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Regulation of Cellular Immune Responses by Selenium

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ABSTRACT

Selenium (Se) is an essential nutritional factor that affects the development and expression of cell-mediated immune responses directed toward malignant cells. These studies have shown that dietary (2 ppm for 8 wk) or in in vitro ($1 \times 10^{-7}M$) supplementation with Se (as sodium selenite) results in a significant enhancement of the proliferative responses of spleen lymphocytes from C57Bl/6J mice in response to stimulation with mitogen or antigen. Se deficiency (0.02 ppm for 8 wk) had the opposite effect. The alterations in the ability of the cells to proliferate, which occurred in the absence of changes in the endogenous levels of interleukin-2 (Il_2) or interleukin 1, were apparently related to the ability of Se to alter the kinetics of expression of high-affinity Il_2 receptors on the surface of activated lymphocytes. This resulted in an enhanced or delayed clonal expansion of the cells, and in an increased or decreased frequency of cytotoxic cells within a given cell population. The changes in tumor cytotoxicity were paralleled by changes in the amounts of lymphotoxin produced by the activated cells. Dietary Se modulations had a comparable effect on macrophage-mediated tumor cytodestruction. The results also suggested that Se exerts its effect 8–24 h after stimulation, and that it most likely affects processes in the cytoplasmic and/or nuclear compartments of activated lymphocytes.

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Index Entries: Selenium and tumor cytodestruction; selenium and cell-mediated immunity; selenium and expression of interleukin 2 receptors; selenium and lymphocyte activation; selenium and macrophage activation for tumor cytotoxicity; selenium and cancer chemoprevention.

INTRODUCTION

The availability of selenium (Se) in the diet affects the function of all components of the immune system. A deficiency in Se appears to impair immune responsiveness, whereas Se supplementation abrogates the depressed responses and results in increased immunologic competence (*1*). Of particular interest is the indication that supplementation with Se inhibits tumorigenesis (2) and may alter the responses of a host to malignant cells (*3,4*).

The immune system actively participates in surveillance against malignant cells, as well as in their destruction, by several nonspecific and specific cell-mediated cytotoxic mechanisms (*5,6*). The control of tumor cell growth by these processes is achieved through the antigenic recognition of the cells as "foreign," which results in the subsequent development and expression of an appropriate immunologic response. The purpose of these studies was to establish whether modulation of the levels of Se in the cell environment affects the development and expression of cell-mediated immunologic responses directed toward malignant cells.

DEVELOPMENT OF THE CELL-MEDIATED IMMUNE RESPONSE

The development of the cell-mediated tumor cytotoxic response begins with the antigenic stimulation of resting T-lymphocytes, which results in their proliferation and the clonal expansion of cytotoxic cells (*7*). The first activation signal for T-lymphocyte proliferation is provided by the interaction of the antigen, presented to lymphocytes in association with the major histocompatibility complex (MHC) Class I or Class II molecules on the surface of macrophages, with the T-cell receptor on the surface of resting T-cells (*8*). The signal from the activated T-cell antigen-receptor complex, which can also be initiated by mitogens, such as concanavalin A (Con A) or phytohemagglutin (PHA), antibodies against various cell surface determinants, or phorbol esters (*9,10*), induces the transition of the cells from the G_0 to the G_1 phase of the cell cycle (*11*). Maximum stimulation, however, requires the presence of the costimulatory factor, interleukin-1 (Il_1), produced by macrophages (*12*). The signal for progression from G_1 to S and mitosis is provided by the interaction of interleukin-2 (Il_2), produced by a specific subset of activated lymphocytes, with the receptor for Il_2 (Il_2-R) on the surface of activated cells (*12*).

To determine whether variations in the levels of Se in the diet affect the development of the cell-mediated immune response, we examined:

1. The ability of spleen lymphocytes stimulated with mitogen or antigen to proliferate and produce Il_2;
2. The ability of macrophages to become activated and produce Il_1; and
3. The ability of activated lymphocytes to express Il_2-R.

