

Selenium and Viral Diseases: Facts and Hypotheses

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Introduction

This review is based upon an article posted to the Internet newsgroup sci.med.aids in August 1995 (at the request of one of the newsgroup moderators), in an effort to clear up some of the confusion surrounding the issue of selenium (Se) and AIDS. It has been updated with new developments and data (theoretical, experimental and clinical) that have emerged in the two years since it was first written. Much of the current interest in the role of Se in viral diseases and AIDS in particular has been stimulated by media coverage of several recent scientific papers, specifically my theoretical paper showing that HIV-1 potentially encodes Se-containing proteins,¹ and more recently the work of Dr. Melinda Beck and coworkers demonstrating that the cardiovirulence of coxsackievirus B3 is highly dependent upon the Se status of the host, and that the virus actually mutates into a more virulent form in Se-deficient mice.² More recently (Christmas, 1996), Se has been in the news in regard to the first definitive clinical cancer study in the USA, by Clark and coworkers at the University of Arizona Cancer Center,³ showing that daily supplementation with 200 micrograms of Se produced a significant chemopreventive effect against several forms of cancer, with a 50% reduction in total cancer mortality over the entire study period. The national media coverage given to all of these papers has drawn much needed attention to the issue of the potential roles of Se in viral diseases and cancer, but naturally has led to some confusion as well, since the science involved is not easily explained in a few para-

graphs. It must also be noted that researchers like Dr. Gerhard Schrauzer have for many years been accumulating evidence for the potential benefits of Se, not only against cancer, but also in viral diseases (and retroviral diseases in particular),⁴ only to be widely ignored by "mainstream" researchers and clinicians. Hopefully, this review will help to rectify that situation. I will begin by summarizing some of the facts about Se, AIDS, and other viral diseases. To keep the number of references within reason, I will generally only include citations directly related to the question of the role of Se in AIDS or other viral diseases.

2. The Facts

2.1 Fact: Se is an essential trace mineral, which can be specifically incorporated into proteins as the rare amino acid selenocysteine (the Se analog of cysteine, which contains sulfur). Se is known to be critical for:

a) Antioxidant defenses, because it is an essential component of glutathione peroxidase (GPx). Along with vitamin E, a form of this enzyme is essential for combating the ubiquitous and harmful process of lipid peroxidation (a result of "oxidative stress"). Another antioxidant protein, selenoprotein P, is the major form of Se in human plasma. Unreversed lipid peroxidation leads to cell membrane destruction and can be induced during apoptosis (programmed cell death).

b) Thyroid hormone function, specifically formation of the active T3 form of thyroid hormone. This hormone is critical in regulating metabolism.

c) Formation of sperm in the male. Sperm have a high Se content, and Se deficiency can lead to infertility in males.

d) Immune function, particularly cellular immunity. Because of the potential

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significance for AIDS, this will be discussed in more detail.

2.2 Fact: Adequate levels of Se are necessary for the immune system, and particularly T-cells, to function properly. 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 (e.g. ref. 5), whereas Se deficiency has the opposite effect, and is commonly associated with impaired immune function. The supporting data have been reviewed by Turner and Finch⁶ and more recently by me.⁷ Correlations between CD4+ T cell counts and plasma Se levels have been documented in animals, the elderly, and, most significantly, in HIV-infected patients (see 2.4). The mRNA for a gene called Sps2, involved in biosynthesis of the Se donor compound required for formation of selenocysteine, is up-regulated upon activation of T lymphocytes.⁸ This shows that selenoprotein synthesis is required for some aspect of T cell function. Comment: because HIV can only replicate in activated T cells, this also suggests that selenoprotein synthesis may be important for HIV.

2.3 Fact: Se potentiates the action of interleukin 2 (IL-2). IL-2 is a cytokine that has recently shown promise in the treatment of AIDS patients, but is unfortunately associated with unpleasant side effects. Se has been shown to potentiate (amplify) the action of IL-2 by upregulating the IL-2 receptor, i.e. increasing the receptor levels.⁹ This suggests that Se supplementation might permit lower doses of IL-2 to be used, thus reducing side effects.

