Effect of Selenium on the Expression of High Affinity Interleukin 2 Receptors (43391)

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Abstract. **Selenium (Se) is an essential nutritional factor that has been shown to affect the development and expression of cell-mediated immune responses. This study shows that dietary (2 ppm for 8 weeks) or *in vitro* (1×10^{-7} *M*) supplementation with Se results in a significant increase in the number of high affinity interleukin (IL) 2-binding sites (K_d of 10^{-11} *M*) on the surface of concanavalin A-stimulated lymphocytes from C57BL/6J mice, whereas Se deficiency (0.02 ppm for 8 weeks) has the opposite effect. Se supplementation or deficiency apparently alters the kinetics of IL-2 receptor expression. Supplementation with Se *in vivo* or *in vitro* resulted in an earlier expression of high affinity IL-2 receptors, whereas Se deficiency resulted in a delayed expression of lower numbers of receptors. To exert its effect on IL-2 receptor expression, Se must be present or absent in the cell environment 8–24 hr after stimulation, and it most likely affects processes in the cytoplasmic and/or nuclear compartments of activated lymphocytes. Thus, in the presence of continuous immunologic stimulation, the presence or absence of Se in the cell environment can result in an accelerated or delayed clonal expansion of immunocompetent lymphocytes, respectively.** [P.S.E.B.M. 1992, Vol 200]

Selenium (Se) is an essential nutritional factor that has been shown experimentally to protect against the development of neoplasms (1). The experimental and epidemiologic data indicate that it may be possible to use Se-containing compounds as cancer chemopreventive agents (2, 3). Of particular interest is the indication that modulation of Se levels in the diet may augment or suppress immune functions and the responses of a host to malignant cells (4–6).

The regulation of immune responses by clonal expansion of immunocompetent cells is influenced by a number of cytokines, among which interleukin (IL) 2 has a central role. T lymphocyte clonal expansion depends on the interaction of IL-2 with its receptor (R) expressed on the cell surface (7). The signals arising from the T cell antigen-receptor complex coordinate the transcriptional activation of both the IL-2 gene and the genes encoding IL-2R (8), and the magnitude and duration of the resulting clonal proliferation depend on the density of IL-2R on the surface of activated cells and the availability of IL-2 (8).

The high affinity IL-2R is a membrane complex composed of at least two different subunits: the α-chain (p55 or Tac) that binds IL-2 with low affinity (K_d of 10^{-8} *M*), and the β-chain (p70 or p75) that binds IL-2 with intermediate affinity (K_d of 10^{-9} *M*) (7, 9). High affinity IL-2R (K_d of 10^{-11} *M*) are composed of p55/p75 held together by noncovalent forces (7) and are formed by fusion of the appropriate IL-2R-containing plasma membranes (10). A critical threshold of triggered high affinity IL-2R must accumulate before a cell is committed to DNA replication and mitosis (11), and the critical signal for progression to the S phase of the cycle appears to be transmitted through the β-chain of the receptor (12).

We have shown previously that supplementation with low doses of Se *in vivo* and *in vitro* or Se deficiency significantly altered the ability of mouse spleen lymphocytes to proliferate in response to stimulation with mitogen or antigen and to differentiate into cytotoxic cells (13, 14). The respective augmentation or inhibition of responses occurred in the absence of significant changes in the endogenous levels of IL-2 or IL-1. The purpose of the present study was to determine whether the observed alterations in proliferative responses were

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related to the ability of Se to alter the expression of IL-2R.

Materials and Methods

Animals and Diet. Male 6-week-old C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were housed in a temperature- and humidity-controlled animal facility with a 12:12-hr light:dark cycle and given water *ad libitum*. Animals were fed at midday and sacrificed between 9 AM and 12 noon to maintain uniformity and avoid circadian variations.

The mice were maintained on either Se-deficient (0.02 ppm Se), normal (0.20 ppm Se), or Se-supplemented (2.00 ppm Se) Torula yeast-based diets for 8 weeks. The diets were prepared commercially (Teklad, Madison, WI), as reported previously, and Se was added as sodium selenite (13). The Se content of the diet was determined fluorometrically according to the method of Spallholz *et al.* (15) using standard solutions of Se (25–250 ng Se/ml, as sodium selenite) to calibrate the assay. The sensitivity of the assay was 10 ng of Se and the Se content in samples of the deficient, normal, and supplemented diets was 0.028, 0.204, and 2.32 ppm, respectively.

