Selenium and vitamin E deficiency impair transferrin receptor internalization but not IL-2, IL-2 receptor, or transferrin receptor expression

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Abstract: Vitamin E and Se deficiency increase the risk of disease by impairing the immune response. To aid in the understanding of how vitamin E and Se deficiency reduce immune competence, this study examined several mechanisms necessary for lymphocyte proliferation. Weanling rats were fed a vitamin E-deficient, selenium-deficient, or control diet for 8 weeks. At this time splenic mononuclear cells were isolated and stimulated with concanavalin A for 48 h. Although the percentage of lymphocytes and monocytes capable of proliferating were consistent among the dietary groups, lymphocyte proliferation was decreased significantly in vitamin E- and selenium-deficient rats. This decrease in proliferation was not associated with alterations in interleukin-2, interleukin-2 receptor, or transferrin receptor expression. However, stimulated cells from vitamin E- and Se-deficient rats internalized few if any transferrin receptors. Reduced transferrin receptor internalization may limit lymphocyte expansion by depleting the intracellular iron stores needed for cellular function and proliferation. J. Leukoc. Biol. 63: 131–137; 1998.

Key Words: antioxidants · immunity · oxidative stress

INTRODUCTION

Selenium (Se) and vitamin E function synergistically to protect against cellular damage by reactive oxygen species [1]. Vitamin E, an integral component of the cell membrane, is in perfect position to disrupt the lipid peroxidation process by scavenging highly reactive intermediates such as alkoxy and alkyl peroxy radicals [1, 2]. Selenium is an essential constituent of the glutathione peroxidase (Se-GSH-Px) active site. This Se-dependent enzyme reduces hydrogen peroxide, as well as other hydroperoxides to less reactive water and alcohols, respectively [1]. Therefore, a deficiency of either micronutrient may compromise the cell’s ability to cope with oxidative stress and thus result in membrane oxidation that can disrupt cell compartmentalization and function.

Vitamin E or Se deficiency can impair host immune responsiveness, thereby increasing the risk of such diseases as bacterial and viral infections, certain types of cancers, as well as atherosclerosis [2–5]. The effects of oxidative stress as a result of Se or vitamin E deficiency on the overall immune response are partially characterized. Both vitamin E and Se deficiency can reduce mitogen-stimulated lymphocyte proliferation [6–8]. In addition, it is thought that vitamin E deficiency may alter T lymphocyte differentiation in the thymus by decreasing the proportion of CD4+ to CD8+ T lymphocytes [9]. Selenium deficiency also was found to decrease B lymphocyte antibody production and cell-mediated immune responses [6, 8]. Although the end results of vitamin E and Se deficiency on immune function have been reported, relatively little is known about the specific mechanisms by which antioxidant deficiency alters lymphocyte proliferation and disrupts the development of an effective immune response. The importance of understanding these mechanisms is critical because nutritional deficiencies are occurring with increasing frequency in certain populations, including the elderly and those with chronic illnesses such as AIDS [10, 11].

Lymphocyte proliferation is regulated by the sequential expression of numerous gene products, three of which are extremely important: interleukin-2 (IL-2), IL-2 receptor (IL-2R), and transferrin receptor (TfR) [12]. The interaction of IL-2 with the IL-2R triggers T lymphocyte proliferation [13, 14]. However, in order for these cells to progress through the cell cycle, TfR surface expression must be increased to internalize the iron required for DNA synthesis and proliferation [15, 16]. Disrupted expression of these critical effector molecules can impair lymphocyte proliferative responses. Several reports suggest that IL-2 activity is not altered during Se deficiency, but that there is a delayed and decreased expression of high-affinity IL-2R necessary for lymphocyte proliferation [17–19]. Therefore, the primary goal of this study was to elucidate the effects of Se or vitamin E deficiency on the expression of IL-2, IL-2R, and TfR and the subsequent effects on lymphocyte proliferation.

MATERIALS AND METHODS

Reagents

Male Long-Evans hooded rats were provided by Charles River (North Wilmington, MA) and the experimental rat diets were formulated by Dyets Inc.

