SELENIUM INTAKE AFFECTS mRNA LEVELS FOR KEY MEDIATORS OF CD4$^+$ T CELL ACTIVATION

Levels of selenium (Se) intake have been shown to modulate T cell responses in both humans and animal models. The mechanisms by which Se levels influence these responses remain unclear. The goal of the study was to evaluate the affect of dietary Se levels on the activation of CD4$^+$ T cell activation. C57BL/6 mice were fed diets containing low (0.08 ppm), medium (0.25 ppm) or high (1.0 ppm) levels of Se. After eight weeks on these diets, splenic CD4$^+$ T cells were isolated and stimulated through the T cell receptor using plate-bound anti-CD3/CD28. Activation of the CD4$^+$ T cells was evaluated by measuring mRNA levels for four co-stimulatory molecules (CD40L, OX40, FoxP3, and RANKL) as well as four cytokines (IL2, IL4, IL10, and IFNγ) after 3 h, 8 h, and 12 h of stimulation.

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BACKGROUND

Selenium (Se) is an important essential trace element that is vital for human health. Se is incorporated into the body as proteins, known as selenoproteins through selenocystine (the 21st amino acid). There are many health benefits from Se including the prevention of certain forms of cancer, heart disease and other disorders. Se supplements are increasingly used as potent nutritional antioxidants. Se also helps to rid the body of reactive oxidative species (ROS) that the immune system creates in order to cause inflammation and destroy invaders. Se affects ROS through a selenoprotein known as glutathione peroxidase enzymes or GPX. This is just one of 25 selenoproteins known to be in humans. Also, it has an indispensable function in development and the immune system. Selenium is incorporated into immune-important organs such as the liver, spleen, and lymph nodes.[1] Se is also incorporated into many other cell types, which makes it not surprising that Se affects immune responses as well as tissues.

CD4$^+$ T cells, T-helper cells, are an important part of humoral and cell-mediated immunity. Se supplementation may also enhance these immunities. A T cell will proliferate and differentiate when its T cell receptor recognizes the major histocompatability complex, an antigen associated with cell-membrane proteins. In this way, the naïve T cell becomes various effector T cells that eliminate foreign microorganisms.[2] Effector T cells send out cytokines, various growth factors which help in the activation of other T cells, B cells, macrophages, and other cells that participate in an immune response. The different cytokines produced also changes the type of immune reaction created. There currently exists a lack of information regarding the effect of Se on activated CD4$^+$ T cells, although it is already known that Se affects immune responses.

METHODS

The primary naïve CD4$^+$ T cells were taken and isolated from the spleens of C57BL/6 mice that had been on diets of deficient (0.08 ppm), adequate (0.25 ppm) or supplemented (1.0 ppm) selenium for eight weeks. CD4$^+$ T cells were isolated using the isolation kit and protocol (from MACS). The cells isolated were then preserved as pellets in an -80° freezer.

The frozen cell pellets were thawed then plated into wells (96-well plate) that had been coated by anti-CD3/CD28 plate-bound antibody to activate the cells through the T cell receptor. The cells were kept on the plates for 0, 3, 8, and 12 hours for a time course analysis. Their RNA was then extracted using the RNeasy Mini kit and the RNase-free DNase I (from Qiagen). The RNA concentration was then measured on the ND1000 Spectrophotometer (NanoDrop Technologies). Synthesis of cDNA was made by using Superscript III (from Invitrogen) and oligo dT primer, with 2 g of RNA per 50:l reaction. Real-time PCR (RT-PCR) was then carried out using 1:l of cDNA per 10:l of reactions with SYBR Green qPCR SuperMix-UDG (from

Table 1. Oligonucleotide primers used for real-time PCR

Name of Target Sequence	Gen Bank Numbers			PCR Product length
Ubc	NM_019639.3	fwd	GAGGGCATCTCCCCTGAC	60
		rev	GCCATCTTCCAGCTGCTT	
CD40L	NM_011616	fwd	ACGTTGTAAGCGAAGCCAAC	61
		rev	TATCCTTTCTTGGCCCACTG	
Foxp3	NM_054039	fwd	AGAAGCTGGGAGCTATGCAG	100
		rev	GCTACGATGCAGCAAGAGC	
RANKL	NM_011613	fwd	TGAAGACACACTACCTGACTCCTG	83
		rev	CCACAATGTGTTGCAGTTCC	
OX40	NM_011659	fwd	AGGACAGCGGCTACAAGC	120
		rev	GGGTCTGCTTTCCAGATAAGGT	
IL-2	NM_008366	fwd	GCTGTTGATGGACCTACAGGA	114
		rev	TTCAATTCTGTGGCCTGCTT	
IL-4	NM_021283	fwd	Proprietary oligos from Superarray, Inc.	
		rev		
IL-10	NM_010548	fwd	Proprietary oligos from Superarray, Inc.	
		rev		
INFγ	NM_008337	fwd	Proprietary oligos from Superarray, Inc.	
		rev		

Invitrogen). The RT-PCR machine used was the LightCycler 2.0 thermal cycler (by Roche Applied Biosystems). The oligonucleotides that were used are shown in Table 1. The conditions for cycling were used as said in the SYBR Green kit instructions and the results were analyzed using Relative Quantification Software (from Roche).

RESULTS

Results demonstrated that CD40L was significantly increased with increased Se uptake. In contrast, differentiation markers including OX40, FoxP3, and RANKL were not significantly affected by Se levels. The mRNA levels for the four cytokines were differentially affected by Se levels. First, IL-2 mRNA was increased for both medium and high Se groups, but remained increased longer for the high Se group. IL-4 mRNA levels were highest for the low Se group, while IL-10 showed no differences between dietary Se groups. Finally, IFNγ mRNA increased with high Se intake, but only at the latest time-point (12 h).

CONCLUSION

Both CD40L and IL2 mRNA levels were most affected by Se intake, with high Se diet resulting in the highest abundance of both mRNAs. CD40L is crucial for T cell activation and differentiation and the affect of Se levels on mRNA for this factor were evident in early periods following T cell receptor stimulation. It has been shown that IL-2 and CD40L are dependent on early increase in calcium; therefore calcium mobilization may be an important mechanism by which Se status in T helper cells affects their activation and differentiation. Future studies will attempt to elucidate the influence of Se levels on calcium mobilization and identify individual selenoproteins that may mediate these affects.

Overall, elucidating specific molecules through which Se modulates CD4 + T cells will allow better use of this potent dietary antioxidant in boosting immune responses.

REFERENCES

1. Hatfield DL, Berry MJ, Gladyshev VN. *Selenium: Its Molecular Biology and Role in Human Health.* 2nd ed. New York, NY: Springer; 2006.
2. Kuby J. *Immunology.* 3rd ed. New York, NY: W. H. Freeman and Company; 1997.