2.4 Fact: A progressive decline in Se levels, paralleling T cell loss, has been widely documented in HIV patients, and Se status is a significant independent predictor of survival in HIV infections. More than 20 papers documenting aspects of this decline, as well as many research abstracts, have been published over the last decade. This has been noted in asymptomatic as well as symptomatic patients, and children

as well as adults. Research groups from New York, California, Florida, Italy, Spain, Germany, France and Belgium, have all reported such observations.^{4,10-31} The obvious and traditional explanation for these observations has been that any HIV-associated decline in plasma Se levels is due to malnutrition and/or nutrient malabsorption, and thus is merely a consequence or feature of the wasting syndrome. However, a number of these authors suggest that something more complex must be taking place. Dr. Brad Dworkin reports that 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".²⁵ 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 GI malabsorption. Similarly, other authors talk about "a surprisingly high prevalence of low levels of Se in early stages of the disease"²¹ (before wasting is commonly detectable), or make comments such as "a low selenium intake seems unlikely, because urinary excretion, which closely reflects the actual selenium intake, was similar in HIV-1 infected patients and controls".²⁸ Most recently, Allavena et al. rule out malabsorption as the underlying cause, correlate Se levels with survival prognosis, and conclude that "the measurement of trace elements, especially Se, may be a useful marker to predict the HIV infection progression".²⁹ In the most recent studies, there is compelling evidence that Se status is actually a significant predictor of outcome in HIV infection,³⁰ and that the relative risk for mortality is much higher in Se-deficient patients.³¹ Thus, at the least, the selenium status of HIV-infected patients appears to be an excellent "surrogate marker" of HIV disease progression.

2.5 Fact: Simple Se compounds DO inhibit HIV-1 in the test tube There is also

other experimental evidence for an effect of HIV upon levels of cellular selenoenzymes, and for Se inhibition of the replication and effects of HIV-1 and other retroviruses. Furthermore, recent work demonstrates a direct effect of Se in regulating the expression of an isoform of an HIV gene *in vitro* (see section 3.4.5). Examples:

2.5.1 “Lipid hydroperoxides induce apoptosis in T cells displaying a HIV-associated glutathione peroxidase deficiency”, Sandstrom et al.³² Quote from abstract: “Since oxidized lipids have been reported to accumulate in oxidatively stressed, HIV-infected individuals, a HIV-associated glutathione peroxidase deficiency may contribute to the depletion of CD4 T cells that occurs in acquired immune deficiency syndrome (AIDS).” Note: Se is an essential component of glutathione peroxidase, so the results show that even in this cell culture model—where malabsorption cannot be blamed—HIV is somehow causing a deficit in the levels of an important cellular selenoprotein.

2.5.2 “Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress”, Sappéy et al.³³ This was a study of effects of Se supplementation on HIV-1 replication induced by oxidative stress in cell culture. Noting that existing data “implicate an HIV-1 mediated antioxidant imbalance as an important factor in the progressive depletion of CD4+ T cells in AIDS”, the authors demonstrate that at concentrations of 25 to 50 micrograms Se per liter as sodium selenite, Se supplementation has the following effects in ACH-2 cells:

- inhibits viral cytotoxic effects and the reactivation of HIV-1 by hydrogen peroxide.
- decreases activation of NF- κ B, an important cellular transactivator of HIV-1.
- protects against activation of HIV-1 by tumor necrosis factor alpha.

2.5.3 Preliminary results from the lab of Dr. Raymond Schinazi from screening

several organic and inorganic Se compounds in a standard assay for anti-HIV activity show that certain simple Se compounds are active vs. HIV-1 at micromolar concentration (abstract published in Antiviral Research).³⁴

2.5.4 Even before the “AIDS virus” was shown to be a retrovirus (i.e. earlier than 1983), it had been demonstrated that simple inorganic Se compounds were able to inhibit other retroviruses both *in vitro* (bovine leukemia virus) and *in vivo* (mouse mammary tumor virus), as referenced in Schrauzer and Sacher.⁴ These early leads have never been pursued by AIDS researchers.