Abbreviations: IL-2, interleukin-2; IL-2R, IL-2 receptor; TfR, transferrin receptor; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid
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Journal of Leukocyte Biology Volume 63, January 1998 131
Experimental animals and diet

Male Long-Evans hooded rats obtained at weaning were fed their respective torula yeast-based diets for 8 weeks: control (+E + Se), vitamin E deficient (−E + Se), and Se deficient (−E − Se). The experimental diets were formulated to provide adequate levels of all known nutrients needed by rats, other than vitamin E and Se. Vitamin E was supplemented at a level of 150 IU/kg as RRR-α-tocopherol acetate and Se at 0.5 mg/kg as sodium selenite. The basal diet containing tocopherol-stripped corn oil and lard as fat sources had undetectable amounts of vitamin E and <0.01 mg/kg Se on analysis [6]. Animals were housed in a temperature and humidity controlled animal facility with a 12:12-h light-dark cycle and given distilled water ad libitum. No overt signs of deficiency were observed and weight gain was similar for all groups after consuming the experimental diets for 8 weeks. Deficiency of vitamin E was ascertained by examining the liver α-tocopherol content, which was 0.006 mg/g liver microsomal protein for rats fed the vitamin E-deficient diet compared with 0.158 mg/g liver microsomal protein for the control diet [20]. Selenium deficiency was confirmed by analysis of blood as well as isolated splenic mononuclear cell Se-GSH-Px activity [21, 22]. The level of Se-GSH-Px activity expressed by splenic mononuclear cells was 6.93, 24.9, and 21.07 nmol NADPH oxidized/min/10^6 cells for the +E − Se, −E + Se, and +E + Se groups, respectively. Blood Se-GSH-Px activity was 15.6, 109.1, and 108.7 nmol NADPH oxidized/min/mg hemoglobin for each of the three experimental groups, +E − Se, −E + Se, and +E + Se, respectively.

Spleen cell isolation

Spleens were collected and combined from five to six rats per diet for each experiment. Three replicate experiments were performed, except when noted. The rats were killed with Nembutal and the spleens removed aseptically. The spleens were teased over a 40-mesh screen submerged in 0.15 M NaCl until analyzed for IL-2 activity. After the final wash, cells were resuspended at 5 × 10^6 cells/mL in binding medium nonessential amino acids, 1% antibiotic/antimycotic, and 1% Na-glutamine. To

Flow cytometry

Isolated mononuclear cells were resuspended at 2 × 10^7 cells/mL in 0.15 M phosphate-buffered saline supplemented with 2.5% heat-inactivated calf serum, 1% goat serum, and 0.01% sodium azide. An equal volume of resuspended cells was incubated for 30 min at 4°C with an equal volume of mouse anti-rat monoclonal antibody specific for CD2 + T lymphocytes (OX34, 1,400), CD4 + T helper lymphocytes (W3/25, 1,5), CD8 + T cytotoxic/suppressor lymphocytes (OX8, 1,200), B lymphocyte common antigen (B; OX33, 1,400), monocyties/macrophages ED1 (mab, 1,400), IL-2Rα (OX39, 1,400), or TfR (OX26, 1,100). The cells then were washed and incubated with a secondary antibody, rat anti-mouse IgG (H+L) fluorescein conjugate for 30 min at 4°C to visualize the bound monoclonal antibody. Appropriate negative controls were conducted to determine the level of background fluorescence. The mononuclear cells were gated by light scatter (forward and side) and assessed for FL1 (green fluorescence) using a Coulter EPICS XL-MCL flow cytometer. Data is expressed as the percentage of positive-staining cells less those stained nonspecifically with the secondary antibody (normally <5%).

The expression of IL-2Rα and TfR by splenic mononuclear cells after stimulation with concanavalin A for 48 h was also determined by flow cytometry. Isolated mononuclear cells were resuspended at 5 × 10^6 cells/mL and stimulated with 5 µg/mL concanavalin A for 48 h. The procedure was performed as outlined above, however, only the antibodies for IL-2Rα and TfR were used. Data are expressed as the percentage of positive-staining cells less background fluorescence. The median channel of fluorescence intensity also was recorded as a measure of receptor density on the cell surface. Four replicate experiments were performed with two rat spleens combined per diet for each experiment.

Culture of isolated splenic mononuclear cells

For all assays, the cells were cultured in RPMI-1640 with 25 mM HEPES supplemented with 5% fetal bovine serum, 5% minimum essential medium non-essential amino acids, 1% antibiotic/antimycotic, and 1% l-glutamine. To minimize the introduction of vitamin E and Se to the cells during culture, a specific lot of fetal bovine serum that contained low levels of these micronutrients was utilized. The fetal bovine serum contained 10 ng/mL Se and 1 µg/mL vitamin E, resulting in a final concentration of 1 ng/mL Se and 0.1 µg/mL vitamin E in the culture media.

