Chapter 53

Immunotoxic Effects of Selenium in Mammals

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Introduction

Although naturally occurring selenosis was recorded by workers at the Agricultural Experiment Stations of South Dakota and Wyoming nearly 70yrs ago (Franke *et al.*, 1934), the recent discovery that human activities such as irrigation or strip mining may mobilize sufficient selenium (Se) to cause toxicity in water-fowl has rekindled interest in spontaneous selenosis. There are very few reports attesting to the immunotoxic effects of Se in mammals (Koller *et al.*, 1986; Larsen, 1988), and none in cattle. There was circumstantial evidence of a likely association between excess dietary Se and immunosuppression in cattle in field studies between 1988-1991. This chapter summarizes a series of trials to explore this association.

In vitro Immunocyte Response to Selenium

Peripheral blood lymphocytes (PBL) from healthy cattle were the basis of a series of experiments utilizing *in vitro* exposure of immunocytes to various forms of Se. Mononuclear cells were harvested and cultured as described by Belden *et al.* (1981). Final Se concentrations in the media were Na_2SeO_3: 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1, 3, or 5ppm Se; selenocystine: 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1, 3, or 5ppm Se; and L-selenomethionine (SEMET): 0.125, 0.25, 0.5, 0.75, 1, 3, or 5ppm Se. Cells in untreated culture media served as controls for each trial. Selenium inhibition thresholds were defined as the concentration of added Se that resulted in greater than 50% inhibition of the function being measured.

Blastogenesis assays were performed as reported (Belden and Strelkauskaus, 1981; Schamber, 1994). Results were expressed as the difference between mitogen stimulated and unstimulated counts. Production and bioassay of interleukin 2 (IL-2)

by PBL were as previously reported (Zelarney and Belden, 1988).

B-cell function was evaluated by measuring the ability of pokeweed mitogen (PWM) stimulated lymphocytes to produce antibody with an enzyme-linked antiglobulin (ELISPOT) assay. Cells were cultured with PWM for 96hrs and plated on antiglobulin treated microtiter plates. After antiglobulin addition and substrate development, the ratio of chromogen positive spots in stimulated and unstimulated wells was expressed as a stimulation index. Interleukin 4-like (IL-4) activity in the supernatant of Concanavalin A (ConA) and Phytohemaglutinin (PHA) stimulated PBL's was also evaluated using the ELISPOT assay.

All three forms of Se produced an obvious dose-related inhibition of both ConA and PHA-induced lymphocyte blastogenesis (Fig. 53.1). The median inhibitory concentrations for Na_2SeO_3 and selenocystine (SECYS) were less than blood or plasma concentrations commonly associated with bovine selenosis; however, that for SEMET was somewhat greater. There was no difference in inhibition between forms of Se at the highest and lowest concentrations. At intermediate concentrations, SEMET was less inhibitory than either Na_2SeO_3 or SECYS, which were approximately equipotent (Table 53.1). This ranking is consistent with the cytotoxic potency observed in other *in vitro* experimental systems (Spallholz, 1994) and probably reflects nonspecific cell death rather than a specific immunotoxic response. These results contrast with reports indicating that SEMET is more immunotoxic than Na_2SeO_3 *in vivo* (Larsen, 1988; Fairbrother and Fowles, 1990). This may be due to the tendency of SEMET to accumulate to higher tissue levels *in vivo*,