2.5.5 The only significant counter-example is a 1983 paper showing that selenomethionine induced the expression of endogenous retroviruses in cultured cells.³⁵ The effect appears to involve the nonspecific replacement of methionine by seleno-methionine (SeMet), because addition of an equivalent amount of methionine (i.e. a 50-50 mixture of Met and SeMet) inhibited the induction by 96%. However, this induction of viral expression was only observed at extremely high (millimolar) concentrations, at which many other Se compounds tested were “highly toxic” to the cultured cells. The selenomethionine concentrations at which induction was observed were at least 100 to 1000 times higher than concentrations observed to inhibit the activity of HIV and other retroviruses in the experiments described above (2.5.2 - 2.5.4), as well as being at least 100 to 1000 times higher than the Se concentration in normal human blood. Such concentrations could never be attained in human plasma unless highly toxic doses were being ingested. Thus, this is not a physiologically significant effect. In the light of all the other evidence cited above, there is no reason to believe that Se supplementation at rational dose levels would have anything other than a beneficial effect in HIV infected individuals.

2.6 Fact: A hypothyroid-like or low T3 syndrome is well-documented in AIDS pa-

tients. A common deficit in thyroid hormone has been widely noted in AIDS patients, and specifically involves reduced levels of T3.³⁶⁻³⁹ The conversion of T4 to T3 depends on a deiodinase enzyme that contains Se, so a reduction in T3 formation would be a logical consequence of Se deficiency. It has been suggested that these thyroid-related abnormalities could be a factor in the AIDS wasting syndrome. Human growth hormone, a current preferred treatment for wasting, is known to stimulate conversion of T4 to T3 by inducing the deiodinase,^{40,41} a process which will be more effective if adequate levels of Se are present.

2.7 Fact: An immense body of evidence demonstrates the role of oxidative stress in stimulating HIV replication, that certain antioxidants can inhibit this process, and suggests the presence of an antioxidant defect in HIV patients. This evidence was reviewed in the symposium on "The place of oxygen free radicals in HIV infections" that was held in France early in 1993, with proceedings published in *Chemico-Biological Interactions*, Vol. 91. In his preface to the proceedings, in regard to oxygen radicals Dr. Alain Favier states "their place in HIV appears as a very strong hypothesis" and that "...the time is right to conduct trials to evaluate the efficacy of antioxidants." Since Se is one of the most critical antioxidant nutrients, a Se deficiency in an AIDS patient could be expected to lead directly to the stimulation of HIV replication, by increasing oxidative stress, and Se supplementation would be expected to counter that process, as has now been shown in the test tube (see 2.5.2).

2.8 Fact: There is extensive evidence of correlations between Se deficiency in humans and animals and the severity of diseases associated with certain other viruses. Examples:

2.8.1 Hepatitis B: in certain low Se regions of China, both hepatitis B viral infection and associated cases of liver can-

cer have been endemic. In extensive 5-year trials of Se supplementation in the human population, Chinese researchers were able to attain significant reductions in the incidence of both viral hepatitis⁴² and liver cancer.⁴³ Note that hepatitis B, a hepadnavirus that encodes a reverse transcriptase, is a close relative of retroviruses.

2.8.2 Keshan disease, a Se-deficiency disease with a viral cofactor: a precedent for HIV? Keshan disease is a classical Se-deficiency disease, named after a county in China where outbreaks occurred due to the very low Se levels in soils of the region. The disease presents as a non-obstructive cardiomyopathy. Due to the seasonal and clustered nature of outbreaks of the disease, Chinese investigators suspected the involvement of an infectious agent or other cofactor, and eventually isolated coxsackievirus from the hearts of disease victims. The probable role of coxsackievirus in Keshan disease is strongly supported by demonstrations that a deficiency of Se can trigger a similar cardiomyopathy in coxsackie-infected mice.⁴⁴ Recently, Beck and coworkers have shown that even a "benign" strain of CVB3 becomes virulent in Se-deficient animals, where it can mutate into a more virulent strain that can produce myocarditis even in Se-adequate mice.^{2,45} This research was recently reported in the popular press, including *Science News* (V.147, p.276), and by Laurie Garrett in *Newsday* (5-1-95, City Edition, News, pg. A27, under the headline "Study: Diet Can Start Virus' Lethal Mutation").

