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Accelerated Article

Selenium and Cellular Immunity

Evidence That Selenoproteins May Be Encoded in the +1 Reading Frame Overlapping the Human CD4, CD8, and HLA-DR Genes

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ABSTRACT

Selenium deficiency can lead to impaired immune function and reduced T-cell counts, as well as various specific disorders. Significantly, in ARC and AIDS patients, a progressive decline in plasma Se, paralleling T-cell loss, has been widely documented. Since evidence now suggests that there is an extremely high turnover of CD4+ T-cells in AIDS patients, with billions of new cells lost and replaced daily, any exceptional requirement for Se in lymphocytes could contribute to this progressive Se depletion. Thus, it may be significant that, overlapping the known genes in the +1 reading frame, the mRNAs of several T-cell associated genes (CD4, CD8, HLA-DR p33) have open reading frames (ORFs) with as many as 10 in-frame UGA codons (CD4, p33), a clustering that is highly improbable by chance alone, and reminiscent of selenoprotein P, the predominant plasma form of Se. The presence of these ORFs, along with potential stem-loop RNA structures displaying consensus selenocysteine insertion sequences, $AUG(N)_mAAA(N)_nUGR$, suggests that these mRNAs may encode selenoproteins, in addition to the known T-cell glycoproteins. If so, the roles of Se in the immune system may be more diverse than previously suspected.

Index Entries: Selenium deficiency; cellular immunity; selenoproteins; T-cell associated genes.

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Although the mechanisms involved have yet to be fully elucidated, it is well established that dietary selenium is important for a healthy immune response. The effects of selenium deficiency can include reduced T-cell counts, as well as impaired lymphocyte proliferation and responsiveness *(1–7)*. These immunological effects are in addition to various specific disorders that have been associated with Se deficiency *(8)*. Given this importance of Se for cellular immunity in particular, it is of considerable interest that a progressive decline in plasma Se has been widely documented in ARC and AIDS patients *(9–17)*. This decline approximately parallels T-cell loss or stage of HIV infection, but seems to be particularly noticeable in the terminal stages of AIDS, where Se deficiency is one of the hallmarks of the disease.

These observations are all the more intriguing in the light of recent evidence suggesting an extremely high turnover of CD4 + T-cells in AIDS patients *(18,19)*, with billions of new cells lost and replaced daily. With such a rate of T-cell loss, and the constant formation of new cells to replace them, it is possible that any exceptional requirement for Se in lymphocytes might contribute to the documented progressive Se depletion, unless Se recycling mechanisms were 100% efficient, which is unlikely.

The clinical evidence for a progressive depletion of Se and the selenoprotein glutathione peroxidase (GPx) in the plasma and tissues of AIDS patients was recently reviewed by Dworkin *(16)*. He points out that "selenium deficiency may be associated with myopathy, cardiomyopathy and immune dysfunction including oral candidiasis, impaired phagocytic function and decreased CD4 T cells" *(16)*. Significantly, most of those are characteristic symptoms of AIDS. Dworkin also reports the results of his clinical studies showing that the reduced plasma Se and GPx levels in ARC and AIDS patients are "significantly correlated with total lymphocyte counts" but that this appears to be "irrespective of the presence or absence of diarrhea or gastrointestinal malabsorption" *(16)*. This suggests that the decline in Se levels parallels the progression of HIV disease (decline in T-cell levels) in a way that cannot be entirely ascribed to nutrient malabsorption. Such observations challenge the common assumption that the decline in plasma Se is exclusively owing to malabsorption, and thus merely a consequence of the wasting syndrome characteristic of AIDS.

Over half of the Se in plasma is in the form of selenoprotein P; its mRNA has 10 UGA selenocysteine (SeC) codons, mostly concentrated in the C-terminal 125 amino acids *(20)*. It has been suggested to serve as an antioxidant and possibly also as a Se transport/storage protein, but it is known to attach to various cell types via a specific receptor, and may also have other functions. Only a few mammalian selenoproteins have well understood roles *(8)*; these include several forms of glutathione peroxidase (GPx) and the type I 5'-iodothyronine deiodinase involved in conversion of T4 thyroid hormone to T3. Thus, Se is critical in humans for the maintenance of glutathione-dependent antioxidant status, and thy-

roid T3 hormone levels, both of which appear to be impaired frequently in AIDS patients (reviewed in ref. *21*).

