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College of Pharmacy
Aug. 31, 1999

Ms. Sandra Thurman, Director,
Office of National AIDS Policy
736 Jackson Place
Washington, DC 20503

Dear Ms. Thurman,

I thought I should add some comments to go with the information I sent you, since it is a rare opportunity for me to communicate with someone who is probably the person most able to influence the direction of AIDS policy (let me guess: despite the intended power of your position, I bet it's not as easy as it should be to do that, when each agency has their own agenda and established bureaucracy...). The one thing I have learned is that few fields of science are as politicized as AIDS research, and when you are outside the mainstream, as I appear to be, one has to try to identify people who are open-minded and not vested in the status quo, and who appreciate the need for practical solutions in the real world. From what I have read about you, I suspect you are such a person.

So where am I coming from? I will be as frank as possible, but in return I ask you to keep this somewhat confidential, as I'm isolated enough as it is, and sometimes the truth irritates people.

In the early '90s, I had a five-year NIH grant for theoretical studies related to drug design and mechanism of action of anti-HIV nucleotides. No doubt had I stayed with that line of research, and cultivated the appropriate ties to the right bio-pharmaceutical companies, I would be enjoying a well funded and successful academic career today. Even though my discovery of selenoprotein coding potential of HIV, and its profound implications for the role of selenium in HIV disease progression, arose from that NIH-funded research project, since I switched my focus to the pursuit of the implications of this finding, my ability to obtain NIH or other extramural funding, and to publish my research, has been drastically compromised. At the time I published the 1994 paper, I naively thought that the leaders of AIDS research at NIH and elsewhere would apply their massive resources to definitively test this theory and either establish it or firmly refute it within six months to a year. What happened was that they did their best to completely ignore it and act as though I and my theory did not even exist. Realistically, they all have their own theories of HIV pathogenesis that they are trying to prove, so why would they want to waste their resources testing mine?

Despite an ever-increasing body of data linking selenium status to HIV disease progression and outcome, and ever stronger theoretical support from my group, coupled with in vitro data generated in my lab (such as proof of several of the predicted novel frameshift sites in HIV), attempts by me and a few collaborators to get NIH or other funding to pursue this have failed, as I pretty well expected. The only ray of light has been the support from senior program staff at NIDA (Jag Khalsa and Henry "Skip" Francis, the Director of their Center on AIDS and Other Medical Consequences of Drug Abuse), who through an administrative mechanism, have managed to get me about $35,000 of direct funding over the last year, with the same amount expected annually for the next few years.

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I could live with this situation, if they (NIH), as more qualified experimentalists, had engaged in a timely and thorough effort to fairly evaluate my hypothesis. However, their most recent effort is too little, too late, and too biased to permit me to have any confidence in their objectivity. A recent PNAS paper by intramural NIH scientists, which actually contains data highly consistent with predictions of the HIV selenoprotein theory, but utterly fails to give a fair assessment of that theory, has only confirmed my belief that these people have taken a dogmatic position, and are going to interpret their results in the light of their preconceptions, until such time as we can beat them over the head with exceptionally hard data. (You may have seen my rebuttal of that paper if you visited my web site).

To show you how bad it can get, I should also mention that I have a collaborator, a virologist formerly at University of Rochester medical center named Benjamin Blumberg, who has obtained preliminary evidence for the existence of a predicted selenoprotein isoform of the HIV-1 nef gene product, which we were preparing for journal submission, after several previous incarnations of the paper, now with much stronger data than the earliest verion, which was rejected the first time we submitted it. Despite provocative preliminary data, Blumberg's grant applications have also been rejected, and a senior virologist told him to hold off on publishing with me until he gets a grant, because it will be politically damaging "because people think Taylor is crazy because he keeps publishing these theoretical papers with no experimental data to back it up". So now that we have some data, we shouldn't publish it? I don't get it. MY lab has experimental data that we're trying to publish, but its not easy in such a climate, I can tell you.

We have cloned one of the predicted HIV seleoprotein genes, expressed it in mammalian cells, and shown it has the predicted enzyme activity. We have even bacterially expressed the protein and purified it to homogeneity and found the same activity. But publishing this in a top journal will be an uphill battle.

Certainly, as you can see from the enclosures I sent you, the *clinical* data on selenium and HIV is now becoming very strong. The set of title pages or abstracts from over 20 such papers shows that the data has been accumulating over the last decade. So there is certainly justification for implementing the use of this nutritional factor in the field, even if my theory continues to be unsupported and unresolved. This is not some untried experimental drug, this is nutrition we are talking about. However I believe that in order to overcome the skepticism and opposition to this type of approach, it would be far better if the molecular basis of the anti-HIV effects of selenium was firmly and definitively elucidated. Even the new work from NIH in the PNAS paper clearly shows that HIV infection depletes the host selenoprotein pool, even though they disagree with my mechanism. I have also enclosed information to you showing that infants are particularly prone or susceptible to selenium deficiency, perhaps explaining why AIDS progresses much more rapidly in young children. I believe this is another justification for implementing a selenium plus multivitamin therapy in at-risk populations without further delay.

I have never claimed that selenium is a cure or effective monotherapy for AIDS (my opponents seem to act as if I have), but the more I have studied the question, the more I've come to believe that selenobiology is a key factor in understanding various aspects of HIV epidemic, including the role of cofactors such as malnutrition, other infections, drug abuse, and the central role of oxidative stress in activating HIV (this is laid out most succinctly in my enclosed 1999 J AIDS - NIDA symposium manuscript). I also believe that there is solid evidence to support the hypothesis that selenium is and has been decreasing in the food chain, particularly in the 20th century, where the levels of fossil fuel burning have increased dramatically (see enclosed items by Dr. Margaret Rayman and Dr. Doug Frost). So I believe that a number of environmental factors, aggravated by widespread malnutrition, may be exacerbating the pathogenic effects of HIV, and that those effects might be considerably attenuated by the appropriate nutritional support.

Because this is likely to be most important in developing countries where economics prohibit the use of expensive antiviral agents, I am beginning to believe that perhaps I should be looking internationally, or to private organizations with international ties, to get the relatively modest funding that I need to try to move this research forward. At the same time, I believe the clinical data are strong enough that, whether I'm right or wrong, this nutritional approach will undoubtedly be of benefit in improving the length and quality of life for many HIV infected people who have few other treatment options. So I am committed to doing all I can to promote and advance such studies or initiatives. I also hope that out of such interactions, people will come to realize the importance of definitively establishing the molecular basis of nutritional approaches, and help me in my efforts to gain support for my small and severely underfunded research team.

I have recently found such allies in Andrew Young and some people close to him, with strong ties to Africa. So it is there that I think the importance and potential scope of nutritional interventions will be fully tested and established.

I want to mention a few of the specific items I sent you. The Africa maps are particularly interesting, if you realize that the one showing HIV incidence is essentially a "snapshot" of how the epidemic was spreading in the early to mid 1990s. Clearly, it has spread much farther and deeper in the African population since the WHO data upon which the map was based. But it is intriguing that the early growth of the epidemic did seem to be correlating with volcanic soil regions, in many of which (e.g. Zaire, Kenya and Malawi) low selenium content of plants has been specifically demonstrated. If you are interested, I have some references documenting low selenium levels in plants and livestock raised on volcanic soils.

My web site, at http://bioinfo.chem.uga.edu/homepage/wtaylor has a few other items that might be of interest, such as a review I wrote entitled "Selenium and viral diseases: facts and hypotheses", which although two years out of date, shows that the potential role of selenium in HIV infection fits into a much larger picture relating to other viruses such as coxsackievirus, which is an established cofactor in the classical selenium deficiency disease called Keshan disease.

Sorry I could not get this out a day sooner, I hope you have time to look it over before we talk. While I would rather that this LETTER was not distributed, that does not apply to the rest of the material. Your interest, and any effort you can make to place this information in the appropriate hands, is greatly appreciated.

I am looking forward very much to talking with you and I am confidant that, contrary to the rumors reaching Dr. Blumberg's virologist friend, you will find that I'm really quite remarkably sane...

Sincerely,

Will Taylor

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- A few of the items may be duplicated in the bundled set of "Appendix" items, from an unfunded proposal I have floating around.

First International Symposium on Human Viral Diseases: Selenium, Antioxidants and Other Emerging Strategies of Therapy and Prevention

19-21 April 1996, Nonnweiler, Germany

BACKGROUND

Why selenium and viral diseases?

Several decades ago selenium (Se) was shown to be an essential trace mineral in mammals. It is a component of the important antioxidant enzyme glutathione peroxidase (GPx), which is critical for defence against lipid peroxidation and other types of oxidative and free radical damage.

A possible link to viral diseases became apparent almost 20 years ago, first with Schrauzer's demonstration that Se is chemoprotective against virally-induced mammary tumours in mice and shortly thereafter with the implication of Coxsackievirus as a probable cofactor in Keshan disease, the classical Se-deficiency disease of humans. This cardiomyopathy was once endemic among peasants in low-Se regions of China, and was essentially eradicated by Se supplementation programmes. In fact, Se has now been shown to have a significant chemoprotective effect against a number of human and animal viruses, including hepatitis B infection linked to liver disease and cancer (1), Coxsackievirus-Keshan disease (2), viral haemorrhagic fever (3) and the mouse mammary tumour virus (4). A link between Se and HIV is still unclear, although low plasma Se is a correlate of disease progression and Se has been shown to inhibit HIV activation *in vitro* (5).

In humans, Se appears to be important not only for antioxidant defences via GPx, but also for thyroid function, reproduction, cellular immunity and aspects of gene regulation. Se acts via 'selenoproteins' that contain the rare amino acid selenocysteine (SeC), which is encoded by the UGA codon when specialized RNA structures are present; otherwise, UGA will direct termination of protein synthesis. In bacteria, SeC is also encoded by UGA, suggesting that this is a very ancient codon usage; thus, it has been proposed that selenoproteins may have been more common early in evolution, before atmospheric oxygen levels increased, and that UGA evolved into a stop codon owing to decreasing bioavailability of SeC. If there is any merit to the concept of RNA viruses as 'molecular fossils' of the RNA world, then one might expect to see some vestigial traces of ancient selenoproteins in such viruses. Intriguingly, it was recently shown that RNA viruses typically have an up to threefold higher incidence than expected of UGA codons in the -1 reading frame overlapping their known genes (6).

Although there is overwhelming evidence of a role for oxidative stress in AIDS pathogenesis, and antioxidants show promise as complementary therapies, the mechanisms involved are poorly understood. HIV infection apparently induces an antioxidant defect in the host (7), and can even produce a deficiency of the mammalian selenoprotein GPx in HIV-infected cells (8). Such data are consistent with the possibility that viral selenoproteins might play a role in the stimulation of HIV replication by oxidative stress or Se deficiency (9,10).

The fact that selenium deficiency has been linked to the incidence, disease progression or virulence associated with a number of viral infections provides ample incentive for a deeper look at these phenomena, and was the basis for convening the 1996 symposium on Se and antioxidants in human viral diseases.

An interdisciplinary group of basic and clinical researchers gathered in Nonnweiler, Germany to review the current status and potential of Se and antioxidants as chemoprotective and therapeutic agents in human viral diseases. In this regard, probably the most striking report of the meeting was that of Jian-Cun Hou (Chinese Academy of Medical Sciences, China). In an outbreak of viral haemorrhagic fever (HF) involving 80 patients, he used oral sodium selenite at 2 mg Se per day for 9 days to achieve a marked reduction in mortality, which in the 'fulminant' category fell from 100 per cent (untreated) to 37 per cent (treated), and from 22 per cent to 0 per cent in the less severe cases. The mechanism remains obscure, but the researchers showed that complement activation was

reduced by the Se treatment. Alternatively, Chandra Ramanathan (University of Georgia, Athens, USA) pointed out that extreme Se deficiency has a pro-clotting and even haemorrhagic effect in animals. Since reading frames with a very high content of UGA codons can be found overlapping the coding regions of various HF viruses, including Ebola, it is possible that the extremely high viral loads seen in HF might cause host Se depletion, either by programmed selenoprotein synthesis, or by low frequency SeC insertion at UGA codons due to random slippage errors during viral transcription and translation.