Lymphocyte proliferation

Isolated mononuclear cells, 2 × 10^6 cells/mL, were incubated with or without 5 µg/mL concanavalin A for 48 h at 37°C, 5% CO2. Unstimulated mononuclear cells were included as a measure of background proliferation. At 48 h, 0.4 µCi [3H]thymidine was added and incubated for an additional 18 h. The cultures then were harvested and the level of [3H]thymidine incorporated into proliferating cells was determined by liquid scintillation spectrometry. The data are expressed as the mean increased counts per minute (cpm) over nonstimulated cultures.

A dose titration of concanavalin A was conducted to determine optimal and suboptimal stimulation of lymphocyte proliferation. This titration ranged from 0.625 to 10 µg/mL concanavalin A. Regardless of concanavalin A dose, cells collected from vitamin E- and Se-deficient rats proliferated less than those collected from control rats. Therefore, in an attempt to clarify the mechanisms responsible for lymphocyte immunosuppression during vitamin E or Se deficiency the optimal stimulatory dose of 5 µg/mL was utilized.

Stimulation and measurement of IL-2 activity

Isolated mononuclear cells were stimulated with or without 5 µg/mL concanavalin A for increasing periods of time. At 12, 24, and 48 h, the culture supernatants were collected and stored at −70°C until analyzed for IL-2 activity.

An IL-2-dependent cell line, CTLL-2, was utilized to determine the IL-2 activity present in supernatants collected from stimulated and unstimulated cultures. Briefly, 1 × 10^5 CTLTL-2 cells were incubated with serial dilutions of culture supernatants for 24 h. At this time, 0.4 µCi [3H]thymidine was added and the cells incubated for an additional 18 h. The cultures then were harvested and the level of [3H]thymidine incorporated into proliferating cells was determined by liquid scintillation spectrometry. The amount of IL-2 activity in the culture supernatants was extrapolated from a standard curve generated with recombinant mouse IL-2. The specificity of this assay for IL-2 was verified by using a mouse anti-rat IL-2 monoclonal antibody that blocked IL-2 activity. In all samples, 95-100% of the biological activity was abrogated.

Iodination of transferrin

Human holo-Tf was iodinated using the chloramine T method [23]. E very 10 µg of holo-Tf was iodinated for 35 s with 5 µCi Na[125I] at a specific activity of 100 mCi/µg. The specific activity of the resultant iodinated protein, [125I]-Tf, was approximately 0.4 µCi/µg protein.

[125I]-Tf internalization

Isolated mononuclear cells were resuspended at 5 × 10^6 cells/mL and stimulated with 5 µg/mL concanavalin A for 48 h. Internalization of [125I]-Tf then was performed similar to the method of Malorni et al. [24]. The cultured cells were washed three times with 0.15 M phosphate-buffered saline to remove any bound Tf. After the final wash, cells were resuspended at 5 × 10^6 cells/mL in binding medium nonessential amino acids, 1% antibiotic/antimycotic, and 1% Na-glutamine, concanavalin A, heat-inactivated calf serum, goat serum, sodium azide, sodium chloride, bovine serum albumin, minimal essential medium nonessential amino acids, and acetic acid were all purchased from Sigma (St. Louis, MO). Fetal bovine serum was obtained from Hyclone (Logan, UT). Monoclonal antibodies for flow cytometric analysis were purchased from Harlan Bioproducts for Science, Inc. (Indianapolis, IN). These antibodies included mouse anti-rat CD2, CD4, CD8, B lymphocyte common antigen, monocyte/macrophage ED1, IL-2Rα, and TfR. The secondary antibody for flow cytometry, rat anti-mouse IgG (H + L) fluorescein conjugate, was provided from Boehringer Mannheim (Indianapolis, IN). [3H]thymidine was acquired from Amersham (Arlington Heights, IL) and Na[125I] from Dupont-New England Nuclear (Boston, MA). The IL-2-dependent cell line, CTLL-2 (TIB24) was provided by ATCC (Rockville, MD). The recombinant mouse IL-2 was purchased from Genzyme (Cambridge, MA) and the human holo-transferrin (Tf) from Calbiochem (San Diego, CA).