Whatever its root causes, to rectify the apparent Se deficit in AIDS, Se supplementation therapy has been recommended repeatedly by those aware of these important biochemical functions of Se. In several very brief studies, Se supplementation has been reported to lead to some symptomatic improvements in HIV patients *(12,14,17)*. It was also demonstrated recently that, in cell culture, Se supplementation inhibits both the activation of HIV-1 by oxidative stress, and the activation of NF-κB, an important cellular transactivator of HIV-1 *(22)*. By providing in vitro evidence of an antiviral effect, these observations substantiate previous claims that Se supplementation can be beneficial in slowing the progression of HIV disease *(17)*.

In order to elucidate fully the molecular basis of the immunopotentiating actions of Se, and thus any possible role for Se in the pathogenesis AIDS, certain fundamental questions must be answered. Perhaps foremost among these is the question of whether the role of Se in leukocytes is limited to its antioxidant function in GPx. In this communication, I present evidence to the contrary. An examination of the CD4 and CD8-β mRNAs revealed that, in the +1 frame overlapping with the main ORF, there are ORFs with start codons and multiple in-frame UGA codons (10 in CD4, 8 in CD8), potentially encoding selenoproteins of 123 and 226 amino acids, respectively. In the mRNA of the invariant chain (p33) of the evolutionarily related MHC Class II molecule HLA-DR, a similar ORF was found in the +1 frame, also with 10 in-frame UGA codons. It must be noted that such a clustering of UGA codons purely by chance is in itself rather improbable. In a "blocked" reading frame overlapping a real gene, on average there should be an essentially random distribution of all three stop codons; so, beginning at an arbitrary position (e.g., at a start codon in the "blocked" reading frame), the probability of finding eight consecutive in-frame UGA codons with no intervening UAG or UAA codons is $(1/3)^8$, or $p < 2 \times 10^{-4}$. For 10 UGA codons, as observed in ORFs in both CD4 and HLA-DR, it is $(1/3)^{10}$, which is $p < 2 \times 10^{-5}$. Significantly, and consistent with the possibility that they encode real proteins, all three of these UGA-rich ORFs have a start codon upstream of the first UGA codon.

Moreover, as shown in Fig. 1, the hypothetical CD4 selenoprotein (CD4-SP) has a highly significant similarity to a Cys-rich region of the sperm mitochondrial capsule selenoprotein, which was the second highest ranked match to CD4-SP in a FastA search of tens of thousands of protein sequences in the PIR 42 database (significance = 7.0 SD in initial scores). There are also several regions of local similarity (e.g., the sequence SCCCXC, C = Cys, or SeC), and 25% identity overall, to the SeC-rich C-terminal of the human selenoprotein P, which, like the CD4-SP ORF, also has 10 UGA codons.

In eukaryotes, efficient selenocysteine incorporation at UGA codons depends on the presence of a cellular protein factor (equivalent to the