2.8.3 Animal viruses: Many examples can be found in the veterinary/agricultural literature linking viral infections with Se deficiency in various animals.

3.0 Hypotheses

Is there a common basis for all these observations? The biochemical roles of Se, and the mechanisms involved in viral pathogenesis, are both sufficiently complex that the apparent antiviral effects of Se are

probably multifactorial in origin. Although the preceding review has focused on evidence for a potential role of Se in AIDS, this is not intended to imply that Se is a cure for AIDS, or to minimize the importance of other factors that contribute to HIV pathogenesis. It is intended to demonstrate that something unusual is probably going on with Se in HIV infections, and that supplementation is likely to be necessary and beneficial, at least in some cases. The question is, why? The following sections briefly outline my theoretical findings that may help explain some of the data reviewed above, as well as new clinical and experimental results that appear to confirm the theoretical predictions. Note that this is not intended to rule out other possible explanations or factors that might also contribute to the observations, or other mechanisms that contribute to HIV pathogenesis.

3.1 Se, HIV and AIDS: the “Viral selenoprotein theory”. On August 19th, 1994 (coincidentally, the day Linus Pauling died; he was the first to suggest antioxidants could be of benefit in viral diseases), my group published a study of the predicted RNA structure of HIV in relation to potential novel open reading frames (protein coding regions) of the virus.¹ This analysis demonstrated the potential for several new genes in HIV, that possibly encode proteins containing selenocysteine (encoded in RNA by UGA codons, which usually cause termination of protein synthesis). We also identified the RNA structural features (e.g. pseudoknots) that would be required for the expression of these genes. If active, such genes would provide the basis of a role for Se in the biochemistry and regulation of HIV. Thus, we must seriously consider the possibility that Se depletion may not only be a correlate of AIDS progression: it may be directly involved in the mechanism by which HIV causes AIDS. Virally-induced depletion of Se in HIV-infected cells, and the potential existence of virally-encoded regulatory selenoproteins,

could help explain the increased susceptibility to oxidative stress characteristic of AIDS. Various observations, some listed in sections 2.4-2.7, are highly consistent with this theory. The theory can also potentially help explain the role of various cofactors that stimulate HIV infection, since many infectious disease states stimulate free radical formation, producing oxidative stress. One source of confusion relates to the question that, if the virus requires Se, why is it that a deficiency of Se appears to be associated with increased viral replication, and Se supplementation inhibits the virus (section 2.5), rather than “feeding” the virus? This is best understood by analogy to a classical example of a nutrient effect on viral replication: the well-documented induction of retrovirus expression in cells cultured in arginine-deficient media. Note that arginine is an essential component of most viral proteins. Thus, paradoxically, in this case also viral replication appears to be triggered by a deficiency of something the virus requires. This would most likely involve some sort of repressor type of mechanism, analogous to known situations in bacteria, like the famous tryptophan repressor. Based on the data that I have reviewed here, it seems quite possible that viruses like HIV and coxsackie B3 may respond to Se deficiency by a mechanism analogous to that involved in this arginine effect. Note that a viral glutathione peroxidase enzyme might also have a repressive effect on viral replication, because it is known that oxidative stress (e.g. H₂O₂ exposure) activates the replication of HIV and other viruses: a viral glutathione peroxidase would reduce oxidant tone, thus reducing viral activation. This is highly significant because genes apparently encoding a selenium-dependent glutathione peroxidase (the prototypical selenoprotein) have now been identified in several viruses, including HIV-1 and hepatitis C virus (see sections 3.2, 3.4.3, 3.4.6, and 3.4.7). A virally-encoded glutathione peroxidase could also

help a virus defend against free radical mediated attacks on infected cells by the immune system, and also increase the extracellular viability of virus particles in the blood stream, because without that enzyme, enveloped virions are more susceptible to membrane lipid peroxidation once they have budded off the host cell and lost the benefit of cellular antioxidant defenses.