Each animal was provided with 5 g/day of the respective diet and body weights were determined on the day of experimental use. There were no statistically significant differences in the weight of animals maintained for 8 weeks on the three diets. The Se concentrations in pooled serum samples (14) from 10 animals from each group were determined fluorometrically as described above (15). The average serum Se concentrations in Se-deficient, Se-normal, and Se-supplemented mice were 7.7, 29.6, and 43.6 μg/dl, respectively.

For the *in vitro* Se-supplementation studies, 14-week-old male C57BL/6J mice were used. The mice were maintained as described above and fed Purina Lab Chow (standard laboratory diet) *ad libitum*. The DBA/2 mice (The Jackson Laboratory) used for the passage of P815 cells were maintained identically.

Culture Media. All cells were cultured in a basic medium of RPMI 1640 supplemented with 25 m*M* HEPES, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 0.1 m*M* nonessential amino acids, and 2 m*M* glutamine (GIBCO, Grand Island, NY), with additional supplementation as specified for each experiment. All cultures were incubated at 37°C with 5% CO_2. The fetal bovine serum (FBS; Hyclone Labs, Logan, UT) used was selected for its low content of 1 μg/dl of Se. In media supplemented to 5% with FBS, this represented 6×10^{-9} *M* Se.

Cell Preparation. Groups of 10 mice were maintained on each of the three diets for 8 weeks. Ten days before the end of the dietary treatment, all animals were immunized by intraperitoneal injection of 5×10^6 freshly isolated P815 mastocytoma cells. At the end of the dietary treatment, pooled spleen cells from two animals (five experiments/group) were prepared as described previously (16), washed once in basic medium supplemented to 5% with FBS, and counted. Spleen cells (2×10^6/ml) were cultured in the same medium for 90 min, and the nonadherent cells were collected and assayed for baseline expression of IL-2R. The remaining spleen cells were cultured in 24-well culture plates at 4×10^6 spleen cells/well in a total volume of 2 ml of basic medium supplemented with 5 μg/ml of concanavalin A (con A; Sigma Chemical Co., St. Louis, MO) and to 5% with FBS.

For the *in vitro* Se-supplementation studies, spleens were removed from animals maintained on the standard laboratory diet. Pooled spleen cells from four animals/experiment (five experiments) were cultured as described above in medium supplemented with 0, 1×10^{-8} *M*, or 1×10^{-7} *M* Se (as sodium selenite). The plates were incubated for 24, 48, and 72 hr, and the lymphoblasts were collected and assayed for expression of IL-2R.

Determination of Intracellular Se Levels. To determine baseline intracellular concentrations of Se, a sample of pooled spleen cells from five animals/diet/experiment (three experiments/diet) was cultured for 2 hr in FBS-free basic medium; the nonadherent cells were collected, separated over lymphocyte separation medium (Organon Teknika Corp., Durham, NC), washed in physiological saline, and counted, and aliquots containing 1×10^7 cells were assayed for their content of Se (15). The remaining pooled cells were washed once in basic medium supplemented to 5% with FBS and counted, and 4×10^6 cells, in 2 ml of basic medium supplemented with 5 μg/ml of con A and to 5% with FBS, were plated in each well of 24-well culture plates. The plates were incubated for 24, 48, and 72 hr, the lymphoblasts were collected, separated over lymphocyte separation medium, washed in physiological saline, and counted, and aliquots of 1×10^7 cells were pelleted by centrifugation at 9000 rpm for 60 sec in an Eppendorf model 5415C microcentrifuge (Brinkman Instruments, Inc., Westbury, NY). The supernatants were removed, the cells were resuspended in 1.0 ml of distilled, deionized water, and the Se content was determined fluorometrically (15).