Another exciting story involves new findings relevant to the Coxsackievirus-Keshan disease link. Orville Levander (US Department of Agriculture, Beltsville, USA) and Melinda Beck (University of North Carolina, Chapel Hill, USA) reviewed their recent work, showing how the cofactor role of Coxsackie B virus (CVB) is strongly supported by their demonstrations that Se deficiency can trigger cardiomyopathy in Coxsackie-infected mice, and that even a 'benign' strain of CVB3 becomes virulent in Se-deficient animals, where it mutates into a more virulent strain that can produce myocarditis even in Se-adequate mice. Similar results were obtained with vitamin E, which has a sparing effect on Se.

These findings have profound implications in regard to the possible role of nutritional status in emerging viral diseases. The underlying mechanisms in both the host and virus may be multifaceted, and so provoked much discussion. Esteban Domingo (Universidad Autónoma de Madrid, Spain) reviewed mechanisms of RNA virus evolution, including hyper-mutability and the viral quasispecies concept, which can explain the rapid mutation of CVB into a more virulent form when the immune system is weakened by nutritional deficiency, unleashing viral replication and permitting the selection of more virulent strains. Ernst Peterhans (University of Berne, Switzerland) amplified this mechanism in terms of the role of reactive oxygen species in cellular responses to viral infections, a recurring theme of the meeting. Luc Montagnier (Pasteur Institute, Paris, France) elaborated extensive evidence for the role of oxidative stress in activating HIV. The involvement of cellular transcription factors like NF-κB in this process was described in detail by Jean-Louis Virelizier (Pasteur Institute).

Alain Favier (UFR des Sciences, La Tronche, France) described multiple antioxidant abnormalities in persons living with HIV/AIDS (PWAs) (including low beta-carotene, zinc and Se), and *in vitro* studies of the roles of trace elements, e.g. showing that Se (as selenite) decreases activation of NF-κB as well as inhibiting the reactivation of HIV-1 by hydrogen peroxide and tumour necrosis factor. Multiple nutrient abnormalities in PWAs, including Se, were also discussed by Marianna Baum (University of Miami, USA), whose most striking finding was that low plasma Se was the highest single risk factor for mortality in the HIV cohort she has been studying, being substantially more significant than low CD4 count (defined as CD4 <200 cells/mm^3) as a risk factor.

MP Look (Medizinische Universitaetsklinik, Bonn, Germany) reported a brief clinical trial of supplementation with Se and *N*-acetyl cysteine (a glutathione precursor) in HIV infected individuals. While a consistent increase in CD4 counts was not seen, favourable shifts in some survival indicators such as T-cell subsets were observed.

Ursulla Cowgill (Department of EPO Biology Carbondale, USA) analysed AIDS death rates in the USA by race and sex relative to Se levels in forage plants in different counties and states. Data for blacks showed a correlation between low Se regions and AIDS mortality, but as might be expected, due to extensive food distribution networks, data for whites generally did not correlate. The hypothesis was offered that since AIDS in blacks is strongly associated with socio-economic factors like poverty, the black HIV population might be more likely to partake of locally grown foods in their diets.

Shu-Yu Yu (Chinese Academy of National Sciences, China) presented data from several extensive studies of human Se supplementation in regions of China where hepatitis B and primary liver cancer are endemic, showing significant reduction in disease incidence even with small daily doses. The mechanistic basis of this effect has yet to be determined.

A classical demonstration of the chemoprotective effects of Se is the work of Gerhard Schrauzer (Biological Trace Element Research Institute, San Diego, USA) with the mouse mammary tumour virus (MMTV). The incidence of mammary tumours consequent to infection with this retrovirus is reduced by increasing dietary Se. The relevance of this animal model to human disease is considerable; aside from the obvious implications for HIV and other retroviral diseases, the implications for human cancer are now quite inescapable. Isabelle Pelisson (Mt Sanai Medical Center, New York, USA) reported the remarkable discovery of sequences highly homologous to the MMTV envelope gene in 38 per cent of human breast cancer specimens. The investigators ruled out any known endogenous retrovirus, and showed a specific localization only to cancerous human breast tissues. This provides a new incentive for research into the role of viruses in carcinogenesis.

My own presentation involved an update on the 'viral selenoprotein theory' as a potential factor in some of the observations documented above. An analysis of the genomic structure of HIV-1 shows the potential for several novel frameshift sites, and shows that certain UGA codons in overlapping reading frames are well conserved in known HIV-1 isolates. Several genomic features required for the expression of such potential selenoprotein genes in HIV-1 have now been experimentally confirmed, such as an RNA pseudoknot in the reverse transcriptase coding region. We now have firm in vitro evidence of activity at a novel frameshift site in the HIV-1 *nef* coding region. Since the -1 reading frame has a highly conserved UGA codon, the possibility that this region encodes a viral selenoprotein must be considered. The most compelling data, highly distinctive GPx-related sequences in several strains of CBV, consistent with a virally encoded GPx enzyme, may relate to the results of Levander and Beck on CVB3. This suggests that a direct utilization of Se by the virus may contribute to observations on CVB3.

This unique meeting was organized by Dr Gerhard Schrauzer, and sponsored by Biosymposia and the International Association of Bioinorganic Scientists. The proceedings will be published in a special issue of *Biological Trace Element Research*, to be co-edited by Dr Schrauzer and Professor Luc Montagnier. Hopefully these proceedings will stimulate the development of new antiviral agents that go beyond simple Se supplementation.

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The Clinical Evaluation of Antiviral Drugs

28 February 1996, London, UK

Introduction

At the end of 1993 the British Society for Antimicrobial Chemotherapy and the Society of Pharmaceutical Medicine agreed to establish a joint working party, to decide upon guidelines and recommendations for the clinical development of new antiviral agents. An inaugural meeting was held on 14 April 1994 at the offices of The Ciba Foundation in London. The working party was chaired by Hugh McDade (Glaxo) and membership included experts in the field from academia, clinical practice and the pharmaceutical industry; sub-committees were established to concentrate on specific topics (regulatory aspects, preclinical considerations, laboratory evaluations in clinical research, and clinical pharmacology) and disease areas (HIV infection, herpesvirus infections, viral hepatitis and respiratory tract infections). Over the following 18 months a series of meetings was held, and draft recommendations were produced by each of the sub-committees and circulated for comment both within the group and to outside experts. A comprehensive document is now in the final stages of preparation.

The meeting, organized by the Society of Pharmaceutical Medicine and held at The Scientific Societies' lecture theatre in London, presented the draft recommendations of the working party to approximately 30 participants from the UK and Europe. It was structured as an interactive discussion meeting, where feedback from the delegates could be incorporated during the preparation of the final recommendations.

Antiviral clinical development

Regulatory aspects of antiviral clinical development were discussed by the sub-committee chair, Dr Flic Gabbay (Gabbay Group Ltd). Potent antiviral agents tend to be toxic, so the risks and benefits to the patient need to be carefully assessed and balanced. 'Fast-track' registration for life-threatening diseases such as AIDS may be possible. Increasingly, pharmaco-economic evaluation of potent, expensive new therapies will be necessary. Companies may well be subjected to substantial pressures to release unlicensed drugs on a 'compassionate-use' basis for people living with HIV/AIDS, which may raise regulatory issues.

Preclinical considerations reviewed by Dr Charles Penn (Glaxo Wellcome) indicated that a greater understanding of the properties and mode of action of antiviral agents is now expected before clinical studies can proceed. Studies should include demonstration of an antiviral effect *in vitro*, assessment of potential interactions, investigations into the mechanism of action and potential for the development of viral resistance, evaluation of possible mechanisms of toxicity and determination of specific toxicity.

Dr Jane Grundy (Royal Free Hospital) considered the question of laboratory evaluations during clinical development. For multicentre studies it is preferable to select a single laboratory to process and report on the specimens. An appropriately qualified member of the laboratory staff should be nominated to take day-to-day responsibility for the processing of trial samples to "Good Laboratory Practice" standards, and in compliance with the study protocol. The choice of clinical sample and timing of collection should be relevant to the natural history of the virus under investigation; high concentrations of the antiviral agent at the time of sampling may interfere with subsequent laboratory investigations.

Initial investigation in humans

Dr Michael Barry (University of Liverpool) addressed the issue of initial investigation in humans. Phase I studies with agents that have the potential to cause serious toxic effects may be performed in seriously ill patients rather

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PAGE PROOFS

Hepatitis C Virus Encodes a Selenium-Dependent Glutathione Peroxidase Gene

Implications for Oxidative Stress as a Risk Factor in Progression to Hepatocellular Carcinoma

W■■■ Zhang, A■■■ G. Cox, Ethan Will Taylor[1]

ABSTRACT

Aim: Using structural bioinformatics methods, the aim is to assess the hypothesis that hepatitis C virus (HCV) encodes a glutathione peroxidase (GPx) gene in an overlapping reading frame, linking HCV expression and pathogenesis to the Se status and dietary oxidant/antioxidant balance of the host.

Methods: The putative HCV GPx gene was identified by searching viral sequence databases, using conserved GPx active site sequences as probes, giving particular weight to the UGA (selenocysteine) codon. Multiple sequence alignments were generated and analyzed to validate the sequence similarity, and to establish the degree of conservation of the identified genomic features in HCV. Molecular modeling was used to assess the structural feasibility of the proposed homology.

Results: The GPx homology region overlaps the NS4 gene, and is well conserved in HCV. The sequence similarity of the conserved active site regions to a set of known GPx is high (4 to 6 SD greater than expected for similar random sequences). The computed strain energy of a molecular model of the HCV GPx is energetically favorable, comparable to the bovine GPx structure.

Conclusions: By linking HCV replication and pathogenesis to the Se status and dietary oxidant/antioxidant balance of the host, the existence of a viral GPx gene could help to explain why HCV disease progression is accelerated by oxidant stresses such as alcoholism and iron overload.

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ZUSAMMENFASSUNG

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The possibility that viruses might encode selenoproteins remained unexplored until 1994 when Taylor et al. published a study of the predicted RNA structure of the human immunodeficiency virus, HIV-1, in relation to potential novel open reading frames (ORFs, protein coding regions) of the virus [10]. That analysis demonstrated the potential for several new gene variants in HIV-1, which might encode selenoproteins, because of the existence of several highly conserved UGA codons (which can encode selenocysteine) in certain regions of HIV. These UGA codons were consistently found to be downstream of potential RNA structural features ("slippery" sequences and RNA pseudoknots) that would be required for the expression of these hypothetical genes by ribosomal frameshifting.

Subsequently, the hypothetical protein encoded by one of these predicted HIV-1 selenoprotein genes was shown to have highly significant sequence similarity to the active site domains of the mammalian selenoprotein glutathione peroxidase (GPx), despite being truncated by several internal and C-terminal deletions [8]. Along the same lines, highly truncated GPx-related sequences were identified in the potentially cardiovirulent Se-dependent B3 strain of coxsackievirus, CVB3 [9], which is the same strain that has been studied by Beck et al. [1] as a putative Keshan disease cofactor.