Mannheim (Indianapolis, IN). [3H]thymidine was acquired from Amersham (Arlington Heights, IL) and Na[125I] from Dupont-New England Nuclear (Boston, MA).
buffer (150 mM NaCl + 25 mM HEPES + 0.1 mg/mL bovine serum albumin) and incubated with 10 nM 125I-Tf for 60 min at 4°C. The cells then were rinsed with 0.15 M phosphate-buffered saline to remove unbound 125I-Tf, resuspended at 5 × 10^6 cells/mL in binding buffer, and 0.2-mL aliquots transferred to triplicate microcentrifuge tubes for each time point. The samples were incubated at 37°C with constant mixing for increasing periods of time. After incubation, the samples were centrifuged, the supernatants that contained free 125I-Tf collected, and the pellets resuspended in 0.3 mL of 0.2 M acetic acid + 0.5 M NaCl (pH 2.4) to remove surface-bound 125I-Tf. After centrifugation, the supernatant containing the acid-sensitive 125I-Tf and the pellet containing the acid-insensitive 125I-Tf were collected. The acid-sensitive supernatant contains the surface-bound 125I-Tf and the acid-insensitive pellet contains the internalized 125I-Tf. The radioactivity of the free, surface bound, and internalized 125I-Tf was determined by counting the level of radioactivity with a gamma counter. The data is expressed as mean counts per minute for each fraction. Four replicate experiments were performed with two rat spleens combined per diet for each experiment, except when noted.

**Statistics**

Data collected from each experiment (n = 3 or 4 per dietary treatment) were analyzed using the general linear model procedure of SAS (SAS Institute, Raleigh, NC). The model included the effect of diet only. Significant differences among the dietary treatment means were determined using Bonferroni's t-test method of multiple comparisons. Differences in least square means were considered significant when P values were < 0.05.

**RESULTS**

**Leukocyte characterization**

Flow cytometric analysis of isolated splenic mononuclear cells from each of the three dietary groups revealed similar percentages of CD2+ T lymphocytes, as well as the CD4+ T helper lymphocytes, CD8+ T cytotoxic/suppressor lymphocytes, B lymphocyte common antigen (B), monocytes/macrophage ED1 (Mφ), IL-2Rα, or TfR. Monoclonal antibody binding was visualized with a secondary rat anti-mouse IgG (H + L)-fluorescein conjugate. The mononuclear cells were gated by light scatter (forward and side) and assessed for FL1 (green fluorescence) using a Coulter EPICS XL-MCL flow cytometer. Data are expressed as the mean percentage of positive-staining cells ± se. Letters represent significant differences among dietary treatments, P < 0.05.

**Fig. 1.** Splenic mononuclear cells isolated from rats fed a vitamin E-deficient (-E + Se), Se-deficient (+E - Se), or control (+E + Se) diet were characterized phenotypically by flow cytometric analysis. This was accomplished by incubating 1 × 10^5 cells with monoclonal antibodies for CD2+ T lymphocytes, CD4+ T helper lymphocytes, CD8+ T cytotoxic/suppressor lymphocytes, B lymphocyte common antigen (B), monocytes/macrophage ED1 (Mφ), IL-2Rα, or TfR. Monoclonal antibody binding was visualized with a secondary rat anti-mouse IgG (H + L)-fluorescein conjugate. The mononuclear cells were gated by light scatter (forward and side) and assessed for FL1 (green fluorescence) using a Coulter EPICS XL-MCL flow cytometer. Data are expressed as the mean percentage of positive-staining cells ± se. Letters represent significant differences among dietary treatments, P < 0.05.

**Fig. 2.** Effects of vitamin E and Se deficiency on lymphocyte proliferation. Splenic mononuclear cells isolated from rats fed a vitamin E-deficient (-E + Se), Se-deficient (+E - Se), or control (+E + Se) diet were stimulated for 48 h with 5 µg/mL concanavalin A. Lymphocyte proliferation was determined through incorporation of [3H]thymidine 18 h after the addition of [3H]thymidine. Lymphocyte proliferation is expressed as mean increased counts per minute (cpm) for each dietary treatment ± se. a,b, Significant differences among treatments, P < 0.05.
Lymphocyte proliferative responses

Splenic mononuclear cells were isolated and stimulated with concanavalin A for a total of 66 h. The stimulated cells isolated from vitamin E-deficient or Se-deficient rats proliferated significantly less than those cells isolated from control rats (Fig. 2). As mentioned earlier, stimulation of lymphocytes with suboptimal doses of concanavalin A (0.625–2.5 µg/mL) gave similar results (data not shown).