IL-2R Assay. The assay was performed essentially as described by Robb *et al.* (17). Lymphoblasts were separated from cellular debris by centrifugation over lymphocyte separation medium and washed in 5 ml of binding buffer (RPMI 1640 with 25 m*M* HEPES and 10 mg/ml of fraction V of bovine serum albumin (Sigma)). The cells were resuspended in 5 ml of binding buffer and incubated in a Dubnoff shaker at 37°C for two 1-hr periods, with extensive washes between incubations, to facilitate removal of bound IL-2. Serial dilutions (0.2 n*M*–6 p*M*) of ^{125}I-labeled human recom-

binant IL-2 (New England Nuclear, Inc., Boston, MA), with and without 500 × molar excess of cold IL-2 (murine recombinant IL-2, a gift from Dr. Gerard Zurawski, DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, CA), were incubated at 37°C with 4–6 × 10^5 lymphoblasts in 150 μl of binding buffer for 15 min. The incubation was carried out in Eppendorf tubes underlain with 150 μl of a 3:1 mixture of dibutyl phthalate (Aldrich Chemical Co., Inc., Milwaukee, WI) to dinonyl phthalate (ICN Biochemicals, Cleveland, OH) (18). After 15 min, 350 μl of ice-cold binding buffer were added to each tube, and the tubes were centrifuged at 9000 rpm for 60 sec in the microcentrifuge. The aqueous phase was collected and the amount of free radiolabeled ligand was determined. The oil mixture was discarded, and the amount of cell-bound radioisotope was determined. Specific binding was obtained by subtracting nonspecific binding from total binding. Computer-assisted Scatchard analyses were used to determine the number of high affinity binding sites per cell and the dissociation constants (K_d).

Effect of Time of Exposure to Se on IL-2R Expression. Pooled spleen cells (five spleens/experiment, four experiments) were prepared from animals maintained on standard laboratory diet, and 4 × 10^6 cells were plated in each well of 24-well culture plates in a total volume of 2 ml of basic medium supplemented with 5 μg/ml of con A and to 5% with FBS. At 0, 4, 8, and 24 hr after the initiation of incubation, the cultures were supplemented to 1 × 10^{-7} *M* Se (as sodium selenite); control cultures received basic medium only. At 48-hr of incubation, lymphoblasts were collected and assayed for IL-2 receptor expression.

Statistical Analysis. The results are presented as the mean ± SE for each control and experimental group. For dissociation constants (K_d), the results are presented as the geometric mean ± SE (19). Differences between the group means were determined using the Student's two-tailed *t* test, and *P*-values ≤ 0.05 were considered to be significantly different.

Results

Expression of IL-2R after Dietary Exposure to Se. The levels of Se in the diet exerted a significant effect on the number of IL-2R/cell and on the kinetics of IL-2R expression on stimulated spleen lymphocytes. Scatchard plots of ^{125}I-IL-2 binding data (0.2 n*M*–6.0 p*M*) on lymphocytes from Se-normal and experimental animals indicated that Se exerted its effect on the expression of high affinity IL-2 binding sites, since the mean K_d ranged from 1.6 to 6.1 × 10^{-11} *M* (Fig. 1; results from a typical experiment). Prior to stimulation with con A, the number of high affinity IL-2R on cells from the three groups was essentially the same, but 24 hr after stimulation with con A, the number approximately doubled, with no significant differences between nutritional groups (Fig. 2). After 48 hr of stimulation, lymphoblasts from animals maintained on the normal diet showed a significant increase (68%; $P < 0.025$) in the number of high affinity IL-2R/cell, as compared with 24 hr of stimulation, and a slight decline, but not statistically significant, after 72 hr of stimulation. At the same time, lymphoblasts from animals maintained on the Se-supplemented diet showed a sharp increase in the number of IL-2R (172%; $P < 0.005$), followed by a significant decline ($P < 0.005$) at 72 hr. Lymphoblasts from animals maintained on the Se-deficient diet showed no significant changes in the number of high affinity IL-2R after 48 hr of stimulation, but an increase (47%; $P < 0.05$) after 72 hr of stimulation. The increase in the number of IL-2R observed after 48 hr of stimulation on lymphoblasts from Se-supplemented animals was significantly higher than the number of sites on cells from Se-normal ($P < 0.05$) or Se-deficient ($P < 0.005$) animals.