The only experimentally verified example of a viral selenoprotein is from a DNA virus, the pox virus Molluscum contagiosum, which has been shown to encode a functional full length GPx enzyme [7]. Zhang et al. subsequently reported that a number of RNA viruses encode GPx-related sequences, including, in addition to the HIV-1 and CVB3 examples mentioned above, potential GPx genes in measles virus and

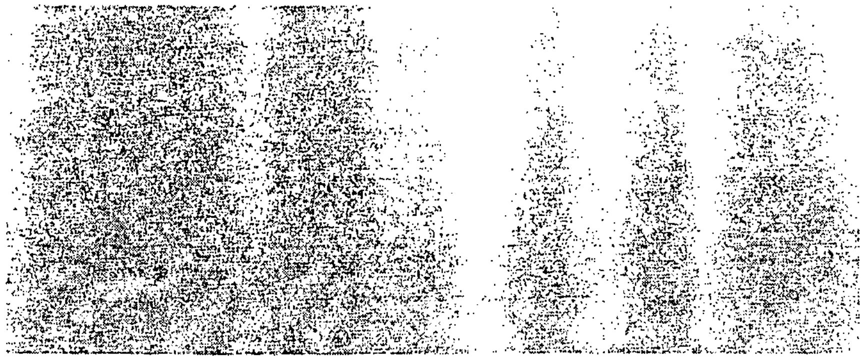
the hepatitis C virus, HCV [11]. Thus, it seems likely that this antioxidant selenoprotein module may prove to be a relatively common gene in both RNA and DNA viruses.

The current paper focuses on the case of the hypothetical HCV GPx gene first predicted by Zhang et al. [11], which is of considerable interest because HCV infection is very prevalent worldwide, is considered an emerging public health threat, and has poorly understood mechanisms of pathogenesis. We will assess the hypothesis that the HCV encoded sequence is a GPx homologue by means of structural bioinformatics methods, including some preliminary results of homology molecular modeling studies of the HCV GPx protein structure, which will be reported in depth in a separate publication [3]. Finally, we will review clinical data and other lines of evidence that are consistent with this hypothesis, and consider its clinical implications.

Methods

❑ *Database Searches and Potential Gene Identification:* As described previously by Zhang et al. [11], the potential HCV glutathione peroxidase (GPx) gene was identified by searching sequence databases with the program TFASTA, using known variants of several different GPx active site sequences as probes, e. g., VASYUGLT (plasma GPx), etc., where U represents the TGA codon in DNA, potentially encoding selenocysteine (Sec). An altered translation table was used, modified specifically so as to distinguish the TGA codon from the other two stop codons, as described in detail previously [6]. Highly ranked database hits were subsequently examined by translating all 3 reading frames in the region of the GPx sequence similarity, to determine the potential for expression of the potential gene by known mechanisms, i. e. a start codon, ribosomal frameshift site, or an RNA editing site.

❑ *Sequence Alignment and Assessment of Statistical Significance of Sequence Similarities:* The BlockAlign program [11] was used to generate multiple sequence alignments; this program produces an optimal, gapped alignment of a probe sequence against an existing sequence alignment. Combined with a randomization routine, it was also used for assessing the statistical significance of the similarity between a query sequence and a prealigned protein family (in this case, a multiple alignment of GPx sequences). Briefly, the method involves 1. the optimal alignment of the query sequence against the prealigned family (GPx), which also yields a similarity score, followed by 2. repeated random shuffling and realignment of the query sequence, 3. calculation of the average similarity score and standard deviation for optimally aligned random sequences of identical composition to the query sequence. The significance of the query-family homology can then be expressed as a shuffling statistic (Z score), calculated as the distance of the actual score from the average random score, in standard deviations (SD). The blosum62 amino acid similarity matrix was used for all sequence alignments, and Sec residues were treated as Cys for the purpose of these calculations.



❑ *Molecular Modeling:* The Sybyl program (Tripos Assoc., St. Louis, MO) was used to build a putative HCV viral GPx homology model, starting from the bovine cellular GPx X-ray crystal structure, file 1GP1 from the Brookhaven Protein Data Base. The model was constructed based on the alignment shown in simplified form in Figure 1 of Zhang et al. [6]. The model was constructed using the biopolymer protein-loop option of Sybyl, which uses a knowledge-based approach to rebuild regions where insertions and deletions are predicted by the alignment.

The resulting model was refined by energy minimization using molecular mechanics; the final minimization used the Kollman all-atom force field as implemented in Sybyl. The Sec residue was modeled as the deprotonated Se^- species. Default program options were used for the minimization, with a ΔE of 0.001 kcal/mol as the termination criterion in the final energy minimization. A