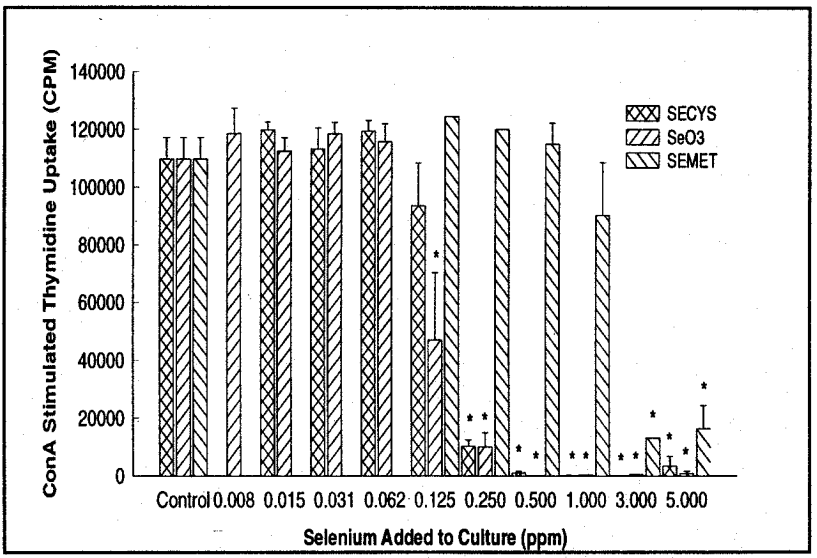


Fig. 53.1. Concanavalin A stimulated blastogenesis in peripheral blood lymphocytes cultured in selenium. Each bar=increased ^{3}H-thymidine uptake as a result of mitogen stimulation. *=significant decrease from control values.

Table 53.1. Selenium inhibition thresholds (ppm, greater than 50% inhibition) in various *in vitro* bioassays.

Form	Blastogenesis	Elispot	Cytokine production
Selenomethionine	3.0	3.0	None
Selenocystine	0.25	0.125	0.5
Selenite	0.125	0.25	0.25

resulting in a larger pool of Se for local conversion to some as yet unidentified proximate toxicant.

In vivo Studies

Mice

Sixteen 5wk-old BALB/C mice were assigned to one of four treatment groups (control, SEMET, SECYS or Na_2SeO_3) and housed by treatment group in plastic cages with rodent chow and deionized water *ad libitum.* Experimental diets were rodent chow plus water to which sufficient of each of the aforementioned forms of Se was added to achieve 7ppm Se. After 14d on Se-treated water each mouse received a single subcutaneous (s.c.) injection of 2mg hen egg albumin (OVA).

After 47d, serum for antibody analysis was collected from each mouse *via* the infraorbital sinus, after which all were euthanized by cervical dislocation. After the spleens were removed aseptically, splenocytes were harvested by mechanical teasing and centrifugation in Hank's balanced salt solution and diluted to $2x10^6$ viable cells/ml in Roswell Park Memorial Institute media (RPMI-1640) +10% fetal bovine serum. Blastogenesis assays were performed as described above for bovine PBLs using ConA, PHA and lipopolysaccharide (LPS) as mitogens. A separate plate was prepared for blastogenesis using OVA as an antigen. B-cell function was assessed in splenocytes using the ELISPOT assay described above. Total Ig concentrations were determined in sera with an antigen trap ELISA, and were calculated by comparison with standards. An indirect ELISA was used to determine OVA specific antibody.

There were few statistically significant differences in mitogen-stimulated blastogenesis between treatment group means. Proliferative response to ConA reflected a slight but non-significant decline from controls for each of the three forms of Se. Immunotoxic potency was $SeO_3^=$ >SECYS >SEMET. B-cell function, as measured by ELISPOT, was significantly decreased from controls by SEMET and SECYS (Fig. 53.2). Total Ig concentration did not vary between treatment groups. Interestingly, there were no differences between Se-treated and control groups in

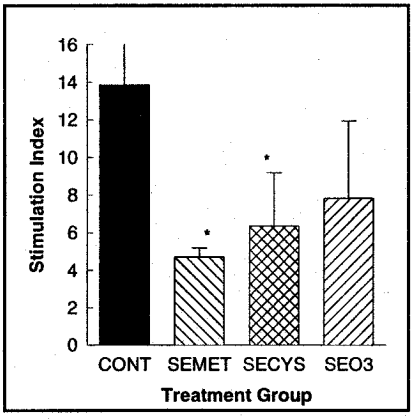


Fig. 53.2. B-cell function in splenocytes from mice fed three forms of selenium for 47d. * denotes less than control ($P<0.05$). Each bar depicts mean±sd ratio of PWM-stimulated to unstimulated splenocytes.