Expression of IL-2R after *In Vitro* Exposure to Se. Stimulation with con A of splenic lymphocytes from animals maintained on normal diet in the absence or presence of exogenous Se (10^{-8} and 10^{-7} *M*) for 24 hr resulted in a similar expression of IL-2R/cell (Fig. 3). Similar to the results with cells from animals exposed to Se *in vivo*, Se exerted its maximal stimulatory effect after 48 hr of incubation with con A. The effect was most evident with cells cultured in the presence of 1 × 10^{-7} *M* Se, which resulted in a 123% increase ($P < 0.02$) in the number of IL-2R sites/cell, relative to 24 hr of stimulation, followed by a significant decline at 72 hr ($P < 0.02$). The increase in the number of receptor sites after 48 hr of stimulation exerted by 1 × 10^{-7} *M* Se was 48% higher ($P < 0.05$) than the increase in the absence of Se supplementation. Analysis of the Scatchard plots (results of a typical experiment are presented in Fig. 4) indicated mean K_d values of 0.7–5.3 × 10^{-11} *M*, which defined the IL-2 binding sites as high affinity.

Effect of Time of Exposure to Se on Expression of IL-2R. The effect of exogenous supplementation with Se on the expression of high affinity IL-2R on lymphocytes from Se-normal animals was determined after 48 hr of stimulation with con A in the presence or absence of Se (1 × 10^{-7} *M*) that was added to the cultures at 0, 4, 8, and 24 hr after initiation of stimulation (Fig. 5). The addition of Se 4 or 8 hr after initiation of stimulation with con A resulted in the same numbers of high affinity IL-2R/cell as on lymphocytes stimulated in the presence of Se from the beginning of culture. In all instances, the increase in high affinity IL-2R was significantly higher than on cells stimulated in the absence of Se ($P < 0.005$–$P < 0.001$). The addition of Se 24 hr after initiation of stimulation with con A had no effect on the expression of IL-2R, as the number of IL-2R/

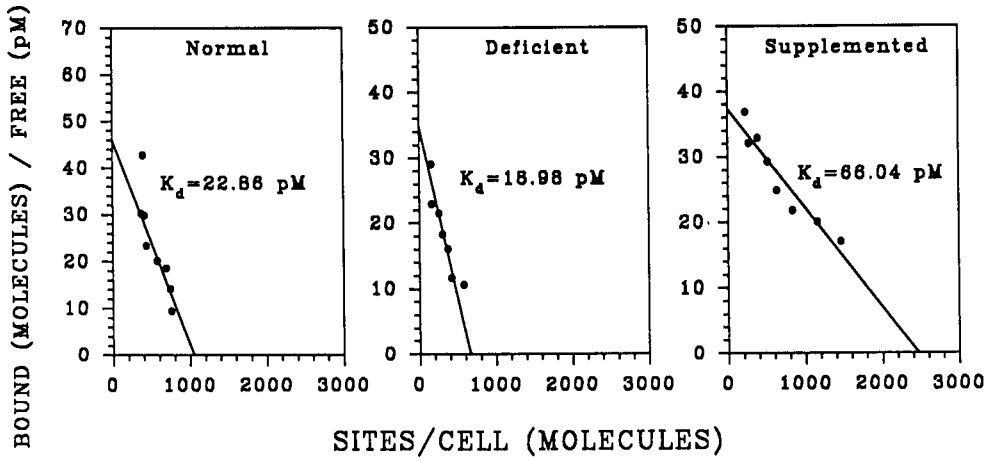

Figure 1. Scatchard plots of the ^{125}I-IL-2 binding data on lymphoblasts derived from C57BL/6J mice maintained on Se-normal (0.20 ppm Se), Se-deficient (0.02 ppm Se), and Se-supplemented (2.00 ppm Se) diets for 8 weeks. The data represent the binding patterns from a typical experiment after 48 hr of stimulation of the cells with 5 μg/ml of con A.