Animals

C57Bl/6J male mice, 6 wk old, were maintained for 8 wk on a commercially prepared basal *Torula* yeast diet (Teklad, WI) supplemented with Se (as sodium selenite) to produce the normal (0.20 ppm Se), supplemented (2.00 ppm), or deficient (0.02 ppm Se) diets (*13*). Each animal was provided with 5 g/d of the respective diet, and body weights were determined on the day of experimental use. There were no statistically significant differences in the weights of animals maintained for 8 wk on the three diets. For each assay, the serum Se levels in pooled blood from 10 each of Se-normal, Se-supplemented, or Se-deficient animals were determined fluorometrically at the end of the 8-wk period, e.g., 27, 48, and 2 μg/dL, respectively (*13,14*).

Stimulation with Mitogen or Alloantigen

Spleen lymphocytes from animals maintained on the three diets were stimulated with PHA-P or with mitomycin C-treated DBA/2 cells in a mixed lymphocyte reaction (MLR) (*13*). The results indicated that dietary supplementation with Se significantly enhanced the ability of resting lymphocytes to proliferate in response to stimulation with either mitogen or antigen (Table 1). In contrast, cells from Se-deficient animals showed a statistically significant decreased capacity to respond. In both assays, the responses were consistently different, even though the cells were cultured for 3–5 d in media that contained 5% fetal bovine serum (FBS), which provided an optimal amount of Se for growth (*15*). This suggested that modulation of the Se levels in vivo resulted in alteration of in the functional properties of the cells that was related to the intracellular Se status of the cells.

Production of Il_2

The ability of spleen cells from animals maintained on the experimental and control diets to produce Il_2 in response to stimulation with Con A was tested as described (*13*). Supernatants from cultures stimulated for 24 h with Con A were assayed for their ability to support growth of the Il_2-dependent CTLL cell line, and the results indicated that there were no statistically significant differences in the amounts of Il_2 produced by activated lymphocytes from animals maintained on the three diets

Table 1
Effect of Selenium on Proliferation of C57B1/6J Spleen Lymphocytes in Response to Mitogen and Allogeneic Stimulation and on Production of Interleukin 1 and 2

Dietary Se content	PHA[a]			MLR[b]			Il_2[c] production, U/mL	Il_1[d] production, U/mL
	H^3-thymidine incorporation	P[e] value	Change, %	H^3-thymidine incorporation	P[e] value	Change, %		
Normal (0.20 ppm)	17,779.6[f] ± 1558.0			9675[f] ± 973			4.59 ± 0.56	20.87 ± 2.64
Deficient (0.02 ppm)	8974.5 ± 1728.5	<0.0025	49.5	6693 ± 1343	<0.05	30.8	5.25 ± 0.52	19.76 ± 2.10
Supplemented (2.00 ppm)	37,454.6 ± 1437.6	<0.005	110.6	20,705 ± 1730	<0.005	114.0	3.99 ± 0.49	23.94 ± 3.26

[a]Spleen lymphocytes (1 × 10^6/mL) stimulated with 10 μg/mL phytohemagglutinin-P for 3 d.
[b]MLR—mixed lymphocyte reaction: 2 × 10^6/mL spleen cells cocultured with the same number of mitomycin-C treated DBA/2 spleen cells for 5 d.
[c]Spleen lymphocytes (5 × 10^6/mL) stimulated with 3 μg/mL concanavalin A (Con A) for 24 h; culture supernatants assayed on CTLL cells.
[d]Immune peritoneal macrophages (1.5 × 10^6/mL) elicited by ip injection of P815 cells 10 d earlier were incubated for 48 h in the presence of 20 μg/mL lipopolysaccharide W from *E. coli*; culture supernatants were tested on C3H/HeJ thymocytes.
[e]Student's two-tailed *t*-test; compared to normal.
[f]$\overline{X}$ cpm ± SEM.

