test unit), mumps (four complement-fixing test units), tetanus toxoid (1:100, vol:vol dilution of a solution containing four flocculation units/0.5 mL), candida (1:100 vol:vol dilution), and trichophyton (1:30 vol:vol dilution). The antigens were diluted with a diluent containing, 3 mL normal human serum and 9 g sodium chloride per liter. Tuberculin purified-protein derivative, mumps, and tetanus toxoid were supplied by Connaught Laboratories Inc, Swiftwater, PA, USA. Candida (Dermatophyton 0), trichophyton, and the antigen diluent were obtained from Hollister Stier, Spokane, WA, USA. Normal saline was injected as a negative control. Response to these antigens was determined by measuring mean induration diameters (mm) at 48 ± 2 h, and again at 72 ± 2 h, after injections. Data are reported as the sum of the maximum induration diameters (measured either at 48 h or 72 h, whichever was larger) for positive responses (“total induration”).

Laboratory measurements

Blood samples were collected in the mornings, after an overnight fast, and serum was separated by centrifugation. Aliquots of serum, plasma, and washed erythrocytes were stored at – 70 °C until analyzed. Se concentrations were measured by HPLC of the fluorescent derivative formed from reaction with diamononaphthalene after digestion in a 5:2 (v/v) nitric-perchloric acid mixture [31]. Glutathione peroxidase activity in erythrocytes and blood plasma was determined using the glutathione reductase-coupled assay [32] with cumene hydroperoxide. Serum tumor necrosis factor-alpha was determined with a LINCOplex immunoassay (Linco Research, St. Charles, MO, USA) on a Bio-Plex Suspension Array System (Bio-Rad, Hercules, CA, USA). Serum immunoglobulins, complement factor C3c and complement factor C4 were determined by colorimetric methods on a Hitachi 902 Analyzer (Roche Diagnostics, Basel, Switzerland). Differential complete blood counts were performed with a System 9000 Diff Model Automated Cell Counter (Serono-Baker Diagnostics, Allentown, PA, USA).

Selenium analysis

Se was measured by HPLC of the fluorescent derivative formed from reaction with diamononaphthalene after digestion in a 5:2 (v/v) nitric-perchloric acid mixture [31]. Calibration standards were prepared with National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3149-Selenium Standard Solution and the performance of each analytical run was validated by analysis of NIST SRM 1577a-Bovine Liver (certified value: 0.71 ± 0.07 μg/g; mean ± SD: 0.705 ± 0.075 μg/g) and duplicate samples of frozen pooled human plasma (within-run RSD: 4.0%, between-run RSD: 8.1%). Samples were analyzed in duplicate. If duplicate samples differed by more than 10%, a second set of duplicates was analyzed and the average of all four measurements was used.

Flow cytometry

Cells were labeled in freshly obtained whole blood with a “lyse and wash” procedure using four-color fluorescent antibodies and reagents supplied by Becton-Dickinson.
(BD Biosciences, San Jose, CA, USA) according to the manufacturer’s protocols and analyzed on a BD FACSCalibur flow cytometer using the recommended settings. Pre-run compensation was performed with FACSScomp software. Three blood samples were analyzed at each visit. The first tube of blood was labeled with MultiTEST CD3 FITC/CD16&56 PE/CD45 PerCP/CD19 APC reagent to enumerate mature T (CD3+), NK (CD3-CD19+) lymphocytes. The second tube was labeled with MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC reagent for analysis of mature T (CD3+), suppressor/cytotoxic (CD3+CD8+) T-lymphocyte subsets, and helper/inducer (CD3+CD4+) T-lymphocyte subsets. Data were analyzed with FlowJo 7.2.2 software (Tree Star, Ashland, OR, USA) by utilizing bivariate density plots such as side-scatter vs. signal height, and gating cells positive for one or more antibodies. Absolute cell counts were calculated using BD TruCOUNT™ recovery marker beads supplied with the MultiTEST assay system. The third blood sample was labeled with a custom CD3 FITC/CD122 PE/CD16&56 Cychrome/CD25 APC reagent and analyzed on the FACSCalibur flow cytometer using Cell Quest software to enumerate interleukin-2 receptor (IL2R)-expressing T-lymphocytes (CD3+CD122+CD25+) and IL2R-expressing NK cells (CD15&56+CD122+CD25+). Because the third sample did not use Tru-Count beads, cells counts were recorded as percentages of lymphocytes and then multiplied by the total CD45+ lymphocyte count to yield total cell counts in whole blood. The cell counts from replicate blood draws for each subject within the same week (or during the baseline period) were averaged and reported as a single value.