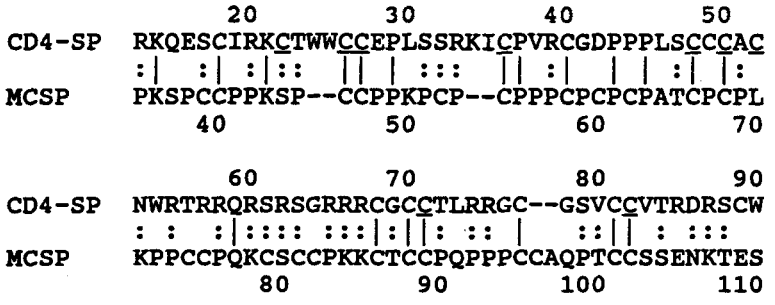

Fig. 1. Sequence similarity (26% identity in a 77 residue overlap) between the central portion of the 123 residue hypothetical CD4 selenoprotein (CD4-SP) and a Cys-rich portion of the mouse mitochondrial capsule selenoprotein (MCSP), which has a total of 197 residues. Potential SeC residues encoded by UGA codons are shown as C. This is the unmodified alignment produced by using CD4-SP as a probe to search the PIR 42 (Protein Identification Resources Data Bank) sequence database using the FastA program, as implemented in the GCG program suite (Program Manual for the Wisconsin Package, Ver. 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, WI 53711). The PAM 250 matrix and other default parameters were used, except the word size was set to 1 for greater sensitivity, and the /OPT parameter was used. This was the second highest ranked hit (significance = 7.0 SD) out of tens of thousands of protein sequences in the database. Note that UGA encodes Trp in mitochondrial DNA, so MCSP, a selenoprotein, is not mitochondrially encoded.

bacterial *selB* protein) and a structural signal in the mRNA 3′-untranslated region (UTR), consisting of a selenocysteine insertion sequence (SECIS) in a characteristic stem-loop structure *(23,24)*. A possibly related stem-loop structure is required in bacteria, but must immediately follow the UGA codon *(25)*. Berry et al. have shown that a SECIS can also function when placed in a 5′-UTR, or even in *trans*, when cotransfected on a separate plasmid; however, the efficiency of SeC insertion is highest when the SECIS is in a downstream 3′-UTR location *(23)*. These results suggest that in-frame UGA codons can be translated as SeC with reasonable efficiency as long as there is an upstream or downstream SECIS in the mRNA. The potential SECIS associated with the UGA-containing ORFs of CD4, CD8, and the p33 invariant chain of HLA-DR (Fig. 2) are either in the coding regions (CD4, CD8) or in a 3′-UTR relative to the ORF encoding the hypothetical selenoprotein (p33). It must also be noted that there is an additional 1.2 kb of the human CD4 3′-UTR that has never been sequenced, and it is quite possible that one or more additional SECIS may be found in that region.

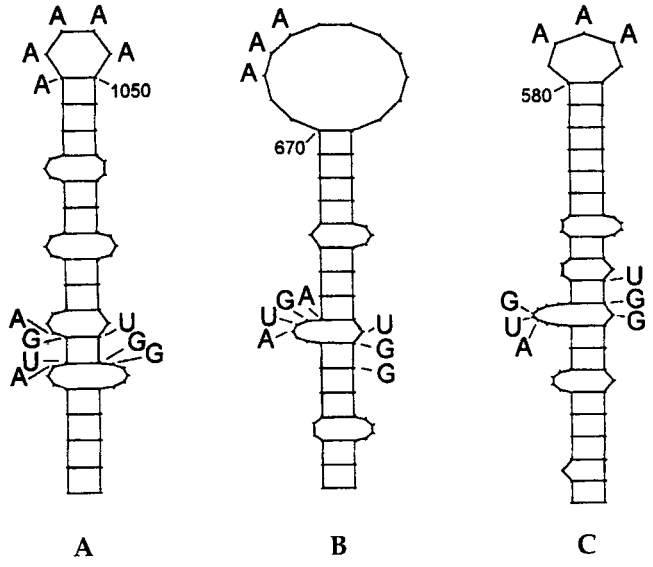

Fig. 2. Potential stem-loop structures displaying consensus selenocysteine insertion sequences (SECIS elements) *(23,24)* found in the following mRNAs: **A.** CD4, bases 1017–1077 in GenBank #m35160; **B.** CD8, bases 647–706 in GenBank #m36712; **C.** HLA-DR invariant chain (p33), bases 544–662 in GenBank #m14765. The potential secondary structures of RNA regions containing the SECIS consensus features (AUG ... AAA ...UGR) were predicted using the FOLD program *(37)* with updated energy parameters as implemented in the GCG software package.

These potential stem-loop RNA structures (Fig. 2) have all the essential features determined from a comparison of known SECIS elements previously identified in mammalian selenoprotein genes: an AUG unpaired or partially paired in a bulge on the 5′ arm of the stem, the unpaired adenine triad on the loop, and UGA or UGG in a bulge on the 3′ arm of the stem *(24)*. These structures also have a near-ideal distance (10–12 bp) between the adenine loop and the AUG/UGR consensus bulge regions; the 9-bp distance in the CD8 SECIS is compensated by a larger adenine loop.