(Table 1). This indicated that the effect of Se on lymphocyte proliferation was not related to the endogenous levels of Il_2. Because the production of Il_2 in response to stimulation with Con A requires intimate contact with accessory cells (i.e., macrophages) and interactions with Class I MHC antigens (*16*), the results also suggested that there were no differences in the abilities of accessory cells to present antigen.

Production of Il_1

Activation of macrophages was achieved by intraperitoneal immunization of animals maintained on the experimental and control diets with allogeneic tumor cells (P815), and production of Il_1 by peritoneal exudate macrophages was stimulated with lipopolysaccharide W from *E. coli* (LPS) as described (*13*). The results indicated that there were no statistically significant differences in the ability of the cells to produce Il_1 (Table 1). Because Il_1 is necessary for maximum stimulation of lymphocytes (*12*), the effect of Se on lymphocyte proliferation was apparently not related to differences in the endogenous levels of Il_1.

Expression of Il_2-R

The clonal expansion of T-lymphocytes into immunocompetent cells, e.g., cytotoxic cells, depends on the interaction of Il_2 with its receptor expressed on the surface of activated cells (*11*). The signals arising from the T-cell antigen-receptor complex coordinate the transcriptional activation of both the Il_2 gene and the genes encoding the Il_2-R (*17*). The magnitude and duration of the resulting clonal proliferation depend on the density of Il_2-R on the surface of activated cells and the availability of Il_2 (*17*).

The high-affinity Il_2-R is a membrane complex composed of at least two different subunits: the α chain (p55 or Tac), which binds Il_2 with low affinity (K_d of 10^{-8} *M*), and the β chain (p70 or p75), which binds Il_2 with intermediate affinity (K_d of 10^{-9} *M*) (*11,18*). High-affinity Il_2-R (K_d of 10^{-11} *M*) is comprised of p55/p75 held together by noncovalent forces (*11*) and is formed by fusion of the appropriate Il_2 receptor containing plasma membranes (*19*). A critical threshold of triggered high-affinity Il_2-R must accumulate before a cell is committed to DNA replication and mitosis (*20*), and the critical signal for progression to the S phase of the cycle appears to be transmitted through the β chain of the receptor (*21*). To determine whether the observed modulation of lymphocyte proliferative responses by Se is related to the ability of the cells to express Il_2-R, the kinetics of Il_2-R expression was examined in the presence or absence of Se.

Spleen cells from immunized animals (5×10^6 P815 cells/animal) maintained on the experimental or control diets were prepared (*13,14*). The cells were stimulated with Con A, and cultured for 0, 24, 48, and 72 h. Cells from control animals (normal diet) were incubated in the pres-

ence or absence of $1 \times 10^{-7}M$ Se (as sodium selenite); the Se content of the culture medium (from FBS) was $6.33 \times 10^{-9}M$. The Il_2-R determination assay was performed as described by Robb et al. (*22*). The results showed that dietary or in vitro supplementation with Se resulted 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 had the opposite effect (Table 2). Supplementation with Se in vivo or in vitro resulted in an earlier expression of higher numbers of high-affinity Il_2-R following mitogenic stimulation, whereas Se deficiency resulted in a delayed expression of lower numbers of receptors. Inasmuch as a critical threshold of triggered high-affinity Il_2-R must accumulate before a cell enters the S phase of the cell cycle (*20*), lymphocytes capable of expressing higher numbers of p55/p75 earlier would replicate and expand faster than other cells in the presence of continuous immunologic stimulation and a constant supply of Il_2. The data also indicated that to exert its effect on the expression of Il_2-R, Se must be present/absent in the cell environment during the period of 8–24 h after stimulation. Se alone (without Con A) had no effect on the expression of Il_2-R (Table 2).