IL-2 activity

Isolated spleen cells were stimulated with concanavalin A for increasing periods of time and analyzed for IL-2 activity. Interleukin-2 activity in concanavalin A-stimulated mononuclear cells did not differ significantly among these dietary groups at 12, 24, and 48 h after stimulation (Table 1). A dose titration of concanavalin A revealed a reduction in IL-2 activity that corresponded with the decrease in concanavalin A concentration. However, the level of IL-2 activity did not differ among the three dietary groups, regardless of concanavalin A dose or stimulation length (data not shown). It should also be noted that IL-2 activity was completely abrogated by the addition of a monoclonal antibody to IL-2R that prevented IL-2 binding (data not shown).

**TABLE 1. IL-2 Activity After Stimulation**

<table>
<thead>
<tr>
<th>Stimulation (h)</th>
<th>Experimental diet</th>
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<tbody>
<tr>
<td></td>
<td>-E + Se</td>
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<tr>
<td>12</td>
<td>52.62 ± 11.22</td>
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<tr>
<td>24</td>
<td>200.99 ± 47.75</td>
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<tr>
<td>48</td>
<td>297.10 ± 59.02</td>
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Rat splenic lymphocytes were cultured in the presence of 5 µg/mL concanavalin A for increasing periods of time, after which the supernatants were collected and analyzed for IL-2 activity using the IL-2-dependent cell line, CTLL-2. Data are expressed as mean IL-2 activity ± SE at each time period for each diet: vitamin E-deficient (-E + Se), Se-deficient (+E - Se), and control (+E + Se).

**DISCUSSION**

Vitamin E or Se deficiency may impair lymphocyte proliferation through several mechanisms. The most obvious means is to modify the proportions of certain cellular phenotypes. However, the current study did not show any gross changes in lymphocyte or macrophage percentages. Although overt signs of altered lymphocyte or monocyte expression were not detected, it is possible that T lymphocyte subpopulations are modified during times of vitamin E or Se deficiency. Fluctuations in the relative proportion of CD4+ Th1 versus Th2 and/or CD8+ T cytotoxic versus suppressor subpopulations may be masked because the overall percentage of CD4+ or CD8+ T lymphocytes did not change. Stimulation of a large proportion of Th2 or T suppressor lymphocytes could increase local IL-4/IL-10 concentrations and indirectly reduce lymphocyte proliferation by preventing or minimizing macrophage functions such as cytokine synthesis, MHC expression, and antigen presentation [25, 26].

**Stimulated expression of IL-2Rα and TfR**

Cells collected from rats fed the vitamin E-deficient, Se-deficient, or control diet were stimulated with concanavalin A for 48 h. Of these stimulated cells, 54–63% expressed IL-2Rα, whereas only 34–40% of the cells expressed TfR, regardless of the diet fed (Fig. 3). The density of IL-2Rα and TfR on the surface of stimulated cells also was comparable for cells collected from rats fed each of these diets (data not shown).

**125I-Tf internalization**

Mononuclear cells isolated from rats fed vitamin E- and Se-deficient diets did not demonstrate any ability to internalize surface bound 125I-Tf (Fig. 4). In contrast, surface-bound 125I-Tf was internalized maximally at 10 min and remained elevated for the control diet. As internalized 125I-Tf increased, the surface bound 125I-Tf decreased concurrently. The levels of free 125I-Tf were similar for each group.

![Fig. 3. Effects of vitamin E and Se deficiency on IL-2R and TfR expression. Splenic mononuclear cells isolated from rats fed a vitamin E-deficient (-E + Se), Se-deficient (+E - Se), or control (+E + Se) diet were stimulated for 48 h with 5 µg/mL concanavalin A and the expression of IL-2R and TfR evaluated by flow cytometry as described in Figure 1.](image-url)
Because the overall proportion of cellular phenotypes did not have an effect on lymphocyte proliferation, we next examined the T lymphocyte growth factor, IL-2. IL-2 activity did not vary with respect to diet, suggesting that an adequate level of IL-2 was present to support lymphocyte proliferation. This conclusion concurs with most other studies of Se deficiency [17, 18]. One exception is the work published by Moriguchi et al. [9], which reported that IL-2 activity was reduced in stimulated thymocytes originally isolated from vitamin E-deficient animals. This is not surprising because thymocytes are antigenically naive and primarily produce IL-2 but not IL-4 or interferon-γ after mitogenic stimulation [27, 28]. As such, a small variation in IL-2 would be more pronounced in thymic cells as opposed to mature T lymphocytes present in the spleen that can synthesize multiple cytokines such as IL-2, IL-4, IL-5, IL-10, IL-12, interferon-γ, and other cytokines upon mitogen stimulation [12, 26, 27].