Statistical analysis

Effects of Se were detected as significant Se × time interactions in two-way repeated measures analysis of variance, representing different time trends between groups. When the Se main effect or the Se × time interaction was significant, the Student–Newman–Keuls multiple comparison test was used to identify significant differences between the groups at individual time points. Alternatively, a t-test of the within-subject changes was used to compare changes between groups when only beginning and ending values were available. Statistical calculations were performed with Sigma Stat (version 2.03, SPSS, Chicago, IL, USA). A two-tailed probability of 0.05 or less was considered significant.

Results

The anthropometric characteristics, dietary intakes of key nutrients, blood counts and humoral immune status of the subjects in each group were similar (Table 1). None of these parameters was significantly different between groups, nor did any change significantly during the treatment period. Se supplementation increased plasma Se from 142±19 to 228±63 µg/L (1 µg Se = 12.7 nmol), and raised erythrocyte Se from 261±35 to 524±141 µg/L, but glutathione peroxidase enzyme activity was not changed in either compartment [29]. Se intake from the diet was estimated at 135±47 µg/d in the controls and 144±63 µg/d in the supplemented group (Table 1), well above the RDA of 55 µg/d.

Total induration from DTH skin responses decreased by 57% in the low-Se group (p < 0.001), but did not change significantly in the Se-supplemented subjects (Fig. 1). Decreased responses to mumps accounted for 49% of the induration change in the low-Se group, while trichophyton and candida accounted for 28% and 15% of the decrease, respectively (Table 2). Tricophyton was the only individual antigen for which there was a significant effect of Se on DTH skin response. However, this was primarily because of higher initial values in the low-Se group. Seventy-four percent of DTH skin tests scored positive for candida, which accounted for 48% of the total induration.

Se supplementation did not affect total lymphocyte, B-cell, T-cell, CD4+, or CD8+ cell counts. However,

| Table 1. Baseline characteristics of 42 men completing the study. |
|-----------------|-----------------|-----------------|
|                 | Low-Se group    | High-Se group   |
| Age (yr)        | 31.7±8.0        | 31.0±9.5        |
| Height (cm)     | 177.3±7.6       | 179.6±7.8       |
| Weight (kg)     | 77.5±12.4       | 76.0±9.4        |
| Se intake (µg/d)| 135±47          | 144±63          |
| Total fat intake (g/d) | 74±23      | 91±37          |
| Polysaturated fat intake (g/d) | 15.2±5.3 | 18.8±8.3 |
| Omega-3 fatty acid intake (g/d) | 1.56±0.57 | 1.85±0.96 |
| Vitamin C intake (mg/d) | 251±404 | 176±121 |
| Vitamin E intake (α-tocopherol equivalents) (mg/d) | 61±135 | 69±230 |
| Hematocrit (%)  | 43.0±2.6        | 43.5±2.7        |
| White blood count (cells/µL) | 5410±1600 | 5441±1121 |
| Lymphocytes (cells/µL) | 1770±294 | 1809±405 |
| Granulocytes (cells/µL) | 3195±1385 | 3205±900 |
| Immunoglobulin A (mg/dL) | 241±147 | 271±136 |
| Immunoglobulin E (IU/mL) | 115±136 | 109±178 |
| Immunoglobulin G (mg/dL) | 1277±393 | 1356±501 |
| Immunoglobulin M (mg/dL) | 125±76 | 119±80 |
| Complement C3c (mg/dL) | 143±51 | 137±39 |
| Complement C4 (mg/dL) | 32.0±15.7 | 31.4±12.7 |
| Tumor necrosis factor-alpha (pg/mL) | 3.28±1.80 | 2.96±1.47 |
the number of IL2R-expressing T-cells (CD3+CD122+CD25+) and IL2R-expressing NK cells (CD15&56+CD122+CD25+) increased in the low-Se group during supplementation (Table 3). Counts of IL2R-expressing cells did not change in the high-Se group.