In all three cases, the start codon for the hypothetical selenoprotein is downstream from that of the "main" ORF encoding the known gene. Thus, selenoprotein synthesis could only be initiated on the relatively rare occasions when ribosomal scanning missed the first Met codon, an event which does happen and indeed has to happen for many viral genes to be expressed in mammalian cells. This inefficient initiation would in fact be desirable in this case, since the ability of cells to synthesize selenoproteins is inherently limited owing to the low abundance of $tRNA^{Sec}$.

Table 1
Translation of UGA-Rich Open Reading Frames, +1 to the Main Coding Region, in CD4, CD8 beta, and HLA-DR p33

CD4

```
  1  MLALETSPWP LKRKQESCIR KCTWWCCEPL SSRKICPVRC GDPPPLSCCC
 51  ACNWRTRRQR SRSGRRRCGC CTLRRGCGSV CCVTRDRSCW NPTSRFCPHG
101  PPRCSQWPCL CWGASPASCF SLG
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CD8

```
  1  MQAGSFSISQ ACSRKTVAST SACSSGAPSC PSGRELSCVW LISFPPLPSP
 51  PRSPPSRREC AGYPGQRPRR AHFVAPSPLA CWWLASWFCW FPWEWPSTCA
101  AGGGEPGFVS CNNFTNKQRI RFWCPATKRH RSVMSTMWKN ERRDTFNPGE
151  FNGCCSCLLF TAARPFCVCC AWEQLVRGSS GILGRRFHCP QGTSQSVLED
201  CVRNAAHATA SGSCAFPELG PLVVAI
```

HLA-DR p33