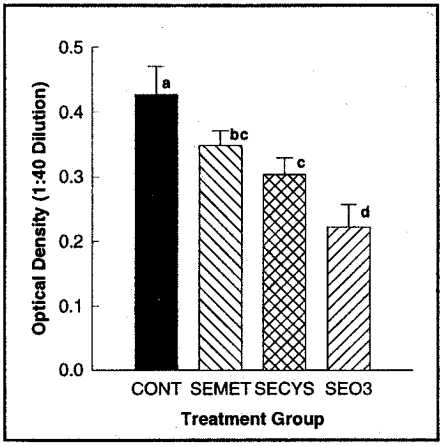


Fig. 53.3. Primary antibody response to OVA in mice treated with 7ppm Se in drinking water for 47d. Superscripts denote statistical similarity (ANOVA, $P<0.05$). Each bar represents mean±sd.

OVA-stimulated blastogenesis, but all Se-treated groups had significantly lower OVA-specific antibody concentrations than did controls (Fig. 53.3).

Cattle

Twenty yearling Hereford-cross steers were housed in concrete-floored pens equipped with heated waterers and electronically gated feed bunks keyed to individual steers. After a 45d acclimation period, steers were divided into one control and six experimental groups. Each experimental steer received Se at 0.15, 0.28 or 0.8 Se mg/kg as either Na_2SeO_3 or SEMET daily. Doses were prepared each day by absorbing aqueous Se solution into 500g ground corn cob and carefully hand mixing with the amount of chopped hay each steer would consume in 18hrs.

After 42d on Se, each steer received a single subcutaneous (s.c.) 40mg dose of OVA. Blood was collected at 21, 42, and 63d post-immunization. Lymphocyte blastogenesis, B-cell function, total Ig and OVA-specific antibody were evaluated as above with proper species-specific reagents. In an effort to duplicate Se content of the cellular microenvironment *in vitro*, the Se (10% of *in vivo* blood levels) form being fed to each animal was added to an another set of cultured cells at 120d.

No significant differences existed between responses to T-cell mitogens, B-cell stimulation, IL-2 or IL4-like activity at any time (data not shown). Addition of SEMET to *in vitro* PBL cultures resulted in no change from the control group or the cultures without added Se. Addition of Na_2SeO_3 to PBL cultures from Na_2SeO_3-treated steers inhibited mitogen-stimulated blastogenesis (Fig. 53.4). Addition of

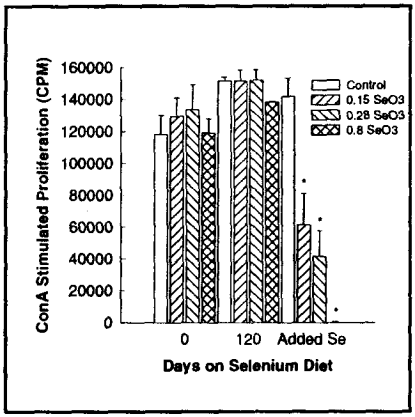


Fig. 53.4. ConA stimulated blastogenesis in bovine PBL from steers on seleniferous diets. PBLs collected at 120d were assayed with and without added Se equivalent to 10% blood Se concentration. * denotes less than untreated ($P<0.05$).

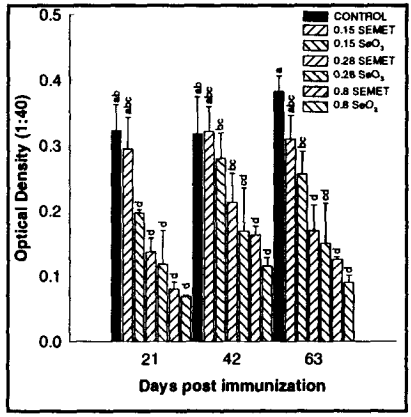


Fig. 53.5. Primary antibody response to OVA in steers fed seleniferous diets. Similar superscripts denote statistical similarity (ANOVA, $P<0.05$). Each bar depicts group mean±sd.