EXPRESSION OF THE CELL-MEDIATED IMMUNE RESPONSE

The expression of the tumor cytotoxic immune response results from the clonal proliferation of activated lymphocytes and their differentiation into cytotoxic effector cells. Tumor cytolysis by activated macrophages is also thought to contribute to the immune control of tumor growth, and it has been suggested that activation of macrophages for tumor cytodestruction is acquired through a series of proliferative and phenotypic alterations that culminate in the development of the cytotoxic state (*23*). To determine whether modulation in the levels of Se affects the expression of the tumor cytotoxic immune response, we examined the ability of: cytotoxic T-lymphocytes (CTL), and tumor cytotoxic macrophages, generated after in vivo or in vitro supplementation with Se, to destroy malignant cells.

CTL-Mediated Tumor Cytodestruction

CTL were generated in animals maintained on the three diets after intraperitoneal immunization with allogeneic cells or in vitro in an MLR with allogeneic cells in the presence or absence of Se (as sodium selenite) (*14*). The ability of the cells to destroy P815 cells was tested in a 4-h ^{51}Cr-release assay, and the number of cells needed to destroy a given number of tumor cells, as well as the cytotoxic cell frequency within each cell population, was determined (*14*). The results indicated that Se supplementation in vivo or in vitro enhanced the ability of CTL within a cell population to destroy tumor cells by means of increasing the cytotoxic

Table 2
Effect of Selenium on the Expression of High-Affinity Interleukin 2 Receptors (Il_2-R) on C57B1/6J Spleen Lymphocytes Activated with Concanavalin A (Con A)

Se content	No. Il_2-R/cell[d]				K_d, $10^{-11}M$	Time Se added	No.[d] Il_2-R/cell at 48 h	K_d, $10^{-11}M$
	0 h	24 h	48 h	72 h				
Diet normal (0.20 ppm)	414 ± 34	817 ± 93	1376[a] ± 173	1269 ± 234	1.5–3.4	No Se	1165 ± 54	2.2 ± 1.7
Diet deficient (0.02 ppm)	303 ± 13	729 ± 44	787[b] ± 44	1077 ± 162	1.6–2.9	0 h	1604[c] ± 73	2.7 ± 5.6
Diet supplemented (2.00 ppm)	358 ± 23	786 ± 65	2137[a,b] ± 256	945 ± 108	0.9–6.2	4 h	1670[c] ± 100	3.1 ± 2.3
In vitro Control; no Se		1204 ± 41	1744[a] ± 98	1472 ± 275	2.2–5.5	8 h	1621[c] ± 91	2.9 ± 0.9
In vitro $1 \times 10^{-7}M$ Se		1156 ± 126	2582[a,c] ± 256	2001[a] ± 123	1.4–6.7	24 h	1254 ± 121	2.0 ± 4.5
						0 h No Con A	473	1.7

[a]Significantly different from respective 24-h sample ($p < 0.02$).
[b]Significantly different from normal at same time period ($p < 0.05$).
[c]Significantly different from control at same time period ($p < 0.05$).
[d]Spleen cells (2×10^6/mL) stimulated with 5 μg/mL Con A for 0, 24, 48, and 72 h. Lymphoblasts were treated with serial dilutions (0.2 n*M*–6 pM) of ^{125}I-labeled human recombinant Il_2, with and without 500 × molar excess of cold murine recombinant Il_2. Specific binding was determined by subtracting nonspecific binding from total binding. Computer-assisted Scatchard analyses were used to determine the number of high-affinity binding sites/cell and dissociation constants (K_d).

cell frequency within that cell population. Se deficiency had the opposite effect (Table 3). The increase or decrease in the number of CTL within each cell population was also indicated by the parallel increase or decrease in the amounts of lymphotoxin released by the activated cells (Table 3).