```
  1  MTSATLSPTM SNCPCWAGAL GPRRASAAAE PCTQAFPSWC LCSSLARPPP
 51  PTSCTSSRAG WTNCQSPPRT CSWRTCACSF PSLPSLCARC AWPPRCCCRR
101  CPWEPCPRGP CRMPPSMATC QRTMCCTCSR MLTPCRCTRH CRGASRRTCD
151  TLRTPWRP
```

UGA codons, potentially encoding selenocysteine, are indicated by C.

In the case of CD4, the UGA-rich ORF in the +1 frame completely overlaps the C-terminal CD4 coding region. The predicted coding sequence (Table 1) is between 947 and 1315 (GenBank #m35160). It is also possible that this selenoprotein is translated from an alternatively spliced RNA, because there is a potential splice acceptor (SA) site (CCCTGCC-CCAG/G) about 10 nucleotides upstream from its initiation codon. In a spliced RNA, this Met would be much closer to the 5′ cap, where it could be initiated more efficiently. If this SA site were spliced to a potential donor (CAG/GT) at position 35, which is upstream from the CD4 start codon, the Met of the CD4-SP ORF would become the first start codon at the 5′ end of the spliced mRNA.

In the CD8-β mRNA, the potential selenoprotein is also encoded in the +1 frame, between 329 and 1006 (GenBank #m36712). It overlaps all but the first 94 N-terminal amino acids of CD8, and extends past its C-terminal by 110 amino acids. Thus, the predicted 226-residue product (Table 1), with 8 UGA codons and 11 Cys, is slightly larger that CD8 itself.

In the HLA-DR p33 mRNA, the UGA-rich ORF in the +1 frame completely overlaps the N-terminal half of the p-33 gene in the zero frame;

the predicted coding sequence is between 60 and 533 (Gen Bank #m14765). The start codon is only 4 nucleotides past that of the p33 gene in the zero frame, and the hypothetical selenoprotein has 10 UGA codons and 15 Cys within its 158 residues (Table 1).

The only precedent for a gene having anything close to that number of in-frame UGA codons is the mammalian selenoprotein P, which also has 10 *(20)*. It may be significant that the Cys and SeC-rich C-terminal region of selenoprotein P has a strong similarity to another protein module found in some cell adhesion molecules: the β chain of integrins and certain leukocyte adhesion proteins like CD18 (both in the top 10 matches, at > 6 SD significance in a PIR database search with this region of selenoprotein P as a probe; Taylor, unpublished). This could be a basis for the observed specific binding of selenoprotein P to cells via an unknown type of receptor *(26)*. Given that β integrin chains are components of a number of leukocyte adhesin proteins and receptors, this potential integrin/selenoprotein P homology suggests a possible evolutionary relationship between selenoproteins and such molecules. This may provide some precedent for the possibility that divergent evolution may link the CD4 gene and known selenoproteins (e.g., Fig. 1).

Because CD4, CD8, MHC proteins, and other members of the immunoglobin superfamily typically share common modules, it is also probable that similar UGA-rich ORFs will be found overlapping other genes that are important in the immune system.

If these ORFs do encode selenoproteins, their functional roles may be of considerable importance. The analysis suggests that, as well as functioning as independent proteins, they may be used as alternate modules that can be attached to part of the protein encoded in the main ORF by alternative RNA splicing or frameshifting. For example, there is a potential programmed –1 frameshift in the CD8-SP (Fig. 3), with a near-ideal heptameric shift sequence (G GGA AGG) and a potential pseudoknot that could permit a frameshift from the +1 ORF into the main ORF just before the membrane proximal domain of CD8. A frameshift here would lead to the formation of an alternate form of CD8 with the usual extracellular domain replaced by a selenoprotein module, which could have a totally different receptor binding specificity. Although this potential shift sequence differs from the "ideal" heptameric pattern (X XXY YYZ) in the sixth position *(27)*, it is a variant that (like the ideal pattern) would still permit two out of three Watson-Crick base pairs in the codon-anticodon interactions of both tRNAs after slippage (Fig. 3). The distance intervening between the shift sequence and the pseudoknot is 4 nucleotides, well within the range observed in known frameshift sites (0–12 bases), and close to the optimal distance of 6 or 7 bases.

A possible advantage to the cell for such selenoprotein modules might be the potential for redox-regulated rearrangement of disulfide/diselenide/selenosulfide bonds, and consequent conformational changes. This might present opportunities for inducing certain specific

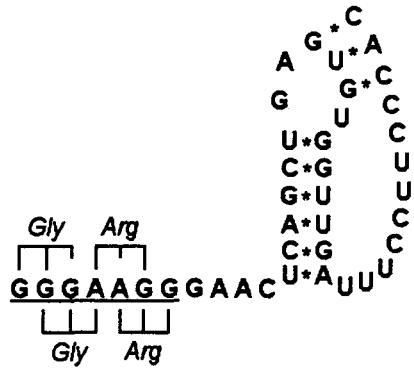

Fig. 3. A potential –1 frameshift sequence in the CD8 β gene (beginning at position 424 in GenBank #m36712), immediately followed by a potential pseudoknot. Codon–anticodon interactions of the P- and A-site tRNAs are shown schematically both before (below sequence) and after slippage (above sequence), emphasizing that 2 of 3 cognate base pairings would still be maintained after slippage. The mismatched pairs would still be purine-pyrimidine in both cases (G:U and A:C). This potential frameshift site is located just before the beginning of the membrane proximal domain in CD8. A frameshift from the CD8-SP ORF into the main CD8 ORF at this point could replace the N-terminal CD8 extracellular domain with a 34 residue selenoprotein module, potentially containing 3 selenocysteines. A similar potential shift site and a significantly larger potential pseudoknot are found beginning at position 535, corresponding precisely to the N-terminal of the transmembrane domain of CD8.

