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Selenium and Immune Functions in Humans

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Earlier animal experiments have shown that selenium depletion may decrease immune functions. In this human study, 40 volunteers from a population with low serum selenium concentrations were supplemented with selenium or placebo for 11 weeks. Blood samples were drawn at intervals for analysis of selenium status and immune function. At the end of the supplementation period, plasma selenium levels were 74 ng/ml in the placebo group and 169 ng/ml in the supplemented group. The improvement in selenium status was associated with a 57% increase in the activity of platelet glutathione peroxidase in the group supplemented with selenium, but there was no increase in the activity of this enzyme in the placebo-treated subjects. Immune function was measured in vitro by tests of lymphocyte and granulocyte activity. Intracellular killing of *Staphylococcus aureus* by granulocytes was slightly lower in the placebo group than in the selenium group at the end of the supplementation period (77.2 compared to 85.2%; $P < 0.05$). No significant changes were observed in phagocytosis, chemotactic factor generation, antibody or leukocyte migration inhibitory factor production by lymphocytes, or proliferative responses to phytohemagglutinin or concanavalin A. These results suggest that the selenium deficiency of the order found in Finland and some other areas of the world has little, if any, influence on the immune functions measured in this study.

The effects of nutrition on immune function are generally recognized in the context of severe malnutrition or vitamin deficiency. Minerals and trace elements such as iron, zinc, and magnesium may also influence immune responses and host defense mechanisms, as reviewed by Beisel (2). One of the interesting trace elements in this respect is selenium. Selenium is an essential part of the enzyme glutathione peroxidase, an enzyme with a central role in the catabolism of tissue hydrogen peroxide. This enzyme is also involved in arachidonic acid metabolism and may affect prostaglandin and leukotriene synthesis (6).

Leukotrienes are produced from arachidonic acid and are thought to be important mediators of inflammation (9). Prostaglandins affect antibody formation and cellular immunity (reviewed in references 5 and 10). Thus, selenium may influence, at least through prostaglandins, the host defense mechanisms. Indeed, studies have been carried out with selenium-depleted animals which suggest that selenium repletion has favorable effects on antibody synthesis (8, 15, 21, 22; S. A. Mulhern, V. C. Mortis, A. R. Vessey, and O. A. Levander, Fed. Proc. **40**:935, 1981), proliferative response to phytohemagglutinin (13), and intracellular killing of microbes (3, 19). To our knowledge, such information is not available concerning humans.

Dietary intake of selenium varies in various parts of the world. Known low-selenium areas are parts of China, New Zealand, and Finland. The daily intake of selenium in Finland is about 30 μg (12), a low value compared to the recommendation by the U.S. National Research Council of 50 to 200 μ. Also, blood selenium concentrations in Finnish subjects are 30 to 50% of those in North American subjects, whose selenium levels in blood range from 150 to 250 ng/ml (24, 25). A selenium supplementation trial carried out in Finland offered us the opportunity to study the effect of selenium on immune functions in humans.

MATERIALS AND METHODS

The design of the selenium supplementation trial has been described elsewhere (O. A. Levander, G. Alfthan, H. Arvilommi, C. G. Gref, J. K. Huttunen, M. Kataja, P. Koivistoinen, and J. Pikkarainen, Am. J. Clin. Nutr., in press). Briefly, 50 healthy men (36 to 50 years old) were randomly divided into four groups, one of which was a placebo group (20 men). Two other groups (total of 20 men) were given a daily supplement of organic selenium, 200 μg/day per subject, as Se-rich

TABLE 1. Effect of selenium on lymphocyte function tests[a]