3.2 Potential selenoprotein genes in other viruses: Coxsackie B3, Ebola Zaire, M. contagiosum, and Hepatitis C Virus. A similar analysis has now been applied to a number of other viruses, yielding consistent and surprising results. There is strong theoretical evidence that similar Se-utilizing genes may exist in coxsackievirus B3 (CVB3), the same strain studied by Beck et al. as a model for Keshan disease (section 2.8.2), and that one of these appears to encode a highly truncated glutathione peroxidase module. These theoretical results regarding CVB3 have been outlined in several papers.^{46,47} A striking example of potential selenoprotein genes in a virus is provided by the highly pathogenic Zaire strain of Ebola virus, where one such potential gene has 16 UGA selenocysteine codons, as well as structural features necessary to express this selenoprotein, which would require 16 Se atoms per molecule.^{48,49} This suggests that infection with Ebola Zaire may place an unprecedented demand for Se on the host, potentially causing a more drastic Se depletion in a matter of days than HIV infection can accomplish in 10 years. Significantly, this gene and related structural features are absent in the Ebola Reston strain, which was essentially non-virulent in humans. A potential role for Se is highly consistent with key aspects of Ebola pathology,⁴⁹ including its effects on Se-rich tissues like blood cells and liver, and the hemorrhaging due to rupture of capillaries obstructed by blood clots (because Se normally plays a role in inhibiting clotting,⁵⁰ and Se deficiency has been associated with thrombosis and even

hemorrhaging in extreme cases in animals). However, the experimental investigations required to confirm this theoretical possibility have not been performed. Nor have indicators of Se status and lipid peroxidation ever been examined in Ebola patients. However, there are some compelling clinical results: Se has apparently been used with considerable success by the Chinese in the palliative treatment of viral hemorrhagic fever caused by Hantaan virus infection. In an outbreak involving 80 patients, oral sodium selenite at 2 mg. per day for 9 days was used to achieve a dramatic reduction in the overall mortality rate, which fell from 38% (untreated control group) to 7% (Se treatment group), thus giving an 80% reduction in mortality.⁵¹ This result, obtained using Se at a dose of about 13 times the RDA as the sole therapy, is all the more striking in light of the fact that, according to conventional medical science, there is no effective treatment for hemorrhagic fever (viral infections with Ebola-like symptoms). Although this did not involve Ebola virus, there are a number of different hemorrhagic fever viruses, and they may share common mechanisms.⁴⁹ This example suggests that pharmacological doses of Se may also have some benefit in infections due to other hemorrhagic fever viruses, including Ebola. Less hypothetical is the recent identification in a DNA virus of a gene that is an obvious homologue of the mammalian selenoprotein glutathione peroxidase. In a paper published in August 1996, the group of Dr. Bernard Moss from NIH published their results on the newly sequenced genome of the pox virus Molluscum contagiosum, where they identified a gene that is 76% identical to glutathione peroxidase at the amino acid level.⁵² While not yet confirmed by functional studies, the high degree of similarity of this sequence to cellular homologues leaves little doubt that this is a real gene (see section 3.4.3). Unmistakable glutathione peroxidase modules have now

been identified by comparative sequence analysis in both HIV-1 (one of the selenoprotein genes I predicted in 1994; see section 3.4.6) and in hepatitis C virus (see section 3.4.7). Thus, this antioxidant selenoprotein module may ultimately prove to be a constituent of a number of RNA and DNA viruses.

3.3 Endemic Kaposi's Sarcoma in Africa Recent work has implicated a new herpes virus in Kaposi's Sarcoma. Ziegler has demonstrated a correlation between the incidence of "endemic" Kaposi's Sarcoma in African subsistence farmers and geographic regions with volcanic soils.⁵³ Significantly, it is well documented in the agricultural literature that plants and animals raised on such soils are typically Se deficient, with regions of Oregon and the East African Rift Valley often cited as typical examples. The increased incidence of Kaposi's Sarcoma in volcanic soil regions in Africa suggests a possible parallel to Keshan disease: a disease with a viral co-factor associated with geographic regions where plants may be low in Se.