cell adhesion reactions or intracellular effects under conditions of oxidative stress, as opposed to others required under more hypoxic conditions.

HLA-DR is an important marker for activated T-cells. Thus, if the gene for its invariant chain also encodes a selenoprotein with 10 SeC residues (suggesting a substantial Se requirement), Se may be an essential lymphocyte nutritional factor that plays a significant role in T-cell function and proliferation. This is consistent with reports that Se supplementation in culture increases the cytotoxicity of killer T-cells as well as the proliferation of T-cells in response to mitogens and antigens *(2,5–7,28,29)*, whereas Se deficiency has the opposite effect, and is commonly associated with impaired immune function *(3,16,30)*. This could also be a partial basis for the extensively documented antiviral and anti-

cancer effects of Se (reviewed in ref. *21*), since cellular immunity is the primary defense against viruses and cancer.

An exceptional requirement for Se in the expression and function of these hypothetical lymphocyte-associated selenoprotein genes could contribute to the decreased levels of CD4+ T-cells in Se deficient animals and humans, as well as the decreased plasma Se levels in ARC and AIDS patients experiencing a high rate of CD4+ T-cell turnover and depletion. The existence of such genes might also help explain observations that have suggested "a priority of Se supply to lymphoid tissues" *(3)*. Finally, under conditions of severe Se depletion, a compromised ability to express these selenoproteins might contribute to T-cell anergy, inappropriate apoptosis, or other defects of T-cell function.

Significantly, Taylor et al. recently pointed out the existence of novel ORFs overlapping known genes in HIV, potentially encoding selenoproteins expressed by ribosomal frameshifting *(21,31)*. Several UGA codons in the –1 reading frame relative to the known coding regions are highly conserved in HIV-1. We also reported similar findings *(31)* in coxsackie B virus (CVB), which has been implicated as a cofactor in Keshan disease, a cardiomyopathy associated with severe Se deficiency. In a mouse model, the dependence of CVB3 cardiovirulence on vitamin E and Se deficiency has been demonstrated by Beck and coworkers *(32,33)*.

It was these HIV findings that prompted the investigation of the CD4 gene for UGA-rich ORFs, because one of the potential selenoprotein genes reported in HIV-1 overlaps the gp41 region of the HIV-1 *env* gene *(21)*, corresponding precisely to a region which has been previously suggested to represent a retroviral acquisition of a cellular gene, owing to a different base composition from the rest of HIV-1 *(34)*. Furthermore, previous work has demonstrated possible evolutionary relationships between regions of HIV-1 *env* and both HLA molecules *(35)* and CD4 itself *(36)*. The discovery of potential selenoprotein genes overlapping HLA-DR and CD4, as well as the HIV-1 *env* gene *(21)*, is certainly consistent with that possibility.

If, as the current findings suggest, T-cells require significant quantities of Se for other important functions in addition to antioxidant protection via GPx, this could help explain how the proposed competition for Se between viral and cellular selenoprotein synthesis *(21)* might further impair T-cell function in AIDS. In addition, as the downward spiral of T-cell loss progresses, the recently demonstrated high turnover rate of T-cells in HIV-infected subjects *(18,19)* could significantly contribute to the observed decline in plasma Se levels *(9–17)*. This might further compromise both helper and killer T-cell functions by inhibiting the formation of the hypothetical CD4, CD8, and MHC-encoded selenoproteins.

In summary, the hypothesis that these UGA-rich ORFs in T-cell associated genes encode selenoproteins is strongly supported by the implications of a number of independent observations:

1. a highly significant clustering of 8–10 UGA codons in these ORFs, always in the +1 frame relative to the known gene;
2. the presence of start codons upstream of the first UGA in all three cases;
3. at least for the CD4-SP ORF, significant sequence similarities to known selenoproteins (e.g., Fig. 1); and
4. the presence of consensus SeC insertion sequences in appropriate potential stem-loop RNA structures in all cases (Fig. 2).

Furthermore, this hypothesis is also consistent with existing literature data on the immunopotentiating effects of Se *(1–3,5–7,28–30)*, and correlations between CD4+ T-cell counts and plasma Se levels that have been documented in animals *(7)*, the elderly *(4)*, and HIV-infected patients *(9–17)*.

If these UGA-rich ORFs do encode selenoproteins, the fact that they overlap extensively with the known genes in a different reading frame would be contrary to the conventionally held views that human mRNAs are always monocistronic transcripts, and that our genes (almost) never overlap. However, such coding density is typical of retroviruses, which in humans just happen to preferentially infect T-cells. Rather than being a mere coincidence, the possibility that virus-like genetic processes may be going on in cellular genes in T-cells could be a reflection of the fact that retroviruses and their animal hosts have had plenty of time to learn each other's tricks during the millions of years that they have been evolving together.

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