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P1 VNVASYUGLTD.QYLELNALQEELGPFGLVILGFPSNQFGKQEPGENSEILPSLKYVRPGGGFVP%WEPMKIHDIR.WNFE.KFLVGP
P2 VNVASYUGLTD.QYLELNALQEELGPFGLVILGFPCNQFGKQEPGENSEILPSLKYVRPGGGFVP%WEPMKIHDIR.WNFE.KFLVGP
P3 VNVASYUGLTG.QYIELNALQEELAPFGLVILGFPCNQFGKQEPGENSEILPTLKYVRPGGGFVP%WEPMKVHDIR.WNFE.KFLVGP
P4 VNVASYUGLTG.QYVELNALQEELEPFGLVILGFPCNQFGKQEPGENSEILATLKYVRPGGGFTP%WEPMKVHDIR.WNFE.KFLVGP
C1 ENVASLUGTTIRDYTEMNDLQKRLGPRGLVVLGFPCNQFGHQENGKNEEILNSLKYVRPGGGFEP%WSPVCRNDIA.WNFE.KFLVGP
C2 ENVASLUGTTVRDYTQMNDLQRRLGPRGLVVLGFPCNQFGHQENAKNEEILNCLKYVRPGGGFEP%WSPVCRNDVS.WNFE.KFLVGP
C3 ENVASLUGTTVRDYTQMNELQRRLGPRGLVVLGFPCNQFGHQENAKNEEILNSLKYVRPGGGFEP%WSPVCRNDVA.WNFE.KFLVGP
C4 ENVASLUGTTVRDYTQMNELQERLGPRALVVLGFPCNQFGHQENAKNEEILNSLKYVRPGGGFEP%WCPVCRNDVS.WSFE.KFLVGP
   └───R1───┘                          └───R2───┘                                └R3┘
```

Figure 1. Simplified multiple sequence alignment (single letter amino acid code) of the HCV encoded GPx homologue vs. a set of Se-GPx sequences. Two HCV genotype 1b strains are shown as V1 and V2; P1-P4 are plasma GPx, C1-C4 are cellular GPx. The symbol # indicates the positions of the conserved catalytic triad of Sec (U), Gln (Q) and Trp (W) in active site regions R1 to R3. Letters on the line above the alignment indicate an exact match between an HCV residue and one or more of the mammalian GPx sequences; an asterisk indicates a chemically similar residue. For brevity, 2 regions of the alignment are not shown: an N-terminal region of 35-40 residues and an internal region at %, of 25-40 residues. The symbol @ indicates an insertion of 11 amino acids in HCV, containing a conserved Trp residue, which, in many HCV isolates where this reading frame is more severely truncated at the 3' (C-terminal) end, may serve to substitute for the function of the active site Trp in R3.

monomer from the bovine GPx structure was minimized similarly for comparison. Additional details regarding these computational studies, the parameters used, as well as further optimization and analysis of the HCV viral GPx model using molecular dynamics, are reported in a separate publication [3].

Results and Discussion

Virus sequence database searches using variants of the Sec encoding region of GPx led to the identification of the GPx homology region in HCV, where the hypothetical GPx gene is in the -1 reading frame overlapping a known nonstructural gene, NS4b, of previously unidentified function. The overlapping region contains one in-frame "stop" codon, UGA, that can potentially encode Sec; this region also lacks a start codon. This may help to explain why this putative gene has not been noted previously, because it does not look like an open reading frame, and a known gene is encoded in the same region of RNA. The Se-dependent GPx sequence and UGA codon are well conserved in HCV genotype 1b (which is predominant in North America), and also in subtypes 2b and 3a. In several other HCV subtypes (1a, 1c), the UGA codon has mutated to an AGA (arginine) codon, a mutation which may be consistent with a non-Se-dependent GPx activity. The same mutation was noted in the putative HIV-1 GPx gene, in subtypes prevalent in Africa and Asia, whereas the UGA codon (Se-GPx) was conserved in the subtypes predominant in Europe and the Americas [8].

As shown in the simplified multiple sequence alignment of Figure 1, the putative HCV GPx sequence has regions of substantial similarity to known GPx sequences; the similarity encompasses the entire sequence, but is strongest in the active site regions. For a sequence block of about 25 residues (beginning VNVAS...) spanning active site region 1, the similarity is statistically significant at > 6 SD relative to random sequences of similar composition. The HCV GPx has features of both the plasma and cellular GPx types.

As discussed in detail previously [11], the HCV GPx is probably expressed via RNA editing rather than the ribosomal frameshift mechanism proposed for the HIV-1 GPx gene [10]. The putative RNA editing site in HCV, consisting of a homopyrimidine sequence (CCUCCCUU) with a triplet of C bases, is just downstream of a known HCV protease cleavage site (FDEMEEC/ASHLP), at the NS4a-NS4b junction in the main polyprotein open reading frame. This implies that, when translated from an edited RNA, the HCV GPx would be cleaved from the viral polyprotein only a few residues upstream from the beginning of the GPx homology region. This processing to form an independent protein suggests a predominantly intracellular and possible intravirion role for the HCV GPx.

A molecular model of the putative HCV GPx homologue was constructed using the HCV subtype 1b sequence shown in Figure 1 as sequence V2 (Genbank accession #D50483f). There is one insertion of about 10 residues in HCV (at the position shown by the symbol @ in Fig. 1), which contains several highly conserved residues, including an Arg and a Trp, that are likely to play an important role in the viral GPx homologue. However, because this has no direct structural equivalent in the bovine GPx structure, we did not attempt to model this insertion explicitly. All of the other insertions and deletions proved to be structurally feasible, because of extensive coiling of the protein backbone in the affected regions. Minimization of the protein model lead to a substantially negative molecular mechanics "strain" energy of –2,002 kcal/mole for 133 residues (-15.1 kcal/mole/residue), which on a per-residue basis is reasonably close to, but not quite as low as, the value obtained by minimizing the bovine GPx crystal structure, -3,619 kcal/mole for 185 residues (-19.6 kcal/mole/residue). Part of the difference arises because the HCV GPx has less electrostatic stabilization than the bovine GPx because the former has a net positive charge, which might be functionally significant (e.g., suggesting association of the viral GPx with the negatively charged backbone of the viral RNA in the virion, or with phospholipid membrane of the viral envelope or infected cell). Furthermore, the geometry of the GPx active site, and distances between active site residues, are not substantially changed in the model (Figure 2). Thus, overall, the molecular modeling results suggest that the proposed homology is structurally feasible.

Several additional lines of evidence support the hypothesis that Se status may play a role in HCV disease progression and outcome:

1. The best direct evidence consistent with an HCV-Se link are 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 Se concentrations compared to those without concomitant HCV-infection" [4].
2. HCV infection has been associated with dilated cardiomyopathy conse-

Figure 2. A comparison of the protein backbone and active site residues of the bovine GPx monomer (left) and a homology molecular model of the putative HCV viral GPx enzyme (right). The protein backbone is shown as a ribbon, and the sidechains of the catalytic triad of Sec, Gln and Trp are shown using a ball and stick rendition. The Se atom of the Sec residue is indicated.

quent to viral myocarditis [9]; this is similar to Keshan disease, the classical Se-deficiency disease, which is characterized by cardiomyopathy. Keshan disease is believed to involve a viral cofactor, coxsackie virus, which shows increased cardiovirulence in Se deficient hosts [1]. Cardiomyopathy has also been linked to Se deficiency in HIV infection [10].

3. Oxidant stresses like alcoholism and iron overload are associated with HCV disease progression, suggesting an antioxidant impairment is involved in pathogenesis. This iron effect is so significant that phlebotomy or intentional bleeding has been found to significantly improve recovery and response to interferon treatment in HCV infection [6]. Unbound iron is a powerful generator of reactive oxygen species via the Fenton reaction. Se protects against such free radicals and peroxides, and Se sequestration in a viral protein could make the host more susceptible to oxidative stress.
4. In several studies, people of low socioeconomic status were observed to have the highest risk of HCV infection; this is consistent with the possibility that malnutrition and consequent Se deficiency can enhance vulnerability to HCV infection, or increase chronicity of the infection by making it more difficult to overcome a primary infection.

In regard to the question of what a virus might gain by encoding a GPx, there are a number of possibilities: 1. A virally-encoded GPx could help a virus defend against free radical mediated attacks on infected cells by the immune system. 2. If associated with the viral particle, a viral GPx could also increase the extracellular viability of virus particles in the blood and extracellular compartments, at least in the case of an enveloped virus such as HCV, which is more susceptible to membrane lipid peroxidation once it has budded off the host cell and has lost the benefit of cellular antioxidant defenses. 3. A viral GPx might also have a regulatory (and potentially repressive) effect on viral replication, because, for example, it is known that oxidative stress (e. g. H_2O_2 exposure) activates the replication of HIV-1 and other viruses: a viral GPx would reduce oxidant tone, thus reducing viral activation. 4) A viral GPx might play a role in attachment to or penetration of target cells.

Conclusions

The prediction of GPx coding potential in HCV is primarily based upon the presence and conservation of significant protein sequence homology, particularly in active site regions, combined with evidence that the gene region in question may be expressed by known mechanisms of RNA editing and readthrough suppression. The specific viral mechanisms or RNA structure features that might be involved in recoding the UGA codon for translation as Sec remain to be identified. The results of the molecular modeling studies, and a number of independent lines of clinical evidence are also consistent with the hypothesis.

The existence of an HCV encoded Se-GPx, well conserved in genotype 1b, may help to explain certain clinical aspects of HCV infection. Significantly, genotype 1b is associated with the highest risk of progression to cirrhosis and hepatocellular carcinoma, and poor response to interferon. The outcome of chronic HCV infection is currently difficult to predict, and many carriers never manifest symptoms of disease, which begs the question as to what individual or environmental factors determine that outcome. Our results suggest that correlations between HCV disease progression and factors such as dietary Se intake and blood levels, markers of lipid peroxidation, etc., should be systematically investigated as potential correlates and predictors of outcome in HCV infection.

In the light of our findings, it is of considerable interest that Berkson has reported the successful treatment of individual cases of HCV-associated viral hepatitis with an antioxidant regimen that includes lipoic acid and Se [11]. Thus, the potential of dietary antioxidants as primary or complementary therapies for HCV infection merits serious investigation.

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(1) J Pediatr 1989 May;114(5):865-870
Selenium deficiency in low birth weight neonates: an unrecognized problem.
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To determine whether selenium deficiency is common among low birth weight infants in our neonatal intensive care unit, we surveyed blood samples from healthy full-term and preterm infants born in our hospital over a 3-month period. Selenium was measured by electrothermal atomic absorption spectrometry. Glutathione peroxidase was measured in plasma by an automated method. Baseline (less than 72 hours postnatal) selenium concentration and glutathione peroxidase activity were significantly lower in low birth weight infants than in full-term babies. Sequential selenium analyses were obtained in 16 sick low birth weight neonates who remained in the intensive care nursery for up to 6 weeks because of lung disease. All were fed parenterally without supplemental selenium, with or without oral intake, for periods varying from 3 to 60 days. All had a marked decrease from baseline selenium levels, and values below the detection limit of our assay were found in seven infants. Selenium deficiency is much more common in small infants than is generally realized, but the clinical significance in neonates is poorly understood.

(2) Acta Paediatr 1997 May;86(5):448-453
Evaluation of full-term infants fed an evaporated milk formula.
Friel JK, Andrews WL, Simmons BS, L'Abbe MR, Mercer C, MacDonald A, McCloy UR
Department of Pediatrics, Memorial University of Newfoundland, St John's, Canada.

The objective of this prospective, cohort study was to compare the nutritional status of full-term infants who were fed human milk (BF, n = 29), formula (FF, n = 30) or evaporated milk formulae (EM, n = 30) for at least 3 months. Infants were seen at enrollment, 3 and 6 months, at which times a blood sample, diet record and anthropometric data were collected. Infants in the EM group received solids earlier (12 +/- 5 weeks) than did FF infants (15 +/- 4 weeks), and both were earlier than BF infants (19 +/- 4 weeks). Only 26% of the EM fed group received iron supplements as ferrous sulphate drops. Seven BF, 12 FF and 20 EM had abnormal ferritin values (< 10 ng ml-1) at 6 months. Copper intake was lower in the EM infants at 3 and 6 months. However, plasma copper and erythrocyte copper zinc superoxide dismutase (ZnCuSOD) levels did not differ between groups. Selenium intake was lower in the EM group (5 +/- 1 and 10 +/- 5 micrograms d-1; 3 and 6 months) than in the FF infants (13 +/- 4 and 19 +/- 7 micrograms d-1; 3 and 6 months). Erythrocyte SeGHSPx levels in EM infants were lower at 6 months (EM, 33.2 +/- 3.4; FF. 35.2 +/- 3.9: BF, 36.1 +/- 3.8 mU mg Hb-1). Thiamin intake (0.99 +/- 0.08 and 1.24 +/- 0.32; 3 and 6 months, mg 1000 kcal-1) was higher in the FF group than in EM infants (0.38 +/- 0.39 and 0.66 +/- 0.38; 3 and 6 months). There were more (13%) abnormal thiamin assays in the EM group at 6 months than in the BF and FF infants (0%). In conclusion, infants fed evaporated milk formula receive adequate copper but may not receive enough thiamin or selenium. Unless supplemented from birth with medicinal iron, intakes of iron will be inadequate.

(3) Am J Clin Nutr 1996 Dec;64(6):860-865
Selenate fortification of infant formulas improves the selenium status of preterm infants.
Tyrala EE, Borschel MW, Jacobs JR
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The purpose of this study was to determine whether selenate fortification of infant formula would improve the selenium status of relatively well, growing, preterm infants during the first 12 wk of enteral feeding. A high-selenium group (n = 7, mean body weight = 1312 g) received selenate-fortified preterm and full-term infant formulas containing 0.36 and 0.22 mumol Se/L, respectively, and a low-selenium group (n = 10, mean body weight = 1262 g) received non-selenium-fortified preterm and full-term infant formulas containing 0.12 and 0.11 mumol Se/L, respectively. There were no significant differences in growth between the two groups throughout the study. The high-selenium group had significantly greater mean selenium intakes than did the low-selenium group from weeks 2 to 12. Plasma selenium concentrations decreased over the study period in the low-selenium group. Plasma selenium-dependent glutathione peroxidase activity was greater in the high-selenium group at week 12 only. Red blood cell selenium concentrations decreased over time in both groups and were significantly greater in the high-selenium group at weeks 4, 8, and 12. Plasma selenium concentrations were significantly