Study group	Plasma selenium (ng/ml)	Platelet glutathione peroxidase (mU/mg of protein)	Immunoglobulin concn (ng/ml)[b]			Plaque-forming
			IgG	IgM	IgA	IgG
Placebo	74 ± 9 (19)	254 ± 50 (19)	1,090 ± 703 (17)	5,030 ± 4,430 (17)	517 ± 316 (17)	19,100 ± 8,400 (8)
Selenium supplemented	169 ± 19 (20)	398 ± 75 (20)	2,180 ± 2,390 (14)	5,740 ± 5,190 (14)	887 ± 797 (14)	16,700 ± 9,000 (7)

yeast or toast made of Se-rich wheat flour for 11 weeks. A fourth group (10 men) obtained the same amount of inorganic selenium. For the present report, we used only the results of the placebo group and the organic selenium group, since the inorganic selenium group achieved only intermediate plasma levels of selenium. Blood samples were drawn before the trial and at 2, 4, 7, and 11 weeks after the start. Informed consent was obtained from all participants. The study protocol was accepted by the ethics committee of the National Public Health Institute. All the analyses were performed blind on coded samples. Plasma selenium levels were measured by direct electrothermal atomic absorption spectrometry (1). In this technique, plasma samples are first diluted 1:10 with a solution containing 0.5% nitric acid and 0.25% nickel nitrate. Platelet glutathione peroxidase activity was determined as described elsewhere (Levander et al., in press).

Preparation of lymphocytes and granulocytes. Blood was taken in tubes containing citrate, and platelet-rich and platelet-poor plasma was collected for other purposes (Levander et al., in press). The remaining blood cells were suspended in an equal volume of Hanks balanced salt solution (HBSS) containing 50 U of heparin per ml and layered onto Ficoll-Isopaque. After centrifugation (15 min at 1,000 × *g*), lymphocytes were recovered from the surface of the Ficoll-Isopaque layer. Erythrocytes and granulocytes were taken from the bottom of the tube and resuspended in HBSS containing Dextran T 150, 0.75%. Cells were left to sediment for 1 h at 37°C, and then the layer on top of the erythrocytes was collected and centrifuged. Granulocytes thus obtained were washed twice with HBSS.

Methods of assessing lymphocyte function. Lymphocytes were cultured for 7 days in the presence of pokeweed mitogen (GIBCO Laboratories, Grand Island, N.Y.), and concentrations of immunoglobulin G (IgG), IgM, and IgA in culture medium were determined by enzyme-linked immunosorbent assay (17). Class-specific antisera were coupled to the microtiter plates and similar but alkaline phosphatase-conjugated antisera were used for detection of trapped immunoglobulins. The range of immunoglobulin standard preparations used in this method was 20 to 200 ng/ml. IgG-, IgM-, and IgA-forming cells were determined by reverse plaque assay (11, 17) with protein A-coupled erythrocytes (7). Pokeweed mitogen culture supernatants were also assayed for leukocyte migration inhibitory factor as described previously (18). Briefly, the migration of leukocytes in the presence of supernatants from pokeweed mitogen-stimulated or unstimulated cultures was assayed by the agarose migration method. The migration index equals the area of migration in the presence of pokeweed mitogen culture supernatant divided by the area of migration in the presence of unstimulated supernatant.

The proliferative response of lymphocytes against phytohemagglutinin (Difco Laboratories, Detroit, Mich.; 5, 10, 20, 40, 60, 80, and 160 μg/ml), and concanavalin A (Pharmacia, Uppsala, Sweden; 2.5, 5, 10, 20, and 40 μg/ml) was studied in U-bottomed microculture trays as described by Oppenheimer and Schecter (16).

Granulocyte function tests. The method of Leijh et al. (14) was slightly modified to investigate phagocytosis and intracellular killing of *Staphylococcus aureus* Cowan I (NCTC 8530). Briefly, 0.5 × 10^6 dextran-sedimented leukocytes, mainly granulocytes, were allowed to ingest 0.5 × 10^6 bacteria for 5 min in a 1.1-ml volume of HBSS (pH 7.3) supplemented with 10% AB serum and 0.1% gelatin. The tubes were immediately chilled, and the cells were washed twice with cold HBSS to remove uningested bacteria. A sample of resuspended cells was frozen for subsequent counting of the number of ingested bacteria. The rest of the cell suspension was incubated in a 37°C water bath for 60 min to let the cells kill the ingested bacteria. Then another sample of the cells was frozen for bacterial count. To count the bacteria, the samples were thawed and frozen three times to disrupt the leukocytes, and colony counts were performed on agar plates. Also, May-Grunwald-Giemsa staining was carried out on the cell suspension, and the proportion of mononuclear cells was counted. This information was used to correct the numbers of ingested bacteria expressed as CFU/10^4 granulocytes.