Macrophage-Mediated Tumor Cytodestruction

Macrophages from animals maintained on the normal or supplemented diets were activated in vivo by intraperitoneal injection of P815 cells, or in vitro by stimulation with LPS and murine γ-interferon, (IFN-γ). Also, the ability of the cells to destroy P815 cells was tested in an 18-h ^{51}Cr-release assay (24). The results indicated that peritoneal exudate macrophages from Se-supplemented animals that were activated in vivo had an enhanced capacity to destroy tumor cells (Table 4). In contrast, peritoneal macrophages from Se-supplemented animals that were activated in vitro did not have an enhanced capacity to destroy tumor cells, indicating that the enhanced tumor cytodestruction by in vivo-activated macrophages from Se-supplemented animals was not related to the ability of the cells to become activated. This conclusion was supported by the observation that the levels of both IFN-γ (Table 4) and Il_1 (Table 1) produced by cells from allogeneically stimulated animals maintained on the normal or supplemented diets did not differ significantly (24).

The results also indicated that macrophages from allogeneically stimulated animals produced significantly greater amounts of tumor necrosis factor-α (TNF-α [24]; Table 4). Immunohistochemical studies have shown that the amount of TNF-α secreted by immunologically elicited macrophages could be directly related to the proportion of activated macrophages within that cell population (25). Consequently, the greater amounts of TNF-α produced by macrophages from Se-supplemented animals may reflect the presence of greater numbers of cytotoxic macrophages within the cell populations activated in vivo. The greater numbers of activated macrophages probably resulted from in vivo interactions between cells and mediators involved in the processes of recruitment and differentiation of cytotoxic macrophages.

CONCLUSIONS

Modulation of Se levels in the cell environment significantly affects the development and expression of the cell-mediated immune response toward malignant cells. The presence or absence of Se resulted in alterations in the proliferative responses of resting lymphocytes, elicited by stimulation with antigen or mitogen in the absence of changes in the endogenous levels of Il_2 and Il_1. The changes in proliferative responses were apparently related to alterations in the kinetics of expression of high-affinity Il_2-R on the surface of activated lymphocytes, which re-

Table 3
Effect of Selenium on Tumor Cytodestruction by C57B1/6J Lymphocytes and on Production of Lymphotoxin

Se content	Lymphocyte-mediated cytotoxicity[a]				Lymphotoxin production[b]		
	No. cells needed to kill 2×10^5 P815 target cells	P[c] value	Change, %	Cytotoxic[d] cell frequency, %	Half-max. U/mL	P[c] value	Change, %
Diet normal (0.20 ppm)	227,934 ± 85,987			6.1	106.21 ± 11.24		
Diet deficient (0.02 ppm)	564,135 ± 128,326	<0.05	99.4	3.5	68.28 ± 14.12	<0.05	35.7
Diet supplemented (2.00 ppm)	151,100 ± 30,107	<0.05	53.9	13.2	234.76 ± 35.35	<0.005	121.0
In vitro Control; no Se	277,501 ± 33,928			7.2	Not measured		
In vitro $1 \times 10^{-7} M$ Se	154,968 ± 10,935	<0.005	44.1	12.9			

[a]Cytotoxic peritoneal exudate lymphocytes generated by allogeneic stimulation with 5×10^6 P815 cells 10 d earlier; 4-h ^{51}Cr-release assay.
[b]Cytotoxic peritoneal exudate lymphocytes (5×10^6) were cocultured with 2×10^6 mitomycin C-treated P815 cells for 4 h; culture supernatants assayed for cytotoxicity on L929 monolayers.
[c]Student's two-tailed *t*-test; compared to normal diet control.
[d]Percent of peritoneal exudate lymphocyte population that was cytotoxic cells; based on the single-hit theory for lymphocyte-mediated cytotoxicity (*14*).