Because IL-2 activity was sufficient for lymphocyte proliferation, the next step was to determine whether altered IL-2R or TfR expression contributed to the decrease in lymphocyte proliferation during vitamin E or Se deficiency. Neither the percentage of cells expressing IL-2R, nor the density of this receptor on the cell surface after stimulation with concanavalin A was affected by dietary Se or vitamin E deficiency. However, Roy et al. [19] reported that Se deficiency decreases and slows the expression of high-affinity IL-2R on the surface of stimulated mouse splenocytes. A possible explanation for this discrepancy is that Roy et al. [19] assessed the density of high-affinity IL-2R based on the $K_d = 10^{-11}$ M calculated from Scatchard analysis of $^{125}$I-IL-2 binding to stimulated mononuclear cells. This high-affinity receptor is the result of at least three IL-2R subunits, IL-2Rα, IL-2Rβ, and IL-2Rγ chains [14]. The current study estimated high-affinity IL-2R density through the surface expression of...
IL-2Rα by flow cytometry. Flow cytometric analysis could overestimate the formation of high-affinity IL-2R by binding to all IL-2Rα chains on the cell surface, including those which have not yet formed high-affinity IL-2R by combining with the IL-2Rβ and IL-2Rγ chains. An inability to form high-affinity receptors may be related to a decrease in membrane fluidity caused by oxidative stress within the cell. Furthermore, a lack of high-affinity receptor formation would limit IL-2-mediated expansion of lymphocyte proliferation by compromising IL-2 binding and subsequent signaling processes.

As with the IL-2R, no differences were observed in the percentage of cells expressing the TFR or in the density of the TFR per cell in rats fed vitamin E- or Se-deficient diets when compared with those rats fed control diets. It is possible that vitamin E or Se deficiency altered TFR internalization by preventing endocytosis of clathrin-coated pits or phosphorylation of the TFR cytoplasmic tail thought to be involved in TFR internalization [29,30]. The hypothesis of reduced TFR internalization is supported by the results of this study, which demonstrated that cells from vitamin E- and Se-deficient rats were able to bind as much 125I-Tf as the cells from control rats, but were not able to internalize the 125I-Tf. This differs from a study by Malorni et al. [24] in which chemically induced oxidative stress led to specific internalization of surface TFR within 10 min of menadione treatment. The results reported by Malorni et al. [24] reflect that acute oxidative stress prevents TFR recycling to the surface by K562 cells, which normally express this receptor during culture [31]. However, those conditions most likely do not accurately reflect what occurs during chronic oxidative stress in lymphocytes that do not express TFR until stimulated. Regardless of the means by which oxidative stress inactivates the TFR, the final outcome is impaired iron uptake.

Dysfunction of lymphocyte TFR can have serious consequences. A reduction or failure to internalize TFR can cause depletion of intracellular iron stores, thereby compromising the ability of cells to enter the S phase of the cell cycle. A recent study by Lucas et al. [32] has demonstrated that iron deprivation of human peripheral blood T lymphocytes by administration of deferoxamine inhibits the appearance of the cyclin A-associated component of the p33cdk2 kinase. This kinase is thought to serve as a restriction point for late G1 phase and may aid in the passage of cells through S phase [33,34]. The expression of IL-2, IL-2R, and TFR in this study without the induction of proliferation suggests that cells from vitamin E- and Se-deficient rats also were halted in the late G1 phase of the cell cycle. The lack of cell cycle progression at this point may be partially related to impaired p33cdk2 kinase activity induced by oxidative stress and subsequent TFR dysfunction in vitamin E- and Se-deficient animals. Future research that examines p33cdk2 kinase activity should determine whether this kinase is impaired in vitamin E- or Se-deficient animals.

In summary, the expression of IL-2, IL-2Rα, and TFR appeared to be normal during both Se and vitamin E deficiency. The evidence does suggest that TFR internalization may be compromised, thereby decreasing lymphocyte proliferation and subsequent participation in the immune response. Future research that examines IL-2R and TFR functional activity in greater detail should provide greater insight into the effects of Se and vitamin E deficiency on the immune response, as well as aid in the development of therapeutic measures in the elderly or chronically ill.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant RO1AI38250.

The authors greatly appreciate the expert technical assistance of Kim Shafer-Weaver, Kristen Aherne, Maria Long, Andy Liken, and George Hildenbrandt, as well as the laboratory animal support staff at the Centralized Biological Laboratory.

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