Treatment of neutrophil leukocytes with the calcium ionophore A 23187 has been shown to produce one of the most potent chemotactic factors known: leukotriene B4 (LTB4) or 5:12 dihydroxy eicosatetraenoic acid (9). We used the chemotactic activity of leukotriene B4 for neutrophil leukocytes to assay it. Leukocytes (5 × 10^6 cells per ml) were exposed to ionophore A 23187, 10 μM, at 37°C for 10 min. The cells were spun down, and the supernatant containing LTB4 was immediately frozen in liquid nitrogen and stored at −70°C until the assay was performed. The assay of LTB4-like activity was essentially that described by Wilkinson and Allan (26). Briefly, neutrophil leukocytes were obtained from normal healthy volunteers (United Kingdom residents assumed not to be selenium deficient) by the method of Böyum (4). One milliliter of a suspension of neutrophils (2 × 10^6 cells per ml) was placed in a polypropylene chamber, the

TABLE 1—*Continued*

cells/10^6 viable cells[c]		Lymphokine synthesis (migration index)[d]	Proliferative response (cpm)[e]		
IgM	IgA		PHA	ConA	Unstimulated
14,900 ± 6,900 (8)	11,300 ± 5,900 (8)	0.50 ± 0.12 (16)	57,600 ± 16,900 (14)	34,800 ± 6,800 (14)	810 ± 380 (14)
11,500 ± 10,300 (7)	12,100 ± 9,100 (7)	0.50 ± 0.15 (14)	57,800 ± 9,200 (17)	32,100 ± 5,400 (17)	1,010 ± 740 (17)

[a] Results are expressed as arithmetic means ± standard deviation. Numbers in parentheses represent numbers of individuals. *P* values: plasma selenium, $P < 0.001$; platelet glutathione peroxidase, $P < 0.001$; all other *P* values were not significant.

[b] Measured in the supernatants of pokeweed mitogen-stimulated lymphocyte cultures.

[c] Immunoglobulin-secreting cells in pokeweed mitogen-stimulated lymphocyte cultures.

[d] Leukocyte migration inhibitory factor measured by the agarose migration method.

[e] PHA, Phytohemagglutinin; ConA, concanavalin A.

base of which was a micropore filter with a pore diameter of 3 μm. The chamber was then placed in a well containing a 1:5 dilution of the supernatant from ionophore-treated cells. After a 4-h incubation at 37°C, the chambers were cooled to 4°C, and the number of cells in the well (i.e., the number of cells passed through the filter) was counted with a Coulter Counter. A control supernatant from neutrophils not treated with ionophore was incorporated in each series of assays, and the number of cells passing through this control filter was subtracted from the numbers obtained when LTB4-rich supernatants were used. The assay, as used, is a measure of chemokinetic and chemotactic activity. Results are given as the corrected number of cells passing through the filter.

A *t* test was used to calculate the statistical significance of the differences.

RESULTS

At the beginning of the trial, the plasma selenium values of both groups were 70 ng/ml (data not shown). At the end of 11 weeks of the selenium supplementation period, the plasma selenium levels in the placebo group remained low, whereas in the supplementation group they had more than doubled (Table 1). Associated with the increase in selenium intake, the activity of platelet glutathione peroxidase was 57% higher in the selenium-supplemented subjects than in the controls (Table 1). The difference in the selenium status was not, however, reflected in changes in the tests of antibody formation, lymphokine synthesis, or proliferative responses against the mitogens phytohemagglutinin and concanavalin A (Table 1). The results of the transformation tests in Table 1 are those obtained with the optimal doses of the stimulants. Neither sub- nor supraoptimal doses revealed differences between the two groups. Some of the immune functions were also studied as a function of time. Thus, transformation tests and immunoglobulin assays were performed before and several times during the supplementation period without any indication of selenium-dependent effects (data not shown). The differences in immunoglobulin concentrations between the groups in Table 1 were shown to remain throughout the study and were not due to selenium.