correlated with plasma glutathione peroxidase activity for all infants on study day 1 and at weeks 4 and 12. Selenium intake of all infants was significantly correlated with plasma glutathione peroxidase activity at 12 wk. Selenate fortification of infant formulas can improve the selenium status of preterm infants. Current selenium contents of infant formulas and recommendations for dietary intakes of selenium for some preterm infants may be inadequate.

(4) Nutrition 1995 Sep;11(5 Suppl):573-575
Selenium and glutathione peroxidase levels in healthy infants and children in Austria and the influence of nutrition regimens on these levels.
Rossipal E, Tiran B
Department of Paediatrics, University of Graz, Austria.

Serum selenium values were investigated in 56 formula-fed and in 18 wholly breast-fed infants. In 14 of these infants, glutathione peroxidase (GSH-Px) was also investigated. Determination of selenium and GSH-Px was also done in umbilical cord blood of seven healthy newborns. In another 109 infants aged 1-15 yr, serum selenium values were investigated. A continuous fall of serum selenium values was noted in the first 3 mo of life. Low levels continued until the age of 6 mo with a mean of 36% of the umbilical cord vein level. Breast-fed babies of GSH-Px showed a less pronounced fall in selenium and had significantly higher levels of GSH-Px. GSH-Px activity was reduced from age 5 to 8 mo. Feeding of beikost caused a rise in the level of selenium. Children in the age groups 1-15 yr still had a significantly lower serum selenium level than adults.

(5) Acta Paediatr 1995 Aug;84(8):859-862
Selenium in German infants fed breast milk or different formulas.
Jochum F, Fuchs A, Menzel H, Lombeck I
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At birth and at 4 months of age, selenium (Se) values of 129 term infants on three different diets were determined: 50 infants were breast fed (HM), 44 received formula based on cow's milk (F) and 35 were fed "hypoallergenic formula" (PHF) (partially hydrolysed whey protein). The Se status of a group of twins (n = 12) fed "hypoallergenic formula" was compared with the respective group of singletons. All infants had low plasma Se values during early infancy. The plasma Se of breast-fed infants remained stable (plasma Se 43 +/- 8 ng/ml at birth and at 4 months), whereas plasma glutathione peroxidase (GSH-Px) decreased (birth: 107 +/- 29 U/l; 4 months: 62 +/- 11 U/l). The formula-fed infants showed a reduction in plasma Se levels from birth to 4 months (38 +/- 10 ng/ml and 29 +/- 9 ng/ml, respectively). The decrease was even more pronounced in infants fed the "hypoallergenic formula". This group presented the lowest Se values (plasma Se 39 +/- 9 ng/ml at birth; 20 +/- 6 ng/ml at 4 months). Renal excretion of Se was found to be lower in the formula-fed infants (F and PHF) compared with the HM group. There was a significant correlation between plasma and urinary Se ($r = 0.62$, $p = 0.0001$). Urinary Se (microgram Se/g creatinine) appeared to be a good indicator of Se intake. Measurements of urine Se might be used as a screening method for the estimation of the Se supply. Weight and length increases in all infants were within the normal range. There were no differences between the different feeding groups.

(6) Nutr Rev 1995 Jun;53(6):159-166
Selenium in human lactation.
Sanz Alaejos M, Diaz Romero C
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The primary factor determining selenium concentration in human milk is the maternal selenium intake. A significant correlation between selenium in human milk and maternal selenium intake has been reviewed in papers from different regions of the world. Infants fed human milk have higher selenium intake than those fed commercially available formula milk or baby foods. Selenium compounds found in breast milk seem to be more biologically available for infant nutrition than those in formulas. Increased requirements of selenium have been observed in pregnant and lactating women. Supplementation of lactating and pregnant women with different selenium compounds has been assayed, and selenium supplementation of soil and cows has been used to increase the selenium status of children fed infant formula made from cow's milk.

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1) Am J Clin Nutr 1993 Nov;58(5):643-648
Selenium status of infants is influenced by supplementation of formula or maternal diets.
McGuire MK, Burgert SL, Milner JA, Glass L, Kummer R, Deering R, Boucek R, Picciano MF
Division of Nutritional Sciences, University of Illinois at Urbana-Champaign.

Plasma selenium of infants fed proprietary formula was significantly less than that in infants fed human milk. Addition of selenite to the formula (0.253 mumol Se/L) increased plasma selenium and activities of glutathione peroxidase (GPx) and total peroxidase (Px). However, erythrocyte selenium decreased significantly during the 12-wk study in infants receiving human milk or formula with or without supplemental selenite. Infants fed human milk from women receiving 0 or 200 micrograms supplemental selenium as selenomethionine or selenium-enriched yeast had plasma selenium that paralleled changes in their selenium intake. Plasma GPx and Px activities were unrelated to human milk selenium intake. Milk from women given either selenium supplement prevented the decline in infant erythrocyte selenium. Results of these studies suggest that the method of feeding modifies the infant's apparent selenium status and that the molecular form of selenium provided and/or its interaction with other milk constituents are determinants of infant selenium status.

8) Am J Clin Nutr 1987 Jan;45(1):49-53
Formula feeding results in lower selenium status than breast-feeding or selenium supplemented formula feeding: a longitudinal study.
Kumpulainen J, Salmenpera L, Siimes MA, Koivistoinen P, Lehto J, Perheentupa J

Thirty-two infants completely weaned by age 3.2 mo were randomized into two groups. Unsupplemented group was fed cow's milk-based liquid formula containing 3-5 micrograms Se/L. Se-supplemented group received the same formula supplemented with 20 micrograms Se/L. A third group consisted of exclusively breast-fed infants (51 at age 4 mo, 41 at 6 mo, 12 at 9 mo). Mean serum Se concentration in unsupplemented group decreased from 41 to 31 micrograms/L during the first 2 mo and remained constant until age 6 mo increasing gradually thereafter. In Se-supplemented group it increased steadily from 41 to 68 micrograms/L at age 6 mo and remained constant while supplemented formula was used. In breast-fed group it increased steadily until age 9 mo, between the levels of the two formula-fed groups, when it reached the concentration of Se-supplemented group. At age 12 mo no significant differences were present among the three groups.

CONCLUSIONS:
- Se def. ↑ or low blood level is common in infants & children (#1, #4)
- Worse in pre-term infants (#1, 3)
- Formula-fed babies will have lower Se than breast fed unless supplemented (#2, 3, 4, 5, 6, 7, 8)
- Se status often declines from birth for first 2-6 months of life. (#4, 5, 7)

HYPOTHESIS: HIV-induced Se depletion (may) and/or increased HIV replication due to reduced Se (↑ or ↓ stress) may be a factor in accelerated HIV disease prog. in children, esp. in malnourished populations.

Selenium: an essential element for immune function

Roderick C. McKenzie, Teresa S. Rafferty and Geoffrey J. Beckett

The importance of selenium for optimal immune function is now apparent. Here, Roddie McKenzie and colleagues describe how selenium is involved in the function of immune cells, and the various immune deficiencies and diseases that result from inadequate dietary intake.

Selenium (Se) was discovered in 1817 by Jons Jacob Berzelius who named it after Selene, the Greek goddess of the moon. It is a toxic metaloid with a wide range of industrial applications, including the production of semiconductors, photocopiers, stainless steel and antidandruff shampoos. In 1957 it became evident that Se was an essential trace element that prevented hepatic necrosis in vitamin-deficient rats[1]. In humans, clinical signs of toxicity (selenosis) appear when dietary intake exceeds 900 μg/day, an intake that can be exceeded in countries where high concentrations of Se are found in the soil. A Se intake of up to 450 μg/day is regarded as safe[1].

The earliest evidence that Se is involved in immune function came in 1959 with the observation that dogs injected with ^{75}Se incorporated the isotope into a leukocyte protein; this protein is now known to be cytoplasmic glutathione peroxidase (cGPX). In sheep and humans, Se is concentrated in tissues involved in the immune response, such as spleen, liver and lymph nodes[2]. Later it was appreciated that the trace element was essential for normal growth and development and could also prevent myopathies associated with nutritional status in farm animals. In areas of China where there is marked Se deficiency, a severe endemic cardiomyopathy known as Keshan disease and a deforming osteoarthritis known as Kashin–Beck disease are found[1]. Both these diseases respond to Se supplementation. Many studies suggest that adequate intake of Se is required to ensure optimal immune function and to prevent malignancy. Various components of the immune system fail to function correctly if dietary Se is deficient[2] (Box 1).

The discovery in 1973 that Se was an integral part of the selenoenzyme cGPX provided a mechanism by which Se could exert its biological actions; this enzyme detoxifies harmful organic hydroperoxides, as well as hydrogen peroxide, which are produced during oxidative metabolism (reviewed in Refs 1–4). However, more-recent work suggests that cGPX is less important in preventing oxidative damage in the absence of stress and may simply provide a buffer to ensure a continued Se supply when dietary intake is limited. Other GPXs and selenoenzymes appear to be essential in preventing oxidative damage to the cell whereas some selenoproteins, such as the iodothyronine deiodinases, have roles that are unrelated to detoxification (reviewed in Refs 1, 3, 4).

Several studies have suggested that the incidence of malignancy and cardiovascular disease is inversely related to Se intake, but the precise means by which Se protects against these diseases is still unclear[1]. If Se does have marked effects on health and well-being, it is of concern that in many countries dietary Se intake is well below recommended levels[5].

Sources of Se

Ultimately, the soil is the source of Se; it enters the food chain through incorporation into vegetable protein as the amino acids selenocysteine and selenomethionine. Thus, if the animal and human population eat predominantly food produced locally, the Se status of the population will reflect soil levels. In many parts of Europe Se intake is low (30–40 μg/day[1,5]). The recommended dietary intake (RDI) for Se in the UK is 75 μg/day for adult males and 60 μg/day for adult females. In many parts of the USA, intake is above the RDI at 90 μg/day. In Finland, the low Se status of the livestock and human population, together with the high prevalence of skeletal muscle myopathy in animals, as well as heart disease and malignancy in humans, prompted a Se supplementation programme whereby inorganic Se was introduced into the food chain via fertilizers. This programme has increased Se intake in the Finnish population to ~125 μg/day.

Food contains organic forms of Se such as selenomethionine and selenocysteine but many experiments involving Se supplementation have used inorganic forms such as selenite. Absorption and use of organic and inorganic Se compounds differ. Organic forms appear to be re-used by the body more efficiently than inorganic forms, apparently because selenomethionine substitutes nonspecifically for methionine residues in proteins but in such circumstances the Se has no bioactivity. For bioactivity and synthesis of specific selenoproteins, the trace element must be present as a selenide-like intermediate that incorporates into specific selenocysteine residues, usually at the active site of the selenoprotein[1,3,4]. Evidence suggests that selenide is more-readily formed from inorganic rather than organic Se. Furthermore, selenite but not selenomethionine reacts readily with glutathione in erythrocytes to form selenodiglutathione[6], a compound that has anticarcinogenic properties and induces apoptosis of human tumour cells[7].

Most Se present in tissues and blood is incorporated into selenoproteins, leaving little free Se. Many *in vitro* experiments have used selenocompounds at concentrations that are nonphysiological in that they reflect total rather than free circulating levels of Se. Circulating Se levels are low in many diseases, but this does not imply that Se is involved in the pathogenesis of the diseases. In many illnesses, trace elements such as zinc fall as part of the acute-phase response.

Selenoproteins and protection from oxidative damage

Selenoproteins are present in every cell type. At least 20–30 selenoproteins exist, but

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Box 1. Effects of Se on immune cell function

Se supplementation

In vivo

↑ Neutrophil migration and O_2^{2-} activity (cow)
↑ High-affinity IL-2 receptor (mouse)
↑ T-cell proliferation and function following age-related decline (mouse)
↑ Natural killer cell activity (mouse, human)
↑ Cytotoxic T-cell activity (mouse)
↑ T-cell response to pokeweed mitogen (cow)
↑ Lymphokine-activated killer cell activity
↑ Enhanced delayed-type response due to better antigen presentation (mouse)
↓ Cell death following paraquat exposure (rat)
↓ UV-induced skin cancers and mortality (mouse)
↓ Erythema following UV exposure (human, mouse)
↑ Vaccine-induced immunity to malaria (mice)

In vitro

↓ HIV long-terminal-repeat activation and HIV replication in T cells (human)
↓ NF-κB activation (human)
↓ B-cell lipoxygenase activity (human)
↑ Antibody responses (primary and secondary) to virus (cow)
↓ Cell death following UV irradiation of skin cells (mouse, human)
↓ DNA damage and lipid peroxidation in UV-exposed skin cells (mouse, human)
↓ IL-6, IL-8 and TNF mRNA following UV treatment of skin cells (human)
↓ Cell death following paraquat exposure (human)
↑ Apoptosis in tumours (human, mouse)
↓ Apoptosis induced by UV in normal skin cells (human)
↑ Phytohaemagluttinin response in lymphocytes (human)
↑ Killing by macrophages (human)
↑ Target killing by cytotoxic T cells (human)

Se deficiency

↑ Platelet aggregation and leukotriene synthesis (atopic human)
↓ IgG and IgM titres (human)
↓ Antibody production by lymphocytes (mouse)
↑ Virulence of Coxsackievirus (mouse)
↓ Neutrophil chemotaxis (goat)
↓ Neutrophil and leukocyte activity (pig)
↓ Candidacidal activity by neutrophils (rat)
↑ $CD4^+$ T cells, ↓ $CD8^+$ T cells, ↓ $CD4^-/CD8^-$ thymocytes (mouse)

Abbreviations: HIV, human immunodeficiency virus; IL, interleukin; TNF, tumour necrosis factor; UV, ultraviolet radiation; ↑, increase; ↓, decrease.