Discussion

Se has been recognized as essential for the normal development and function of the immune system for many years. Nevertheless, the precise roles and functions of Se in the immune system remain unclear. Most effects of Se on the human immune system are observed during development, nutritional deficiency, old age or disease, rather than in healthy well-nourished adults. The results of the present study are consistent with this trend insofar as the subjects were healthy and well-nourished, and most indices of immune status were not affected by the treatments. However, DTH skin responses unexpectedly decreased in the low-Se yeast group. DTH anergy predicts sepsis and related mortality in intensive care and trauma patients, is associated with adverse outcomes from infection, burns, or surgical trauma, and is used to forecast postoperative complications and severity of malnutrition [33–36]. DTH skin response has been used to detect effects of nutrition on immune function [37]; however, its sensitivity to moderate changes in diet has been challenged [38]. Because DTH anergy developed only in the low-Se group and did not develop in subjects taking an identical yeast tablet supplemented with Se, prevention of DTH anergy may be attributed specifically to Se supplementation.

In retrospect, we might have predicted that yeast would affect DTH responses. Various components of yeast are known to induce oral tolerance in mice [39–41], and S. cerevisiae β-glucan suppresses cell-mediated immune responses when fed to weanling pigs [42]. Oral administration of β-glucan decreased rhinoconjunctivitis and rhinitis symptoms in seasonal allergy sufferers [43], demonstrating that yeast antigens can induce tolerance in human cell-mediated immune responses. Selenoprecise yeast contains approximately 7.5% of its dry weight as β-glucans (37.5 mg/d). It seems reasonable, therefore, to conclude that DTH anergy in the low-Se group resulted from immunotolerance engendered by oral administration of yeast antigens.

Maintenance of a strong DTH response to a recall antigen requires effective long-term immune memory and robust proliferation of lymphocytes. After the initial immune response subsides, a surviving sub-population

Fig. 1. Delayed-type hypersensitivity skin responses. The maximum diameters of blisters in response to five recall antigens at 48 or 72 h were summed into a total induration score for each subject. The bars on the left represent the average total induration in each group at baseline and the bars on the right represent induration after 48 weeks of treatment. Solid black bars represent the high-Se yeast group and open white bars represent the low-Se yeast group. Error bars represent SEM. Bars not sharing a common superscript were significantly different at p<0.05 in a Student–Newman–Keuls multiple comparison test.

| Table 2. DHS skin responses to common recall antigens in healthy men consuming high-Se yeast or low-Se yeast. |
|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| | Low-Se yeast (mean ± SD, N = 20) | High-Se yeast (mean ± SD, N = 22) | Se effect1 (p) |
| Tuberculin (PPD) (mm) | 1.0 ± 4.4 | 0 ± 0 | 0 ± 0 | 0 ± 0 | NS3 |
| Trichophyton (mm) | 4.0 ± 8.2a | 0.7 ± 2.0b | 1.1 ± 3.6ab | 1.9 ± 5.1ab | 0.018 |
| Mumps (mm) | 11.7 ± 12.2 | 3.3 ± 5.5 | 7.3 ± 7.7 | 2.9 ± 5.4 | NS |
| Tetanus (mm) | 2.0 ± 3.7 | 2.0 ± 3.5 | 4.0 ± 6.8 | 2.0 ± 4.3 | NS |
| Candida (mm) | 9.9 ± 8.2 | 7.5 ± 4.6 | 10.8 ± 7.9 | 9.9 ± 10.5 | NS |
| Number of positive reactions, N | 1.9 ± 1.0 | 1.5 ± 1.0 | 1.8 ± 1.0 | 1.3 ± 0.9 | NS |

1P-value for selenium × time interaction in repeated measures ANOVA (different trends over time between groups). Values in the same row not sharing common superscripts are significantly different at p<0.05. Student–Newman–Keuls multiple comparison test.