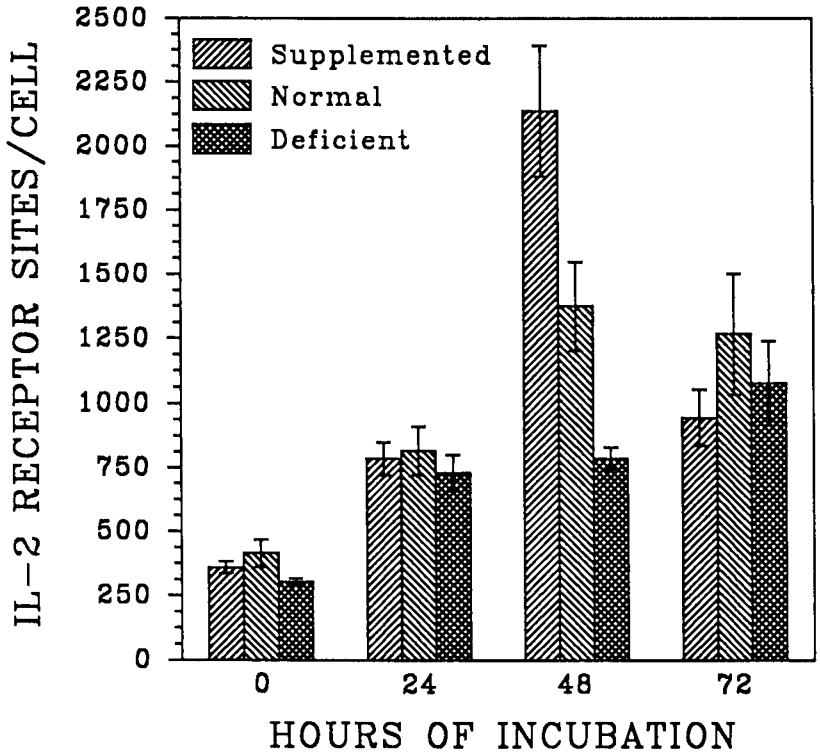

Figure 2. Number of IL-2 binding sites per cell for lymphoblasts derived from C57BL/6J mice maintained on Se-supplemented (2.00 ppm Se), Se-normal (0.20 ppm Se), and Se-deficient (0.02 ppm Se) diets for 8 weeks before incubation (0 hr) and after 24, 48, and 72 hr of stimulation with 5 μg/ml of con A. Data are presented as the mean IL-2R sites/cell ± SE.

cell was the same as on cells stimulated in the absence of Se. Se alone (in the absence of con A) had no effect on the expression of IL-2R.

Intracellular Se Levels after Dietary Exposure to Se. The baseline levels of Se in freshly isolated spleen lymphocytes from Se-supplemented animals were 89.1% higher ($P < 0.05$) and those in lymphocytes from Se-deficient animals were 72.1% lower ($P < 0.05$) than the levels in cells from Se-normal animals (Fig. 6). After 24 hr of incubation, the Se content of cells from Se-supplemented animals declined to levels equivalent to those from Se-normal animals and remained at that level during the 72 hr of incubation. The sharp decline in the intracellular Se level is most likely due to release related to the difference in the concentration of Se in the medium, i.e., 6.33×10^{-9} *M*, and the intracellular environment. As shown by others, removal (decrease) of Se from the growth medium results in a 52–83% reduction of intracellular content by 24–48 hr after withdrawal (20). Lymphocytes from Se-deficient ani-