The experiments described above were designed to measure the immune responses of the lymphocytes. To study nonspecific mechanisms of host resistance, we chose to measure phagocytosis and intracellular killing of *S. aureus* by granulocytes. Cells from both groups showed no difference in the ability to ingest bacteria, but killing was decreased by 9.4% in the low-selenium group (Table 2). The difference was statistically significant ($P < 0.05$). Generation of chemotactic activity (LTB4) after stimulation of granulocytes with the ionophore A 23187 was not different in the two groups.

DISCUSSION

The present results suggest that relative deficiency of selenium (as shown by low plasma selenium levels and low activity of platelet glutathione peroxidase) is not associated with changes in immune function but may influence the killing of bacteria by leukocytes in humans. Our results are apparently in disagreement with many of the previous animal studies. Thus, in vivo antibody response against sheep erythrocytes in mice (21–23; Mulhern et al., Fed. Proc.), chickens (15), and dogs (20) and the response against *Plasmodium berghei* vaccine in mice (8) was increased by repletion of selenium and/or vitamin E. In chickens, the effect was influenced by antigen dosage and the age and sex of the responding animals. Larsen and Tollersrud found that the response in pigs to phyto-

TABLE 2. Effect of selenium on granulocyte function tests[a]

Study group	Phagocytosis of *S. aureus* (CFU/10^4 granulocytes)	No. of ingested bacteria viable after 1 h	Killing (% of ingested bacteria)[b]	Leukotriene B4[c]
Placebo	2,870 ± 860 (19)	570 ± 410 (19)	77.2 ± 15.5 (19)	9,690 ± 3,330 (19)
Selenium supplemented	3,220 ± 710 (20)	460 ± 210 (20)	85.2 ± 6.5 (20)	8,610 ± 4,530 (20)

[a] Results are expressed as arithmetic means ± standard deviation. Numbers in parentheses represent numbers of individuals. $P < 0.05$ for killing; all other P values were not significant.

[b] $\frac{\text{Ingested bacteria} - \text{ingested bacteria viable after 1 h}}{\text{ingested bacteria}} \times 100.$

[c] Number of cells that migrated through the filter in the chemotaxis assay.

hemagglutinin in transformation tests was increased by selenium or vitamin E (13). The animals in these studies, however, were severely depleted of selenium. It may well be that the selenium status of the individuals in our study was sufficiently high to maintain normal functions in the tests used. Another difference is that, for obvious reasons, we used in vitro tests of immune function and mitogen stimulation, in contrast to many of the animal studies, in which the in vivo response to antigenic challenge was investigated. Furthermore, our subjects had a normal vitamin E status, and this may have counteracted the effects of selenium deficiency.

The less effective killing of bacteria by leukocytes obtained from individuals with low selenium intake is in agreement with studies in experimental animals. Boyne and Arthur (3) and Serfass and Ganther (19) found normal phagocytosis but decreased killing of yeast cells by rat or cattle leukocytes. It should be noted, however, that the difference between selenium-deficient animals and control animals was much larger than that observed in this study. On the other hand, the intake of selenium in these studies was extremely low, in contrast to the relative deficiency observed in the participants of the present investigation. If confirmed, the impaired killing of bacteria by granulocytes may have some bearing on the host resistance against microbial infections in people living in the low-selenium areas of the world. The mechanism by which selenium deficiency might bring about this change is still largely unknown. It has been suggested that glutathione peroxidase, by its hydroperoxide-destroying activity, may protect cytosolic components and membranes from the peroxides diffusing from the phagolysosomes (19).

It has recently been reported that glutathione peroxidase depletion modifies in vitro the lipoxygenase pathway leading to the synthesis of leukotrienes (6). No change was observed in this study in the amount of leukotriene B4 released by granulocytes after selenium supplementation. This suggests that the changes in glutathione peroxidase of the order seen in this study do not influence the leukotriene production in granulocytes in humans.

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