only about 12 have been either partially or completely characterized (reviewed in Refs 1, 3, 4) and most appear to catalyse oxidoreduction reactions[1,3,4]. It is widely held that changes in selenoprotein expression explain many of the biochemical and clinical manifestations of selenium deficiency, although direct effects of selenium compounds on cells cannot be excluded. Immunologically, the ability of selenoproteins to protect the host from oxidative stress is vitally important, since many host defence systems rely on the microbiocidal effects of macrophage- or neutrophil-generated free-radical species. Oxidative species are generated through general metabolism, during the metabolism of xenobiotics and during exposure to ultraviolet radiation (UV) in sunlight. Inflammation as a process to clear infection and damaged tissue also generates great oxidative stress. If antioxidant systems are not functioning correctly, host cells will be damaged.

Much is known about the functions of the family of GPXs. The membrane-bound phospholipid hydroperoxide GPX (PHGPX) detoxifies phospholipid hydroperoxides and, along with vitamin E, helps prevent oxidative damage to membranes. The PHGPX may be more important than the cGPX in protecting the cell from oxidative stress. Elimination of peroxides in the extracellular fluid is dealt with by the extracellular or plasma form of GPX. Peroxynitrites, products of superoxide and nitric oxide, are produced in skin cells during exposure to UV and cause single-strand DNA breaks. The synthetic Se compound ebselen functions as a GPX and prevents these breaks[8]. Ebselen also inhibits the proinflammatory enzymes nitric oxide synthase and protein kinase C (Ref. 9). In addition, the GPXs play a vital role in the synthesis of arachidonic acid metabolites. The lipoxygenase and cyclooxygenase pathways produce hydroperoxyeicosatetraenoic acids, which must be reduced for lipoxin, prostaglandin and leukotriene synthesis[2]. Eicosanoid synthesis is depressed in Se deficiency (see Ref. 2). Furthermore, accumulation of lipoperoxides impairs prostacyclin synthesis and promotes thromboxane accumulation, which can increase platelet aggregation in cardiovascular disease[5].

Other selenoproteins include the iodothyronine deiodinases (types I, II and III), which regulate the metabolism of thyroid hormones in all tissues. Clear functions are still being sought for other selenoproteins such as selenoprotein P and selenoprotein W. The former may be involved in Se transport; the latter is lost in Se-deficiency-induced myopathy[1,3,4].

The discovery that thioredoxin reductase (TDR) is a selenoenzyme is of great interest. One of its substrates is the 12 kDa thiol-protein thioredoxin[10] on which it acts as a protein disulphide reductase. TDR acting alone or in conjunction with thioredoxin can thus affect the redox regulation of a variety of key enzymes, transcription factors and receptors including ribonucleotide reductase, the glucocorticoid receptor, AP-1 and NF-κB. Thioredoxin is classified as a T-cell leukaemia survival factor because it stimulates expression of the α subunit of the interleukin 2 (IL-2) receptor[10]. In addition to

Box 2. Human diseases affected by Se or correlated with Se status

Se deficiency

Keshan disease (endemic cardiomyopathy)
Atopic asthma – low platelet glutathione peroxidase
Kashin–Beck disease (endemic deforming arthritis)
Coronary heart disease – correlates directly with low serum Se
AIDS – low serum Se correlated with rapid disease progression
Spontaneous abortion – correlates with low Se status
Psoriasis – severity and duration of disease correlated directly with decreased blood Se levels
Skin cancers – abnormally low serum Se in T-cell lymphoma and malignant melanoma
Myxodematous cretinism – low serum Se

Se supplementation

Reduction in gastrointestinal, prostate and lung cancers (Se-replete population)
Depressed neutrophil activity and increased monocyte chemoattractant protein in the elderly
Protection against hepatitis B-induced hepatoma
Improved sperm motility in subfertile men
Decrease in lipid peroxidation following UV exposure

For abbreviations, see Box 1.

reducing thioredoxin, TDR can break down hydroperoxide and lipid peroxides in the presence of NADPH more efficiently than GPX can. Some anticarcinogenic effects of Se appear to be mediated via TDR (Ref. 11).

Effects of Se on immune function

Se deficiency depresses the effectiveness of immune cells generally, with diverse specific effects listed in Box 1. Supplementation with Se appears to boost cellular immunity by three mechanisms. First, it upregulates the expression of the T-cell high-affinity IL-2 receptor[12] and provides a vehicle for enhanced T-cell responses. Since the T cell is a key component in providing B-cell help for antibody synthesis, this may explain the stimulatory effects of Se on antibody production (Box 1). In fact, age-related decreases in cellular immunity can be partially reversed by Se supplementation increasing responsiveness to IL-2 (Ref. 13). Second, it prevents oxidative-stress-induced damage to immune cells. Third, it alters platelet aggregation by decreasing the ratio of thromboxane to leukotriene production. Human diseases associated with Se deficiency are listed in Box 2 and below.

Selenium and viral diseases

In mice, the coxsackievirus mutates to a virulent cardiotoxic form when it is passaged through Se-deficient hosts[14], possibly because the host immune system is weakened. The fact that human Keshan disease is seen in Se-deficient areas of China led to the hypothesis that it may be caused by a coxsackie B virus that mutates to a cardiotoxic form in Se-deficient hosts. This disease responds to Se-dietary supplementation. Similarly, in China, the incidence of hepatitis-B-induced hepatoma fell after Se supplementation[15]. In AIDS patients, Se status is predictive of survival times[16]. There is also the intriguing observation that virulent strains of influenza virus evolve in Se-deficient areas of China and the suggestion that human immunodeficiency virus (HIV) may have crossed the species barrier into man in Se-deficient areas of Africa. Selenite also seems to protect mouse cells in culture from the transforming effects of murine mammary tumour virus. Interestingly several viruses (molluscum contagiosum, HIV-1, coxsackie B and Ebola) contain sequences with homology to GPX (Ref. 17); perhaps these viral GPXs might be involved in protecting the virus from host-cell-derived peroxides.

Se and protection from UV-induced damage and cancer

UV is the most ubiquitous environmental carcinogen. Skin exposure leads to DNA, lipid and protein damage by direct and free-radical-mediated effects. In mice, dietary Se supplementation reduces incidence of and mortality from UV-induced non-melanoma skin cancers. Se supplementation prevents DNA adduct accumulation, cell death, lipid peroxidation and inflammatory cytokine induction in skin cells in culture following UV irradiation[18]. Transfection with a viral GPX also improves keratinocyte survival following UV exposure[19].

An exciting finding is that doses of 200 μg/day selenomethionine reduce the overall incidence of prostate, lung and gastrointestinal cancers by 39% (Ref. 20). These effects may result from a number of mechanisms including preventing oxidative damage to DNA, maintaining effective immune defences and inhibiting tumour proliferation.

Conclusion

In conclusion, adequate Se is essential for immune function and can protect the immune system from oxidative damage. Dietary Se supplements hold promise as a means of treating inflammatory conditions and rejuvenating the ageing immune system, and in protection from carcinogenesis.

We apologize to authors not quoted in the text because of space constraints; references for studies not listed in our reference section can be obtained from the authors. We thank G. Priestley and J. Arthur for their help in preparation of the manuscript. Our work is supported by the British Foundation for Skin Research, the Foundation for Skin Research, and the Sir Stanley and Lady Davidson Trust. T.S.R. holds a Faculty of Medicine Scholarship from the University of Edinburgh.

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HIV-1 Encodes a Sequence Overlapping *env* gp41 With Highly Significant Similarity to Selenium-Dependent Glutathione Peroxidases

To the Editor: A progressive decline in plasma or serum selenium levels paralleling the loss of $CD4^+$ T cells has been widely documented in HIV-1 infections. As reviewed (1), such findings have been reported in more than 20 papers published during the last decade originating from independent laboratories in at least six different countries and have been observed for asymptomatic and symptomatic HIV patients, for children and adults (2–7).

The observations of several investigators suggest that this HIV-associated decline in selenium levels cannot be explained simply by malnutrition or nutrient malabsorption. According to Dworkin (3), levels of plasma selenium, cardiac selenium (at autopsy), and the antioxidant selenoprotein glutathione peroxidase in patients with AIDS-related complex and AIDS are "significantly correlated with total lymphocyte counts," but selenium depletion can occur "irrespective of the presence or absence of diarrhea or gastrointestinal malabsorption" (3). This suggests that the decline in selenium levels that parallels the progression of HIV disease cannot be attributed entirely to malabsorption. Similarly, other researchers have pointed out "a surprisingly high prevalence of low levels of selenium in early stages of the disease" (2) well before wasting is usually observed and found that ". . . a low selenium intake seems unlikely [as a cause of low plasma selenium], because urinary excretion, which closely reflects the actual selenium intake, was similar in HIV-1 infected patients and controls" (5). Allavena et al. (6) exclude malabsorption as the underlying cause, correlate selenium levels with survival prognosis, and conclude that "the measurement of trace elements, especially selenium, may be a useful marker to predict the HIV infection progression." Similarly, in a 12-month study involving 95 patients, Constans et al. (7) show that serum selenium is a statistically significant predictor of outcome in HIV infection.

The dramatic results of the study by Baum et al. (8), a 3.5-year longitudinal study of 125 HIV-positive intravenous drug users, show that selenium deficiency is an exceptionally high risk factor for HIV-associated mortality. The relative risk (RR) for mortality associated with selenium deficiency (RR = 10.8, $p < 0.002$) is far higher than that for any other nutrient deficiency and 15 times higher than that associated with a low CD4 count (defined as <200 cells/mm^3; RR = 0.69, $p < 0.04$).

In light of this body of clinical evidence, we would like to report an observation of potential significance for the molecular mechanisms underlying any interaction between HIV and dietary selenium. Taylor et al. (9) previously pointed out several regions of HIV-1 with the potential to encode selenoproteins due to the existence of potential frameshift sequences and conserved UGA codons (potentially encoding selenocysteine) in several locations overlapping known HIV genes. One of these overlaps the HIV-1 *env* gp41 coding region in the −1 reading frame and contains a single UGA codon near the middle of a highly conserved novel open reading frame (ORF) of about 120 amino acids, following a conserved −1 frameshift signal (A AAA), consistent with the established "P-site" slippage mechanism.

We have identified the sequence encoded in this ORF, which we call *env-fs*, as a homologue of glutathione peroxidase (GPx), the prototypical eukaryotic selenoprotein. The database search method we used in 1994 was not sensitive enough to pick up this similarity, in part probably because of several deletions in the active site region and the high mutation rate of retroviral sequences, leading to a low overall similarity to any single GPx sequence.

Nonetheless, the presence in this HIV-1 genomic region of a common variant of the GPx active site consensus sequence, spanning the catalytic selenocysteine, is apparent on examination of a multiple alignment of GPx sequences (Fig. 1: YUGAT in HIV-1 versus YUGLT in GPx, where U = selenocysteine). This similarity occurs precisely at the location of the single conserved UGA codon in the −1 reading frame of the gp41 coding region of HIV-1. This UGA codon is highly conserved in the predominant North American–European HIV-1 strains (subtype B), but other HIV-1 subtypes and most HIV-2 subtypes have a conserved arginine codon (AGA) at this position. This substitution is also highly consistent with peroxidase activity, because arginine is the active site residue of many peroxidases (e.g., horseradish peroxidase), and arginine variants of the GPx active site motif (e.g., SLRG rather than SLUG or SYUG) are common in non–selenium-dependent peroxidases.

Using more sensitive sequence comparison methods, a highly significant similarity between this HIV-encoded sequence and GPx can be demonstrated. For example, we have assessed the similarity of *env-fs* to the aligned set of GPx sequences by a variation on the standard method for comparing two sequences, which we implemented in a computer program developed in our laboratories. In brief, the program produces an optimal alignment of the probe sequence (*env-fs*) compared with a fixed multiple alignment (GPx) by optimizing a scoring function that correctly takes into account linear gap penalties in the multiple alignment. The score of the optimal alignment of the probe sequence is the best possible value of the sum of the scores of the pairwise alignments induced between the probe sequence and the rows of the input multiple alignment. Then the probe sequence is randomly shuffled (50 times in this case) and realigned by the same algorithm. The average alignment score and the standard deviation (SD) for random sequences of identical composition are then calculated, and the *significance* of the actual score is expressed as a Z score (i.e. the number of SD from the "expected" random score).

Using a truncated GPx alignment as shown in Fig. 1, the blosum62 similarity matrix, gap creation penalty of 5, and gap extension penalty of 2, the *env-fs* sequence is aligned essentially as shown, with a significance score of 5.0 SD. Using the full GPx alignment (including the region shown as % and extending at the C terminus), with slightly different gap parameters, a Z score of 4.9 SD is obtained, producing an alignment that is somewhat looser in the C-terminal region but otherwise identical to that in Fig. 1 up to the YUGLT active site region.

The possibility of a virally encoded selenium-dependent GPx gene is not unprecedented. A GPx homologue was reported in the molluscum contagiosum virus (10). We have also identified GPx-related sequences in coxsackie B virus, the cofactor in Keshan disease, a classic selenium-deficiency disease (1).

Because a GPx with a UGA codon at the active site is a selenoprotein, the existence of this sequence in HIV-1 strongly suggests that a conserved UGA codon in HIV may encode selenocysteine, rather than a conventional amino acid, which can also occur. This possibility merits serious consideration in