3.4 Summary of key data consistent with predictions of the viral selenoprotein theory. Based on evidence that has emerged in the last few years, there is now little reason to doubt that some viruses encode selenoproteins. Recent developments and confirmations of the theoretical predictions include the following:

3.4.1 My 1994 prediction that Se levels should be a factor in disease progression in AIDS1 has now been amply confirmed in several recent papers, e.g. Constans et al. (1995), "Serum selenium predicts outcome in HIV infection",³⁰ as well as other current papers by several groups (of course, these are only the most recent of a series of over 20 papers published over the last decade documenting Se depletion in HIV/AIDS; see section 2.4). Dr. Marianna Baum of the University of Miami has been studying nutrient abnormalities in HIV/AIDS for some years, and had ear-

lier reported such Se abnormalities in several papers.^{21,26} Her latest analysis of a cohort of HIV+ IV drug users shows that low serum Se is 15 times higher (more significant) than low CD4 count as a risk factor for mortality.³¹ The pathology of muscle weakness in HIV infection (myopathy) has also recently been associated with Se deficiency in AIDS.⁵⁴ Furthermore, Se has been shown to inhibit HIV in vitro by at least two independent labs (see section 2.5).

3.4.2 The RNA pseudoknot that I predicted overlapping the active site coding region of HIV-1 reverse transcriptase has now been experimentally verified by enzymatic and chemical stability studies, published in a recent paper and thesis from Dr. Barbara Carter's group at University of Toledo.⁵⁵

3.4.3 My 1994 proposal that some viruses may encode selenoproteins, initially received with considerable skepticism, is no longer in doubt, although it has yet to be definitively proved in the case of HIV. The most indisputable example of a viral selenoprotein is the homologue of glutathione peroxidase (GPx) recently identified by Moss and coworkers in Molluscum contagiosum virus.⁵² My group has also demonstrated GPx-related sequences in coxsackie B virus, the cofactor in Keshan disease, a classical Se-deficiency disease.⁴⁶ More recently, we have shown that one of the potential selenoprotein genes we predicted previously in HIV is a GPx homologue (see section 3.4.6), and we have now identified the same gene (GPx) in hepatitis C virus (see section 3.4.7).

3.4.4 The growing body of evidence that Se has apparent chemoprotective effects vs. a number of viral infections including HIV was attested by and documented in the recent conference on selenium and human viral diseases held in Germany in April 1996, with proceedings (edited by G. Schrauzer and L. Montagnier) published in a peer-reviewed journal, Biological Trace Element Research (Vol. 56 #1).

3.4.5 In my lab, we have now obtained

firm in vitro evidence for a novel-1 frameshift site associated with highly conserved UGA codons (potentially encoding selenocysteine) in the HIV-1 nef gene coding region, that we predicted previously.^{46,47} Furthermore, Dr. Benjamin Blumberg, a collaborating virologist at University of Rochester Medical Center, has obtained in vitro and immunocytochemical evidence for the predicted nef variants in post-mortem HIV+ brain tissues (where nef is overexpressed). This is particularly significant because one of the reactive antisera was to a peptide located downstream of a highly conserved UGA codon at the 3' terminal of nef, proving that readthrough of that UGA codon must be taking place, as we first proposed in 1994.¹ Most significantly, the results of in vitro translation experiments show that this event is Se-dependent: addition of small amounts of Se to the medium greatly enhances the production of this novel HIV-1 gene product, and 75Se incorporation in an isoform of the HIV nef protein can be demonstrated.⁵⁶

3.4.6 In a recent paper,⁵⁷ we show that a potential selenoprotein that we previously identified in HIV-1, overlapping the envelope gene coding region, is in fact a homologue of glutathione peroxidase (GPx), the prototypical eukaryotic selenoprotein. The sequence encoded in this HIV-1 gene region contains a common variant of the GPx active site consensus sequence, spanning the catalytic selenocysteine. The similarity score of this novel HIV sequence vs. an aligned group of GPx sequences is five standard deviations (SD) above the average similarity score of randomized sequences of identical composition; thus, the probability of obtaining this degree of similarity purely by chance is less than one in a million. This gene has now been cloned for experimental verification of GPx activity.