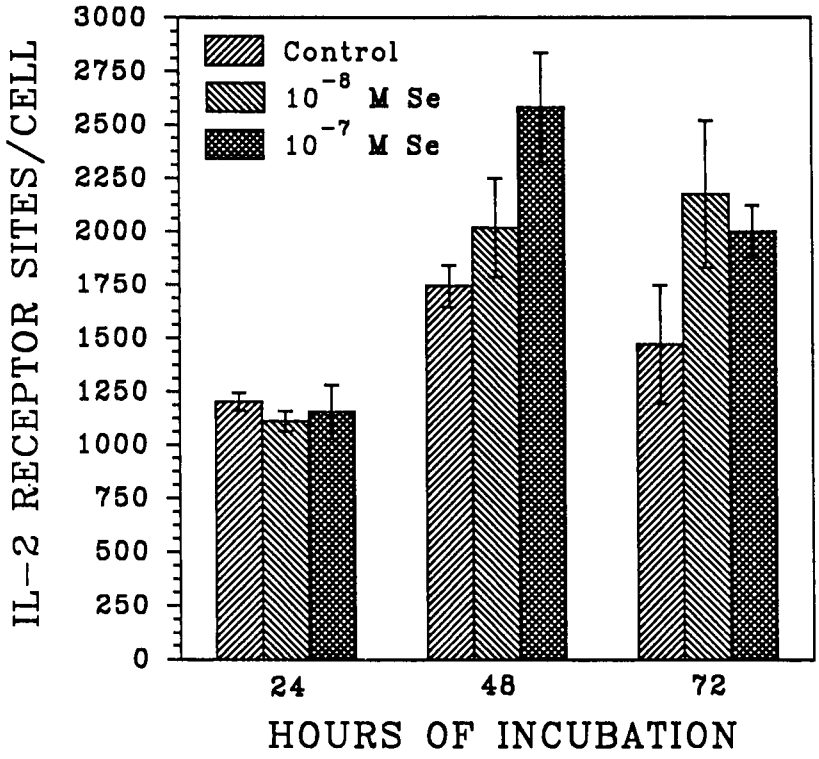

Figure 3. Number of IL-2 binding sites per cell for lymphoblasts derived from C57BL/6J mice maintained on normal laboratory diet after stimulation with 5 μg/ml of con A in the presence or absence of exogenous Se. Data are presented as the mean IL-2R sites/cell ± SE.

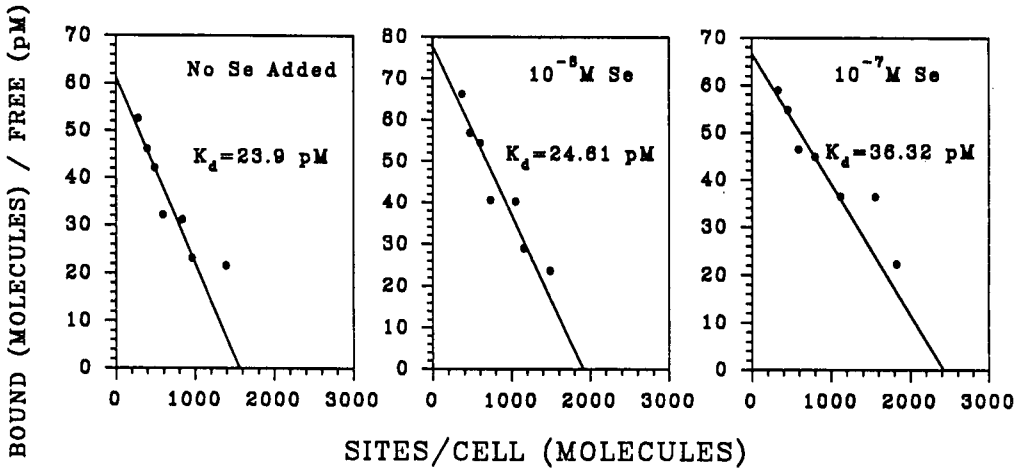

Figure 4. Scatchard plots of the ^{125}I-IL-2 binding data on lymphoblasts derived from C57BL/6J mice maintained on normal laboratory diet. Data represent the binding patterns from a typical experiment after 48 hr of stimulation of the cells with 5 μg/ml of con A in the presence or absence of exogenous Se.

mals displayed depressed intracellular Se levels, relative to cells from Se-normal animals, throughout the entire incubation period.

Discussion

The results of these studies have shown that dietary (2 ppm) or *in vitro* ($1 \times 10^{-7} M$) supplementation with Se results in a significant increase in the number of p55/p75 (K_d of $10^{-11} M$) sites on the surface of activated lymphocytes, whereas Se deficiency has the opposite effect. Se supplementation or deficiency apparently alters the kinetics of the expression of IL-2R. Supplementation with Se *in vivo* or *in vitro* results in an earlier expression of higher numbers of high affinity IL-2R after mitogenic stimulation, whereas Se deficiency results in a delayed expression of lower numbers of receptors. Inasmuch as a critical threshold of triggered high affinity IL-2R must accumulate before a cell enters the S phase of the cell cycle (11), lymphocytes capable of expressing higher numbers of p55/p75 earlier will rep-