Table 4
Effect of Selenium on Tumor Cytodestruction by C57B1/6J Peritoneal Macrophages and on Production of Tumor Necrosis Factor (TNF)

	Macrophage-mediated cytodestruction of P815 cells					TNF[c] production			IFN-γ[f] production
	In vivo activation[a]			In vitro activation[b]					
Dietary Se content	Cytotoxicity, %	P[d] value	Change, %	Cytotoxicity[c] %	P[d] value	U/mL	P[d] value	Change, %	U/mL
Normal (0.20 ppm)	49.17 ± 2.77			45.29 ± 2.70		339.54 ± 52.67			23.96 ± 0.35
Supplemented (2.00 ppm)	58.16 ± 3.79	<0.05	18.2	46.55 ± 2.00	<0.07	595.70 ± 75.88	<0.01	75.4	23.79 ± 0.37

[a]Immune macrophages elicited by ip injection of 5×10^6 P815 cells 10 d earlier.

[b]Nonimmune macrophages elicited by ip injection of 1.5 mL of 10% proteose peptone 3–4 d earlier; macrophages were incubated with 3 ng/mL lipopolysaccharide W from *E. coli* (LPS) and 1 U/mL recombinant murine γ-interferon for 24 h.

[c]18-h ^{51}Cr-release assay; 10:1 effector: target cell ratio.

[d]Student's two-tailed *t*-test; compared to normal diet.

[e]Immune macrophages cultured for 4 h in serum-free medium supplemented with 1 ng/mL LPS; culture supernatants assayed for cytotoxicity on L929 monolayers.

[f]5×10^6/mL spleen cells stimulated with 2 μg/mL Con A for 48 h; culture supernatants tested for inhibition of cytopathic effects of encephalomyocarditis virus on L929 cells.

sulted in an enhanced (Se supplementation) or delayed (Se deficiency) entry of the cells into the S phase of the cell cycle. Cells capable of expressing higher numbers of p55/p75 earlier, replicated and expanded faster than other cells, which resulted in an increased frequency of cytotoxic lymphocytes within the cell population and in an enhanced tumor cytotoxic response (*14*). A deficiency of Se had the opposite effect.

As demonstrated by the in vitro studies, Se exerted its effect 8–24 h after lymphocyte activation, and the effect was restricted to low concentrations, i.e., 1×10^{-8}–$1 \times 10^{-7}M$, of Se (*14*). Inasmuch as Se alone, without mitogen stimulation, had no effect on the expression of Il_2-R, and since both the α and β subunits m-RNAs are already present in the cytoplasm at peak or close to peak levels, 8 h after lymphocyte activation (*23–28*), Se apparently does not directly affect gene activation. Moreover, because expression of Il_2-R protein at the cell surface reaches its peak 42–72 h after activation (*28*), it is also unlikely that Se affects the aggregation of Il_2-R at the cell surface. Although the data do not elucidate the mechanism(s) involved in the enhancing or inhibitory effects of Se on the expression of Il_2-R, they do suggest that Se may act by binding to and modulating the function of specific cytoplasmic proteins or nucleotides during the period of 8–24 h after stimulation with mitogen or antigen. Since Se also enhances the growth of other cell types, e.g., fibroblasts, epithelial cells, within the same low concentration range (*15*), the mechanism(s) responsible for the effect must be common to the growth regulatory mechanisms of all affected cell types.

On the basis of the presented data, it can be postulated that modulation of cellular immune responses by Se intake may have direct clinical applications. Se is an essential nutritional factor that has been shown experimentally to protect against the development of neoplasms (2), and experimental and epidemiologic data indicate that it may be possible to use Se-containing compounds as cancer chemopreventive agents (*29,30*). The ability of an individual to generate CTL or lymphokine-activated killer cells (LAK) declines with advancing age (*31–33*), and this age-associated reduction in the proliferative potential of T-lymphocytes is linked to an impaired expression of high-affinity Il_2-R (*34*). Consequently, a restoration of the ability to express Il_2-R in the elderly can be of clinical significance, since the incidence of malignant disease is highest in the aging population. The participation of the immune system in cancer prevention and treatment appears to be related, in part, to the ability of a host to generate natural killer cells, CTL, and LAK, which emphasizes the potential of Se as a cancer chemopreventive agent through enhancement of the clonal expansion of immunocompetent cells.

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