3.4.7 We have now identified the same gene in hepatitis C virus (HCV), a very common infection in the U.S. (about 1.5% or 4 million people are seropositive). In both

HIV and HCV the GPx gene is in the -1 reading frame overlapping a known gene (the NS4a gene in the case of HCV), contains an in-frame "stop" codon, UGA, that can also encode selenocysteine, and also lacks an apparent start codon, thus explaining why these genes have escaped detection up to now. The putative HCV GPx sequence is highly similar to known GPx sequences; the similarity encompasses the entire enzyme active site region, and is statistically significant at 6.2 SD relative to random sequences of similar composition, or 6.7 SD if compared only to the mammalian extracellular plasma GPx enzymes (Taylor and Zhang, paper in preparation). The HCV GPx (active site amino acid sequence VQVASPUGLLG) is most similar to the human plasma GPx (active site sequence VNVASYUGLTG, where U signifies the selenocysteine codon). The Se-dependent GPx sequence and UGA codon are highly conserved in HCV genotype 1b, which is predominant in North America. Significantly, genotype 1b is associated with the highest risk of progression to cirrhosis and hepatocellular carcinoma, and poor response to interferon. An HCV-encoded GPx gene may help explain why oxidant stressors like alcoholism and iron overload are associated with HCV disease progression. The best direct evidence consistent with an HCV-Se link is the clinical data of Look et al., who found that in HIV+ patients, the progressive decline in Se levels characteristic of HIV infection was greater in those with HCV co-infection, who "showed markedly lower selenium concentrations compared to those without concomitant HCV-infection".⁵⁸

4. Clinical Implications

My theoretical findings outlined in section 3 provide a new theory as to why Se may be critical in HIV infection and other viral diseases - but even before that theory was developed, there was already abundant evidence supporting the idea that

Se supplementation could be of benefit to HIV-infected patients. Even if the HIV-selenoprotein theory proves to be incorrect (which now seems very unlikely!), the facts listed in section 2 cannot be denied. Thus, based on currently available data, it seems advisable to seriously consider some level of supplementation, at least as a precautionary measure. However, patients are strongly advised to consult with their physicians on this question, particularly if they are in a symptomatic stage of the disease. It is important to realize that when we talk about Se we are fundamentally talking about nutrition, not a drug. Furthermore, some physicians already recommend the use of Se supplements to their HIV-infected patients, and such recommendations can also be found in literature published by various AIDS activist and self-help groups, so this is nothing new or untried. In several very brief clinical trials, symptomatic improvements in ARC and AIDS were reported.^{12,16,19} A leading US research group has already completed preliminary studies for a new, double-blind, placebo controlled clinical trial of Se supplementation in HIV patients who are not Se deficient. Because research has shown that there are problems in nutrient absorption even in asymptomatic HIV+ individuals, the suggestion has been made that HIV patients need to take larger amounts of vitamins than uninfected individuals to attain the same blood levels.⁵⁹ Since the USDA states that nutritional supplementation in the range of 50-200 mcg of Se daily is safe and effective for healthy individuals, a dose of 400 mcg seems reasonable for HIV infected individuals, if they do have impaired absorption. For an AIDS patient who is demonstrably deficient in Se, an even higher daily dose (up to 800 mcg) for a brief period of time (say several weeks) to get their blood levels up, followed by a decrease to 400 mcg, is an effective strategy that was used in one published clinical study involving AIDS patients.¹² This question of dose level natu-