```
Match: G SR  Y  TV DADG *   *  Y * A  F**  *YUG*TAS *A  S  *GHQ* PGKN        KGS  PG  G*L K  L** C LEC
env-fs GSSRKHYGRTVNDADGTGQTIIVWYSAAAEQFAE..GYUGATAS.VATHS..LGHQAAPGKNPGCGKIPKGSTAPGDLGLLWKTHLHHCCALEC
S05317 AAQSTVYAFSARPLTGGEPVSLGSLRGKVLLIENVASLUGTTIR%LGFPCNQFGHQV.YGARW....VALGSRLPRA.GHRLLSPLV..LQLEC
A53395 RCARSMHEFSAKDIDG.HMVNLDKYRGYVCIVTNVASQUGKTEV%LAFPCNQFGRQE.PGSDAEI....KE...FAA.GYNVKFDMFSKICVNG
S20501 SKPQSIYDFTVKDAKG.NDVDLSIYKGKVLIIVNVASQCGLTNS%LAFPCNQFGGQE.PGSIEEIQN.MVCTR......FKAEYPIFDKVDVNG
Jq0476 GISGTIYEYGALTIDGEEYIPFKQYAGKYVLFVNVASYUGLTG.%LGFPCNQFGKQE.PGENSEILPTLKYVR.PGG.GFVPNFQLFEKGDVNG
A55086 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%LGFPSNQFGKQE.PGENSEILPSLKYVR.PGG.GFVPNFQLFEKGDVNG
Jx0176 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%LGFPCNQFGKQE.PGENSEILPSLKYVR.PGG.GFVPNFQLFEKGDVNG
Jx0280 GVGGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTG.%LGFPCNQFGKQE.PGENSEILATLKYVR.PGG.GFTPNFQLFEKGDVNG
S18912 DEKGTIYDYEAIALNKNEYVPFKQYVGKHILFVNVATYCGLTA.%LGFPCNQFGKQE.PGDNKEILPGLKYVR.PGG.GFVPNFQLFEKGDVNG
A47367 DVKGTIYDYEALSLNGKEDIPFKQYRGKHVLFVNVATYCGLTI.%LGFPCNEFGKQE.PGDNLEILPGLKYVR.PGK.GFLPNFQLFAKGDVNG
B40464 GVAGTVYEYGANTLDGGEYVQFQQYAGKHILFVNVASFCGLTA.%LGFPCNQFGKQE.PGKNSEILLGLKYVR.PGG.GFVPNFQLFEKGDVNG
A45207 FIAKSFYDLSAISLDG.EKVDFNTFRGRAVLIENVASLUGTTTR%LGFPCNQFGHQE.NQQNEEILNSLKYVR.PGG.GYQPTFTLVQKCEVNG
S21119 VAQSTVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTR%LGFPCNQFGHQE.NGKNEEILNSLKYVR.PGG.GFEPNFTLFEKCEVNG
S06304 AAAQSVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTVR%LGFPCNQFGHQE.NAKNEEILNSLKYVR.PGG.GFEPNFMLFEKCEVNG
S03723 AAAQSVYSFSAHPLAGGEPVNLGSLRGKVLLIENVASLUGTTVR%LGFPCNQFGHQE.NAKNEEILNSLKYVR.PGG.GFEPNFMLFQKCEVNG
                                +++++++++ ++++++++++++++++++
```

FIG. 1. Sequence alignment of hypothetical HIV-1 *env-fs* sequence compared with various selenium-dependent glutathione peroxidases. The HIV-1 sequence shown as *env-fs* is as given in Table 1 of Taylor et al. (9), beginning right after a predicted putative protease cleavage site. It is aligned with a set of eukaryotic glutathione peroxidases (GPx) from various sources (single letter amino acid code, with U as the symbol for selenocysteine [Sec], encoded by the UGA codon in RNA). A match to one or more of the aligned GPx sequences is shown as a letter in the match line above the alignment; similar residues are indicated by an asterisk. Active site regions of GPx are indicated by ++++++ below the alignment. Compare the matches in the region of the conserved catalytic selenocysteine, with *env-fs* residues YUGATA, with YUGLTG in GPx sequence Jq0476 (here, UGA means Sec-Gly-Ala, not the UGA codon). The most common variant in the predominant North American–European HIV-1 subtype B is SYUGAT instead of GYUGAT, an even better match to many of the GPx; the active site motif usually is SYUGLT or SLUGTT. After a 19 residue deletion in the HIV sequence (shown as % in the alignment), a serine (S) in *env-fs* aligns with a conserved cysteine (also one GPx has S rather than C), followed by GHQ . . . PGKN, which are exact matches to several GPx sequences. The deletion (at %) in *env-fs* between these two conserved regions (YUG and GHQ) is not part of the GPx active site and thus is not a problem structurally. The N terminus of the mature GPx protein begins at the fourth residue in the alignment, and one human GPx variant (S05317) ends just beyond the end of the alignment; thus, a known GPx variant is truncated almost identically as this putative HIV GPx homologue. The total similarity score of the *env-fs* sequence compared with the aligned GPx sequences is 5 standard deviations above the average similarity score of randomly shuffled sequences of identical composition, each optimally aligned by the same algorithm. Assuming a normal distribution of random scores, this significance level (5 SD) means that the probability of obtaining this degree of similarity purely by chance is less than 1 in 1 million.

light of the high statistical significance of the *env-fs* similarity to the GPx sequence and the clinical data strongly linking HIV disease progression to the host's selenium status.

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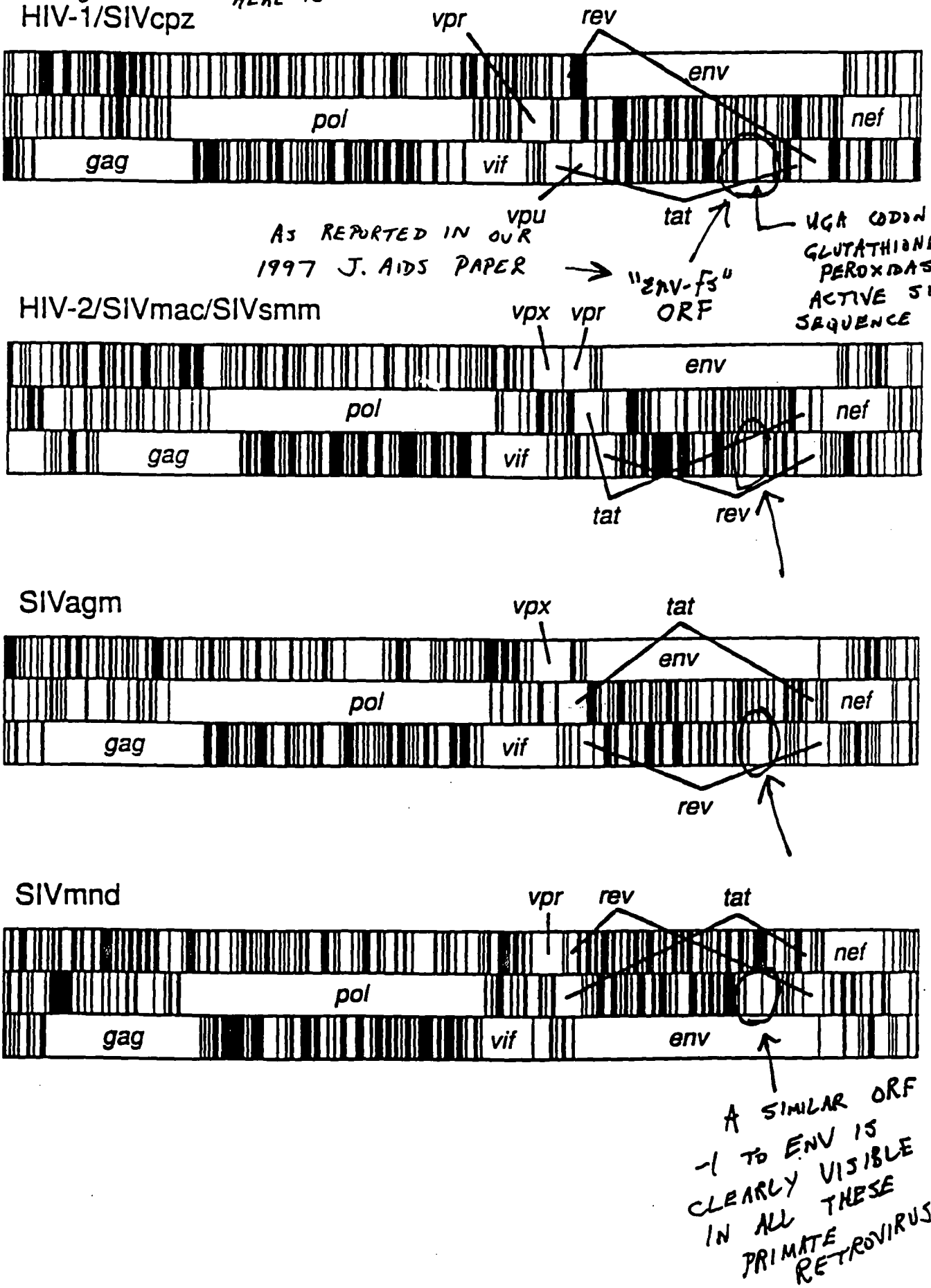
– Fig. FROM GIBBS & DESROSIERS, IN HUMAN RETROVIRUSES, ED. CULLEN, 1993

Fig. SHOWS KNOWN HIV GENES. HERE IS ONE THEY MISSED!



FYI - These are some of >20 papers published over the last decade linking Se levels to HIV disease progression.

1995
Constans et al. France

Serum Selenium Predicts Outcome in HIV Infection

To the Editor: Recent studies suggest that serum selenium levels decrease in HIV-positive patients and are lower in patients with advanced disease (1,2). We mea-

DISCUSSION

Serum selenium decreases in several chronic diseases, particularly in HIV-positive patients (1,2). In the present work, we found that selenium decreases like the CD4 cell count. Moreover, serum selenium is related to prognosis, irrespective of CD4 cell count. The functions of selenium

ELSEVIER SCIENCE IRELAND
Chemico-Biological Interactions 91 (1994) 181–186

New York

Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS)

Brad M. Dworkin

Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology
15:370–374 © 1997 Lippincott-Raven Publishers, Philadelphia

U. Miami - See attached press release

High Risk of HIV-Related Mortality Is Associated With Selenium Deficiency

*Marianna K. Baum, *Gail Shor-Posner, †Shenghan Lai, *Guoyan Zhang, *Hong Lai, †Mary Ann Fletcher, ‡Howerde Sauberlich, and *J. Bryan Page

Eur J Clin Nutr 51 (4): 266-272 (Apr 1997)

U. Bonn, Germany See attached

Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection.

Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, Sudhop T, Hoch J, Stockinger K, Spengler U, Sauerbruch T

SHORT REPORT
1997

Key words: selenium; oxidative stress; human immunodeficiency virus; muscle; zidovudine

MUSCLE NERVE 20:386–389 1997

France

MUSCLE INVOLVEMENT IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS IS ASSOCIATED WITH MARKED SELENIUM DEFICIENCY

PATRICK CHARIOT, MD,[1,2,4*] MARIE-LAURE DUBREUIL-LEMAIRE, MD,[3]
JIANG YAN ZHOU, PhD,[1] BOUCHRA LAMIA, MSc,[1,4]
LAURENCE DUME, Pharm D,[1] BLANDINE LARCHER, PharmD,[1]

University of Miami Press Release

STUDY FINDS SELENIUM AFFECTS SURVIVAL IN HIV/AIDS

EMBARGO UNTIL SEPTEMBER 30, 1997

MIAMI, Florida, September 30, 1997 - Scientists at the University of Miami School of Medicine, Center for Disease Prevention, reported today in the Journal of AIDS that deficiency of selenium, an essential trace element for maintaining a healthy immune system, has a profound effect on survival in HIV infected men and women. The researchers, led by Dr. Marianna K. Baum, found that HIV-1 infected patients with selenium deficiency were 19.9 times more likely to die of HIV related causes than those with adequate selenium levels.

The dramatic results of this NIH funded study in 125 HIV infected men and women, published on September 30 in the Journal of Acquired Immune Deficiency Syndrome, demonstrate that selenium plays a critical role in HIV-1 disease. The researchers also found that while other nutrients (vitamins A, B_{12}, and zinc) affect survival, these nutrients produce a substantially lower risk for mortality. In fact, when the nutrients were examined together, SELENIUM had the strongest impact upon mortality.

The authors suggest that the striking relationship between selenium deficiency and mortality could be related to selenium's antioxidant function and/or action in gene regulation which may affect HIV replication. As proposed in a report of Dr. Will Taylor, of the University of Georgia, published in the same issue of the Journal of AIDS, selenium, as part of selenoproteins, could have an important role in regulating HIV expression. He has proposed a novel viral mechanism that contributes to a decline in selenium levels accelerating disease progression, while adequate selenium would be expected to prevent HIV replication, and thus, delay the course of disease progression.

Based on this research, Dr. Baum's team is developing a study to determine whether selenium treatment can slow disease progression and improve survival over time in HIV infected men and women. The study has been approved and is under consideration for funding by NIH Institutes, including the National Institute on Drug Abuse and the Fogarty International Center.

Selenium Deficiency Characterizes HIV Infection (April 1997 Reuters report)

WESTPORT, Apr 22 (Reuters) - Selenium deficiency is common among individuals with advanced HIV infection, according to German researchers. Whether this is the result of malnutrition or opportunistic infection, they believe this selenium deficiency needs to be corrected.

Dr. M. P. Look of the University of Bonn and colleagues evaluated the antioxidant defense status of 104 HIV-positive patients. "Evidence...suggests that an imbalance between diminished host antioxidant defenses and increased formation of oxygen radicals and proinflammatory cytokines create a state of 'oxidative stress' in HIV disease, Dr. Look explains.

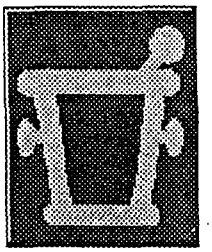
Because selenium is a key element in the antioxidative enzyme glutathione peroxidase, Dr. Look's group measured the subjects' serum selenium, erythrocyte glutathione peroxidase (GSH-Px) activity, plasma thiol (-SH) and glutathione (GSH) concentrations. In addition, they assessed clinical stage and surrogate markers of HIV disease for each patient.

Overall, they found that selenium levels positively correlated with CD4 cell counts, and inversely correlated with markers that indicated progressing HIV disease. "Serum selenium and GSH-Px activity in hospitalized AIDS patients was significantly lower as compared to asymptomatic patients and healthy subjects, whereas plasma SH and GSH concentrations were lower in both asymptomatic and AIDS patients, than in the controls."

Based on these results, they recommend that controlled studies should be aimed at maintaining maximum levels of micronutrient and vitamin status, to suppress oxidative stress and perhaps also to help suppress HIV replication.

European J Clin Nutrition 1997;51 :267-272.

British researcher urges increased selenium intake



It is time that we begin to take the declining intake of the trace mineral selenium seriously, says the British researcher Dr. Margaret P. Rayman, in an editorial in the February 1997 issue of the British Medical Journal. Among the problems that she sees associated with the low selenium levels in blood are infertility, cancer and heart disease.

"Declining sperm quality" and "increased incidence of cancer" are headlines that are becoming more and more common, and they are among the problems that the British university researcher Dr. Margaret P. Rayman says can be explained by reference to the drastic fall in the public's intake of the trace mineral selenium. In England alone, where Dr. Rayman lives and works, the average selenium intake has declined by half in the course of the past 22 years. Today it lies under the recommendation established by British public health authorities.