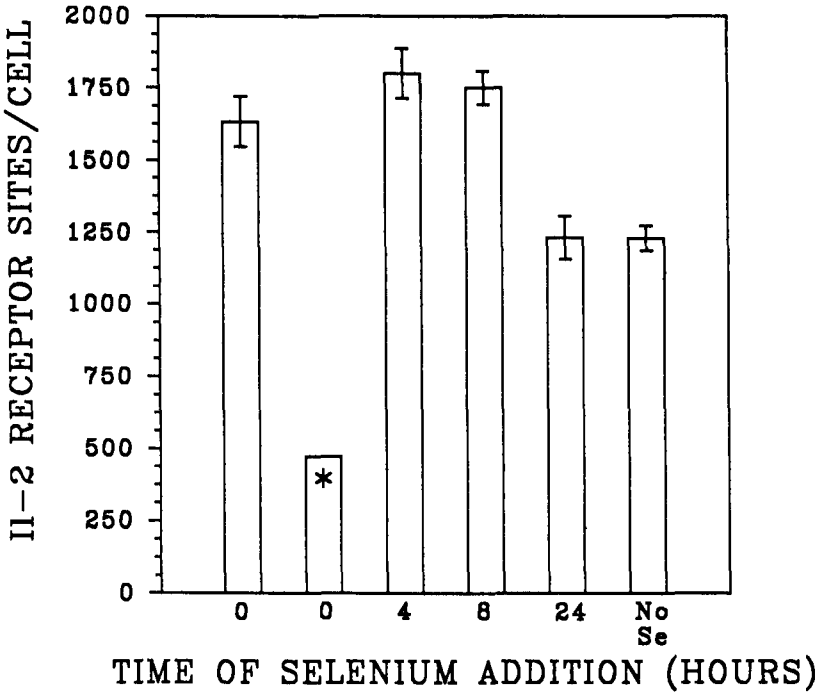

Figure 5. Number of IL-2 binding sites per cell for lymphoblasts derived from C57BL/6J mice maintained on normal laboratory diet after 48 hr of stimulation with 5 μg/ml of con A. Data are presented as the mean IL-2R sites/cell ± SE for lymphoblasts exposed to $1 \times 10^{-7} M$ exogenous Se at the initiation of culture (Time 0) or 4, 8, or 24 hr later. Control cultures: con A stimulated, no Se supplementation (No Se); Se supplemented at 0 hr, no con A stimulation (*).

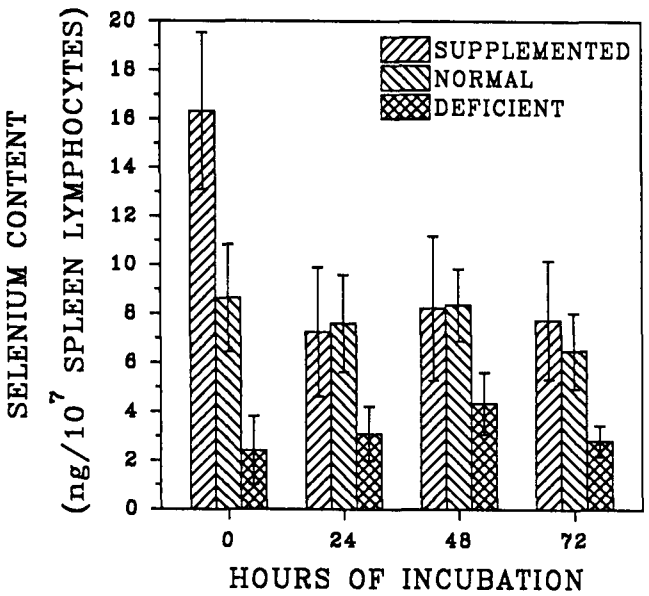

Figure 6. Intracellular concentrations of Se in lymphocytes derived from C57BL/6J mice maintained on Se-normal (0.20 ppm Se), Se-deficient (0.02 ppm Se), and Se-supplemented (2.00 ppm Se) diets for 8 weeks at 0, 24, 48, and 72 hr of stimulation with 5 μg/ml of con A. The data are presented as mean ng Se/1 × 10^7 cells ± SE.

licate and expand faster than other cells in the presence of continuous immunologic stimulation and a constant supply of IL-2. This conclusion is supported by our previous studies (13, 14), which indicated that supplementation with Se *in vivo* or *in vitro* results in an enhanced proliferation and increased frequency of cytotoxic lymphocytes within a given cell population (an increase from 6.1% to 13.2%) in response to stimulation with antigen or mitogen and in the absence of significant changes in the endogenous levels of IL-2 or IL-1. Se deficiency had the opposite effect.