rally arouses concerns, because in the past so much has been made of the potential toxicity of Se. I believe that the danger of serious toxicity with Se supplementation has been exaggerated. The threat of serious acute toxicity with supplementation is in my opinion nonexistent at doses less than 1000 mcg per day, and in several studies people in certain geographical locations have been shown to be ingesting from 600 to over 700 mcg per day for extended periods of time without evidencing any ill effects - in northern Greenland, as much as 1000 mcg per day in some individuals. Thus, doses in the 400 mcg range are undoubtedly safe. In any case, the signs of chronic Se toxicity - garlic odor of breath and sweat, metallic taste in mouth, brittle hair and fingernails - are very distinctive, and easily reversed by lowering the dose. In regard to Se and viral diseases in general, I find myself in the position of Linus Pauling in regard to the anticancer and antiviral benefits of vitamin C: I believe that there is a sufficient body of clinical and basic research data to support the conclusion that Se has not only anticancer benefits, but also chemoprotectant effects vs. a broad spectrum of viral infections. Furthermore, Se may have not only preventive, but also therapeutic potential in active viral infections - even some that can be acutely lethal - because the life-saving benefits of a brief course of treatment with reasonable pharmacological doses (i.e. in the milligrams per day range) have been demonstrated in at least one case.⁵¹ The full potential of Se therapy in the treatment of HIV infections has yet to be rigorously assessed in a large-scale study. Considering that Se deficiency is associated with increased incidence of various cancers, and increased morbidity and mortality due to infectious diseases like AIDS, we must seriously consider evidence suggesting that there may be a global trend towards a decrease of Se in the food chain, caused by various factors, including modern agricul-

tural practices, fossil fuel burning and acid rain (primarily because SO₂ reacts with Se compounds in soil, forming elemental Se that plants cannot absorb⁶⁰). Studies have shown that Se levels in the British diet have decreased by almost 50% over the last 22 years.⁶¹ If dietary Se levels have decreased so drastically over 22 years in Britain, a wealthy and highly developed nation, then what is the situation in rapidly developing Third World countries? In light of the evidence showing that Se deficiency is associated with adverse outcomes in viral infections, and can foster the emergence of more virulent viral strains, any localized or global depletion of Se in the food chain could be a significant factor contributing to our increased susceptibility to emerging viral diseases, as well as to recent increases in cancer mortality rates in developed nations.

Conclusion

A considerable body of evidence supports the hypothesis that some viruses may encode selenoproteins. Much of the evidence at present is still theoretical. We have found potential selenoproteins encoded in HIV and other retroviruses, some strains of coxsackievirus, definitely in hepatitis C virus, and possibly in Ebola Zaire, hepatitis B, and several human herpes viruses.^{1,34,46-49,56,57} A similar theoretical analysis by an independent research group has revealed an unmistakable glutathione peroxidase gene in the human pox virus, *M. contagiosum*.⁵² At least in the case of coxsackievirus, there is substantial in vivo evidence that Se plays a role in regulating viral pathogenicity.^{2,45} The evidence for Se deficiency as a high risk factor for HIV disease progression and mortality is now very strong,^{29-31,54,58,62} and there is firm evidence that Se compounds can inhibit HIV cytopathicity and the activation of HIV by oxidative stress (section 2.5). Although such results do not prove that Se inhibits HIV by the mechanism I have proposed (i.e. that viral selenoproteins are involved), they

are highly consistent with the predictions of the theory. Our recent identification⁵⁷ of a glutathione peroxidase homologue in HIV-1 leaves little room for doubt that a direct interaction between HIV and Se can occur, particularly since the same gene has now been identified in several other viruses. Despite all the compelling evidence regarding a central role for oxidative stress in HIV activation and AIDS pathogenesis, no one has previously explained how the virus produces the well-documented "HIV-1 mediated antioxidant imbalance".³³ Nothing could be simpler than the depletion of Se in infected cells due to the formation of virally-encoded selenoproteins, the mechanism I have proposed. Two of the potential selenoprotein genes that we identified in HIV have now been cloned, and experimental evidence of their function, if any, should be available in the near future. Let me conclude with a quote from Dworkin's 1994 paper:²⁵ "Selenium deficiency may be associated with myopathy, cardiomyopathy and immune dysfunction including oral candidiasis, impaired phagocytic function and decreased CD4 T-cells." To that I would add: hypothyroid (low T3) syndrome, increased risk of thrombosis,⁵⁰ and psoriasis.⁶³ Do any of those symptoms sound familiar? Think about it.⁶

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