2Values at the end of the baseline period before starting supplementation.

3NS – not significant. Neither the Se main effect nor the Se × time interaction was significant and between-group comparisons were therefore not tested.

of antigen-specific T-cells becomes quiescent and acquires the characteristics of stem cells. Immunological memory fades over time and may disappear altogether, depending on the nature of the antigen, the strength and duration of the initial immune response, and the frequency of re-exposure. Mumps is an infrequently encountered antigen and was responsible for most of the decrease in DTH skin responses in the low-Se group, consistent with a loss of immunological memory due to a lack of re-stimulation by mumps antigen. It seems plausible that Se supplementation may have prevented DTH anergy by improving the survival and/or proliferation of memory T-cells. Se is known to augment the survival and proliferation of mouse and human lymphocytes [44]. Our prior study in men fed low- and high-Se foods offers some support for the notion that immunological memory depends on dietary Se. A strongly enhanced antibody response (which depends on immunological memory) was engendered upon secondary vaccination with diphtheria vaccine in men fed high-Se foods, but was absent in men fed low-Se foods [27].

T-cells are required to initiate the DTH reaction upon re-exposure to a recall antigen [45]. Upon re-exposure, the T-memory cells are activated by antigen-presenting cells bearing their specific antigen, whereupon they begin expressing IL2R and proliferating. The proliferating T-memory cells differentiate into various lineages that migrate to the injection site and interact with the vascular endothelium to attract neutrophils and monocytes to produce the early hallmarks of inflammation. Se supplementation improved in vitro neutrophil chemotaxis in goats [15] and cancer patients [46]. Thus, improved immune cell migration is another plausible way in which Se supplementation could have sustained a strong DTH reaction.

Oral administration of S. cerevisiae antigens induced DTH anergy in the low-Se group, a phenomenon known as "bystander suppression". DTH anergy is a reflection of changes in one or more of the T-cell sub-populations responsible for the response [47]. Bystander suppression of DTH through oral tolerance is mediated by Foxp3+CD25+CD4+ regulatory T-cells, which secrete anergy-inducing cytokines and/or directly kill nearby effector T-cells specific for unrelated antigens [48,49]. We did not observe any change in CD25+ T-cells or CD122+ T-cells in the present study (data not shown); however, CD25+ CD122+ T-cells did increase in the low-Se group. It is tempting to speculate that this may represent a population of activated regulatory T-cells mediating DTH anergy. Se added in vitro specifically attenuated the generation of suppressor cells, suggesting that inhibition of regulatory T-cells may contribute to Se’s blocking of DTH anergy [50].

Immunological memory, re-initiation of DTH and bystander suppression are all T-cell-mediated processes. Recently, microarray analysis of lymphocyte mRNA profiles identified T-cell receptor (TCR) signaling as a major biological target of Se [51]. TCR is the antigen-specific cell-surface receptor that regulates the proliferation, survival and differentiation of the T-cells that control immune memory and peripheral tolerance [52]. Therefore, modulation of TCR signaling is a common molecular mechanism that could underlie any of the above T-cell-mediated processes by which Se might plausibly sustain DTH responses. Further work will be