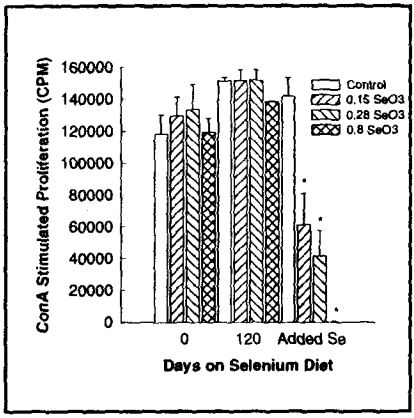
0.27ppm Se (high dose group) resulted in cytotoxicity like that seen *in vitro* (Table 53.1). In the groups that received Na_2SeO_3 at 0.15 and 0.28mg/kg much less Se than the *in vitro* toxic threshold, proliferation was inhibited (Fig. 53.4).

Mean total Ig concentrations did not vary between treatment groups (data not shown). Primary antibody response to OVA exhibited a dose-related inhibition, with larger Se doses resulting in lower OVA specific antibody concentrations (Fig. 53.5). Greater inhibition of antibody formation resulted from Na_2SeO_3 than SEMET.

Antelope

Eight captive-raised pronghorn antelope (*Antelocapra americana*), randomly assigned to two groups, were housed in concrete-floored pens with heated waterers. The control group received a ration (0.3-0.5ppm Se) of native grass hay and alfalfa. The experimental group received a ration (13-16ppm Se) prepared similarly from seleniferous grass (Raisbeck *et al.*, 1996). Both diets were fed free choice for 167d.

After 28d on Se, each antelope received a s.c. injection of 40mg OVA. A battery of tests similar to that used previously with the steers was applied to blood taken at 3wk intervals after immunization. There were no significant differences between treatment groups in PBL response to T-cell mitogens, B-cell function, or total Ig concentration. Primary antibody response to OVA, was less in the Se-fed group than controls at all sampling intervals after immunization (data not shown).



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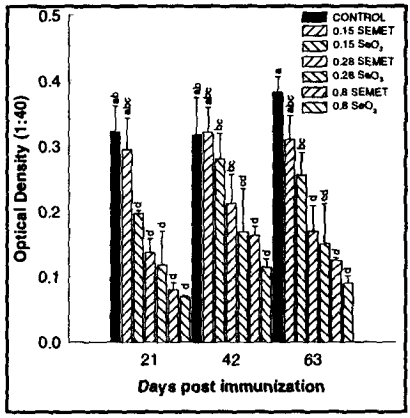

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Summary

Exposure to dietary Se at concentrations that did not result in clinical selenosis compromised immune function in rodents, cattle and antelope. This effect was manifested as decreased primary antibody response *in vivo* to antigen administered while the animal was on a seleniferous diet. With the possible exception of ConA-stimulated blastogenesis in Na_2SeO_3-treated mice, there were no significant Se-related effects in any assay that relied on *in vitro* manipulation of immunocytes. These results are in contrast to Larsen (1988), who reported that lymphocytes from sheep fed 1.3ppm SEMET exhibited decreased ConA-stimulated blastogenesis, but lymphocytes from sheep fed Na_2SeO_3 did not.

Inhibition of various immunocyte functions by Se compounds *in vitro* corresponded with overt cytotoxicity and cell death. Cell death does not explain the depressed *in vivo* primary antibody response in these experiments, as the latter occurred in Se-treated animals despite normal circulating lymphocyte counts. Interestingly, Se concentrations that inhibited various functions in Na_2SeO_3- or SECYS-treated PBL cultures were within the range of plasma concentrations commonly accepted as "adequate," but the SEMET concentration required was an order of magnitude higher. This difference in potency is consistent with the relative cytotoxic potential of these compounds using other *in vitro* endpoints such as chemiluminescence (Spallholz, 1994).

These observations re-emphasize the artificial nature of *in vitro* bioassays. It is unlikely that the majority of blood Se exists in either of these forms *in vivo*. In all probability the proximate toxicants in selenosis are highly reactive moieties with relatively short half-lives (Burk, 1991). Without better data on Se toxicokinetics and metabolism at toxic doses it is impossible to model Se immunotoxicity realistically *in vitro*.