The mechanism and regulation of the expression of IL-2R are not well defined. Resting human and murine T lymphocytes express the p75 chain constitutively, whereas the p55 chain is not detectable (21–23). Upon stimulation with antigen or mitogen, there is a 2.5-fold increase in the expression of mRNA coding for the β-chain that peaks at 24 hr after stimulation, followed by a gradual decline (24). With human peripheral blood lymphocytes, this results in a 3- to 4-fold increase in the expression of the corresponding surface protein with a peak at 3 days after stimulation (25). The inducible expression of the p55 gene is apparently regulated at a posttranslational level by activation of KB-specific DNA-binding proteins that interact with the IL-2R gene promoter (26). The mRNA coding for p55 appears within 5 hr of activation (26) and subsequent to the appearance of mRNA coding for IL-2 (27). The cellular expression of the corresponding protein reaches a peak 24 hr after stimulation of tonsillar lymphocytes, with a decline after 48 hr; the surface expression of the protein continues to increase beyond the peak of cellular protein expression (28). Thus, as demonstrated with human peripheral blood lymphocytes, the peak (8–24 hr)

and decline in IL-2R mRNA levels precede those of cell surface IL-2R expression (48–72 hr) by more than 24 hr (29).

A number of processes involved in the expression of IL-2R at the surface of stimulated lymphocytes may be potential targets for regulation by Se. Se may exert its effect at the cell surface, by affecting the assembly of receptor components on the membrane or signal transduction, or on the cell interior, by affecting transmission of signals to the nucleus, gene activation, or posttranscriptional modulation of the production and trafficking of receptor subunits. Inasmuch as Se in the absence of mitogen stimulation had no effect on the expression of IL-2R (Fig.5), and it is known that very little Se accumulates within the nucleus of a cell (1), it is unlikely that Se has a direct effect on gene activation. The addition of 1×10^{-7} *M* Se to lymphocyte cultures 24 hr after stimulation with con A had no effect on the expression of IL-2R, indicating that Se probably does not modulate the aggregation and presentation of receptor subunits at the cell surface since the expression of IL-2R at the surface reaches its peak 48–72 hr after stimulation (29). Because the mRNA coding for the α- and β-subunits are already present in the cytoplasm at peak or close to peak levels 8 hr after lymphocyte activation (24, 26, 29), and the addition of Se to activated lymphocytes 8 hr after stimulation resulted in a significant increase in the expression of IL-2R at the surface, it is also unlikely that Se affects signal transduction from the surface to the cell interior. Therefore, our data suggest that to exert its effect on the expression of IL-2R, Se must be present or absent in the cell environment 8–24 hr after stimulation and it most likely affects processes in the cytoplasmic and/or nuclear compartments of activated lymphocytes.

Evaluation of the Se content of spleen lymphocytes cultured in media containing 6.33×10^{-9} *M* Se (from the FBS supplement) indicated an 89.1% higher and a 72.1% lower Se content in cells from Se-supplemented and Se-deficient animals at initiation of culture; at 24 hr of culture, there were no significant differences in the Se contents of the cells. These results indicate a difference in the availability of intracellular Se during the early hours of culture. As shown by Sayar *et al.* (28), the steady state of IL-2R expression at the cell surface depends on the continuous active expression of the α-gene and the *de novo* synthesis of α-chains. Inasmuch as the p55 mRNA appears within 5 hr of activation (26), the availability of Se in the cytoplasm thereafter may affect the rate of mRNA processing and degradation, and, thus, affect the rate of expression of high affinity IL-2R. The existence of a number of mammalian Se-binding proteins other than glutathione peroxidase (30, 31), as well as selenocysteine tRNA (32), has been shown and the possibility of their involvement in the regulation of several intracellular metabolic processes has been proposed (30, 32). Although the results from the present study do not elucidate the mechanisms involved in the enhancing and inhibitory effects of Se on IL-2R expression, they do suggest that Se may act by binding to and modulating the function of specific cytoplasmic proteins and/or nucleotides during the period of 8–24 hr after stimulation with mitogen or antigen.

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