### Table 3. Cellular immune status in healthy men consuming high-Se yeast or low-Se yeast.

<table>
<thead>
<tr>
<th></th>
<th>Low-Se yeast (mean ± SD, N = 20)</th>
<th>High-Se yeast (mean ± SD, N = 22)</th>
<th>Se effect1 (p)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline2 48 weeks 96 weeks3</td>
<td>Baseline2 48 weeks 96 weeks</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (CD45+)</td>
<td>1862 ± 324 1841 ± 423 1724 ± 360</td>
<td>1883 ± 610 1709 ± 494 1721 ± 468</td>
<td>N.S.4</td>
</tr>
<tr>
<td>B-lymphocytes (CD45 + CD19+)</td>
<td>322 ± 109 286 ± 110 365 ± 112</td>
<td>289 ± 133 293 ± 104 373 ± 121</td>
<td>N.S.</td>
</tr>
<tr>
<td>T-lymphocytes (CD45 + CD3+)</td>
<td>1399 ± 246 1386 ± 338 1251 ± 271</td>
<td>1399 ± 449 1269 ± 349 1280 ± 322</td>
<td>N.S.</td>
</tr>
<tr>
<td>Helper/inducer T-cells (CD4+)</td>
<td>858 ± 180 784 ± 147 718 ± 130</td>
<td>822 ± 355 755 ± 266 766 ± 262</td>
<td>N.S.</td>
</tr>
<tr>
<td>Suppressor/cytotoxic T-cells (CD8 +)</td>
<td>476 ± 180 457 ± 203 425 ± 175</td>
<td>493 ± 214 415 ± 121 431 ± 109</td>
<td>N.S.</td>
</tr>
<tr>
<td>NK cells (CD16&amp;56+)</td>
<td>270 ± 116 337 ± 158 343 ± 147</td>
<td>352 ± 126 304 ± 120 312 ± 120</td>
<td>N.S.</td>
</tr>
<tr>
<td>IL2R + T-cells</td>
<td>4.70 ± 1.64a 6.81 ± 1.47b 5.69 ± 2.18a</td>
<td>5.40 ± 1.25a 5.40 ± 1.97a 5.60 ± 0.92a</td>
<td>0.006</td>
</tr>
<tr>
<td>IL2R + NK cells</td>
<td>1.48 ± 0.67a 1.99 ± 0.97b 1.17 ± 0.49a</td>
<td>1.61 ± 0.95abc 1.08 ± 0.44ac 1.16 ± 0.63abc 0.010</td>
<td></td>
</tr>
</tbody>
</table>

Cells were fixed in fresh whole blood, stained with antibodies specific for the indicated protein markers, and counted in a flow cytometer. Values represent cells per microliter whole blood. Each subject was sampled twice on different days during the same week and the average value was used.

1P-value for Selenium × Time interaction in repeated measures ANOVA (different trends over time between groups). Values in the same row not sharing common superscripts are significantly different at p<0.05, Student–Newman–Keuls multiple comparison test.

2Values at the end of the baseline period before starting supplementation.

3Values at the end of the follow-up period, 48 weeks after end of supplementation.

4N.S. – not significant. Neither the Se main effect nor the Se × Time interaction was significant and between-group comparisons were therefore not tested.
required to clarify the relationship between Se’s functions in T-cells and prevention of DTH anergy. DTH anergy in the low-Se group might also be related to the increase in circulating NK cells expressing both subunits of the functional high-affinity IL2R (CD3−CD16&56+CD25+CD122+). NK cells have potent immunoregulatory properties that can promote tolerance induction by several mechanisms, including killing of activated T-cells, secretion of inhibitory cytokines, and antigen-specific mechanisms independent of B- and T-cells [53–57]. IL2R-expressing NK cells correspond to the “CD56 bright” subpopulation of NK cells that mediate tolerance induction [58], and thus may be responsible for inducing DTH anergy. As noted above, Se can attenuate the generation of suppressor cells [50]. Our data are consistent with a role of IL2R+ suppressor NK cells in inducing DTH anergy in response to orally administered fungal antigens and suggest that Se supplementation may block activation of suppressor NK cells.

Our data are not sufficient to delineate the mechanism(s) by which Se sustained strong DTH responses. However, the data suggest that Se supplementation of healthy people may modulate development of immunotolerance. This could be important in self/non-self recognition, allergic reactions, transplant rejection and cancer immunosurveillance. Further research is needed to understand how Se supplementation prevents DTH anergy and identify the underlying mechanisms.

A final note of caution is in order regarding high-level selenium supplements. Both large randomized trials of selenium supplementation conducted to date in the US have reported an increased incidence of Type II diabetes mellitus [59,60]. Although diabetes was a secondary endpoint and the increases were small and of questionable significance, these reports serve as a reminder that Se has a relatively narrow margin of safety for a nutrient and can be toxic when consumed in excessive amounts.

Acknowledgements

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