In vitro tests on PBLs from Se-exposed animals in these trials further support this conclusion. These assays were essentially negative despite the fact that PBLs being assayed came from animals in which primary antibody response was inhibited. Finch and Turner (1989) reported that lymphocytes from Se-deficient lambs regained normal function during the course of a typical blastogenesis assay if exposed to adequate Se in culture media. Cells from Se-intoxicated animals should also regain normal function by equilibrating with normal-Se media.

In the intact animal, Se somehow interferes with the cellular events responsible for an immune response. Elevated Se has been shown to promote peroxidative damage in *in vitro* and *in vivo* systems (Spallholz, 1994; Bjornstedt *et al.*, 1996). Lymphocyte cell membranes are especially susceptible to free radical damage by virtue of their relatively high unsaturated fatty acid content. Selenium-induced free radicals could also increase the oxidation potential (pE) of the immediate cellular microenvironment or the pE of the cytosol, inhibiting these functions by a similar mechanism. Removal to a normal-Se environment, i.e. a typical *in vitro* bioassay, would decrease oxidant stress and thus permit recovery of apparently normal lymphocyte function.

Obviously, none of these questions will be answered until there is an understanding of the forms of Se in the cellular microenvironment and how the *in vivo* redox environment influences lymphocyte function and immunity. This will necessarily require additional *in vivo* and *in vitro* examination of Se immunotoxicity.

References

Belden, E.L. and Strelkauskas, A.J. (1981) Mitogen and mixed lymphocyte culture responses of isolated bovine lymphocyte subpopulations. *American Journal of Veterinary Research* 42, 934-937.

Belden, E.L., McCrosky, J.K. and Strelkauskas, A.J. (1981) Subpopulations of bovine lymphocytes separated by rosetting techniques. *Veterinary Immunology and Immunopathology* 2, 267-270.

Bjornstedt, M., Odlander, B., Kuprin, S., Claesson, H. and Holmgren, A. (1996) Selenite incubated with NADPH and mammalian thioredoxin reductase yields selenide, which inhibits lipoxygenase and changes the electron spin resonance spectrum of the a active site iron. *Biochemistry* 35, 8511-8517.

Burk, R.F. (1991) Molecular biology of selenium with implications for its metabolism. *FASEB Journal* 5, 2274-2279.

Fairbrother, A. and Fowles, J. (1990) Subchronic effects of sodium selenite and selenomethionine on several immune functions in mallards. *Archives of Environmental Contamination and Toxicology* 19, 836-844.

Finch, J.M and Turner, R.J. (1989) Enhancement of ovine lymphocyte responses: A comparison of selenium and vitamin E supplementation. *Veterinary Immunology and Immunopathology* 23, 245-256.

Franke, K.W., Rice, T.D., Johnson, A.G. and Schoening, H.W. (1934) *Report on a Preliminary Field Survey of the So-Called "Alkali Disease" of Lvestock.* US Department of Agriculture Circular No. 320, pp. 1-10.

Koller, L.D., Exon, J.H., Talcott, P.T., Osborne, C.A. and Henningsen, G.M. (1986) Immune responses in rats supplemented with selenium. *Clinical and Experimental Immunology* 63, 570-576.

Larsen, H.J. (1988) Effect of selenium on sheep lymphocyte responses to mitogens. *Research in Veterinary Science* 45, 11-15.

Raisbeck, M.F., O'Toole, D., Schamber, R.A., Belden, E.L. and Robinson, L.J. (1996) Toxicologic effects of a high-selenium hay diet in captive adult and yearling pronghorn antelope (*Antilocapra americana*). *Journal of Wildlife Diseases* 32, 9-16.

Schamber, R.A. (1994) *Selenium Immunotoxicity.* MS Thesis, University of Wyoming.

Spallholz, J.E. (1994) On the nature of selenium toxicity and carcinostatic activity. *Free Radical Biology and Medicine* 17, 45-64.

Zelarney, P.T. and Belden, E.L. (1988) Bovine interleukin 2: Production by an E-rosette-defined lymphocyte subpopulation. *Veterinary Immunology and Immunopathology* 18, 297-305.