Somewhat the same situation prevails in the Scandinavian countries and in the rest of Europe. Dr.Rayman sees the falling selenium intakes as a threat to public health, because selenium is one of the most important elements in the body's defences against cancer and heart disease. The rare trace mineral is also decisive for human reproduction. This relationship was made clear when the Scottish researcher Dr. Alan MacPherson showed some years ago in a double-blind, placebo-controlled trial that selenium supplementation could increase infertile men's sperm quality by 100 percent. [*MacPherson A et al. 1993*] It has been demonstrated that "relatively harmless viruses can become virulent by passing through a selenium deficient host" which raises new questions about the prevention of viral diseases in a period in which HIV and new influenza types are flourishing globally.

"It is time to act"

As Dr. Rayman sees it, we must act now. Selenium deserves more attention than it has formerly received. Dr. Rayman wonders why it is that British farmers have been giving their animals selenium supplements for years in order to prevent the occurrence of disease but no one has introduced a similar policy for humans.

"Perhaps it is time to consider measures such as those adopted in Finland" in 1984, she says in her editorial. (In Finland, a program for selenium enrichment of the soil through selenium-enhanced fertilizers has been in operation for some years.) Alternatively, she suggests that we could fortify various foodstuffs with selenium, eat Brazil nuts (the best food source of selenium) or take selenium as a supplement.

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Molecular modeling studies and *in vitro* activity of the expressed protein are consistent with the hypothesis that HIV-1 encodes a functional glutathione peroxidase gene

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ABSTRACT Based on theoretical evidence, it has been proposed that HIV-1 may encode several selenoprotein modules, one of which (overlapping the *env* gp41 coding region) has highly significant sequence similarity to the mammalian selenoprotein glutathione peroxidase (GPx). The similarity score of the putative HIV-1 viral GPx homolog relative to an aligned set of known GPx is 6.3 SD higher than expected for random sequences of similar composition. Based on that alignment, a molecular model of the HIV-1 GPx was constructed by homology modeling from the bovine GPx crystal structure. Despite extensive truncation relative to the cellular GPx gene, the structural core and the geometry of the catalytic triad of selenocysteine, glutamine and tryptophan are well conserved in the viral GPx model. All of the insertions and deletions predicted by the sequence alignment proved to be structurally feasible, because of extensive coiling of the protein backbone in the affected regions. The model is energetically favorable, with a computed molecular mechanics strain energy close to that of the bovine GPx structure, when normalized on a per-residue basis. To validate the predictions of the computational chemistry and biology methods, the hypothetical HIV-1 gene was cloned, and found to encode functional GPx activity when expressed as a selenoprotein in mammalian cells. In transfected canine kidney cells, the increase in GPx activity ranged from 21 to 43% relative to controls (avg. 30%, n=9, $p<0.0001$), whereas in transfected MCF7 cells, which have low endogenous GPx activity, a near 100% increase was observed (avg. 99%, n=3, $p<0.05$).

Abbreviations: GPx, glutathione peroxidase; Sec, selenocysteine; NF-κB, nuclear factor kappa B; SECIS, selenocysteine insertion sequence; 5'-DI, 5'-deiodinase; TDR, thioredoxin reductase.

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As various genome projects have continued to expand the number of entries in nucleic acid sequence databases, there has been an increasing demand for computational biology and computational chemistry methods capable of solving several fundamental problems (1). These include the prediction of: i) the existence, location and architecture of genes, ii) the functions of the encoded proteins, and, ultimately, iii) the structures of the encoded proteins. Advances in comparative sequence analysis, including methods for the identification of remote homologs (1, 2), coupled with advances in protein structure prediction and molecular mechanics (3-5), have now brought all of these objectives within reach, at least when there is some degree of homology between a novel gene and known examples in databases. The ability to identify remote homologs and predict their protein structures are still major challenges for computational chemists and biologists.

The need for such advanced computational methods should not be underestimated, because their use can lead to the identification of genes whose existence or function is not obvious and which can be missed even after extensive analysis by conventional methods. To illustrate exactly such a case, we present here an example involving a complex retroviral genome, that of the human immunodeficiency virus type 1 (HIV-1). In 1994, based on theoretical evidence, Taylor et al. proposed that HIV-1 might encode several selenoprotein modules (6). Subsequently, one of the proposed HIV selenoprotein genes, potentially expressed by a ribosomal frameshift from the *env* coding region (thus named *env-fs*) was demonstrated to have highly significant sequence similarity to the mammalian selenoprotein glutathione peroxidase (GPx), which contains a catalytic selenocysteine (Sec) residue, encoded by the UGA codon in RNA (7). There is no mystery as to why this potential gene was not identified earlier, because the UGA codon generally serves as a stop signal for protein synthesis, and only rarely as a Sec codon (8); furthermore, like the retroviral *pol* gene, the putative HIV-1 GPx gene lacks a start codon, and thus does not appear to be an "open" reading frame. However, it does have a well conserved potential -1 frameshift sequence (A AAA) consistent with the established "P-site slippage" mechanism (7, 9).

In the current study, based on a revised multiple sequence alignment (Fig. 1), we have generated a full 3-D protein model of the hypothetical HIV-1 selenium (Se) dependent GPx, refined the structure using molecular mechanics, and applied various structural and energetic criteria to assess the model. In order to validate the predictions of the theoretical studies, we have cloned this hy-

pothetical HIV-1 gene, expressed it as a selenoprotein in several mammalian cell lines, and assayed for functional GPx enzyme activity in the transfected cells.

MATERIALS AND METHODS

Sequence analysis. The statistical significance of the similarity between the hypothetical HIV-1 *env-fs* protein (putative GPx) as a query sequence and a prealigned set of mammalian GPx sequences was assessed using the BlockAlign program of Zhang et al. (10). This program produces an optimal, gapped alignment of a probe sequence against an existing sequence alignment. By random shuffling of the query sequence, the average similarity score for optimally aligned random sequences of identical composition can be calculated. Briefly, the method involves i) the optimal alignment of the query sequence against the prealigned family (GPx), which also yields a similarity score, followed by ii) repeated random shuffling and realignment of the query sequence, iii) calculation of the average similarity score and standard deviation (SD) for optimally aligned random sequences of identical composition to the query sequence. The significance of the sequence similarity between the query and the given sequence alignment can then be expressed as a shuffling statistic (Z score), calculated as the distance of the actual score from the average random score, in SD. For the alignment of Fig. 1, the blosum62 amino acid similarity matrix was used, with gap creation and gap extension penalties of 6 and 2, respectively; 100 random shufflings were used for the significance calculation. Sec residues were treated as Cys for the purpose of these calculations, because no amino acid similarity matrix has yet been developed that includes Sec: as discussed previously (10), this will lead to a slight *underestimation* of the significance of alignments involving Sec residues in conserved positions.

Molecular modeling. The Sybyl program (Tripos Assoc., St. Louis, MO) was used to build the putative HIV-1 viral GPx homology model, starting from the bovine cellular GPx X-ray crystal structure (11), file 1GP1 from the Brookhaven Protein Data Bank. Based on the alignment of Fig. 1 (in which the bovine GPx sequence is shown as #P00435), the model was constructed using the *biopolymer protein_loop* option of Sybyl, which uses a knowledge-based approach to rebuild regions where insertions and deletions are predicted by the alignment. Both insertions and deletions were handled by deleting one or more residues on either side of the affected region, and then building a new loop that included those residues, plus or minus the required number of residues

(e.g., for a single residue insertion, delete 2, rebuild 3). The resulting model was refined by molecular mechanics energy minimization using the Kollman all-atom force field as implemented in Sybyl. A notable difference between Cys and Sec residues is the increased acidity of the selenol group, which has a pKa around 6, as opposed to approximately 7 for the SH group of Cys. Thus, the Sec residue was modeled as the deprotonated Se^- species. Force field parameters and partial atomic charges for the Sec residue were estimated by analogy to the Kollman parameters for the Cys residue, combined with the results of HF/STO-3G *ab initio* molecular orbital calculations on Cys, Sec, methyl thiol and methyl selenol, using the Gaussian 94 program (Gaussian, Inc., Pittsburgh, PA. The absolute change in charge was noted on going from the S to the Se compounds. Kollman partial charges were assigned to the Sec residue in such a way that the *net* change from the Cys charges were zero, to preserve electrical neutrality (charge conservation). The results of the molecular orbital calculations on Cys and Sec indicated that changing S to Se had little effect on the charge on the amino acid α-carbon; thus, the partial charges on the Sec backbone atoms, including the α-carbon, were set to the same values as those for Cys. The total charge on the neutral side chain is then 0.016, as calculated by Kollman for Cys. Adding a minus one formal charge to the side chain for the ionized state gives a total charge for the sidechain of -0.984. The calculated *ab initio* partial charges for the C, two H and Se atoms of deprotonated methyl selenol (which summed to -0.987) were then scaled to sum to the required total (-0.984), resulting in partial charges that are almost identical to the *ab initio* partial charges for the same atoms from the Gaussian calculation. Thus the partial charges used were: Se, -0.706; βC, -0.254; βH, -0.012. All of the force field parameters and partial charges are listed in supplemental material and posted on our web site, http://bioinfo.chem.uga.edu/ homepage/wtaylor. For the electrostatic potential energy term, the default distance-dependent dielectric model was used (i.e., dielectric constant = r, where r is the distance in angstroms between nuclei). A ΔE of 0.001 kcal/mol was used as the termination criterion in the final energy minimization. The model was initially minimized using a set of distance constraints to maintain the geometry of the three residues involved in the catalytic triad, Sec (U), Gln (Q) and Trp (W). In previous computer simulations of various GPx active site intermediates modeled from the bovine GPx crystal structure, the Q and W residues were shown to act as H-bond donors to the ionized Se atom (12). Thus, the interatomic distances from the Se atom to the Trp N1 (3.5 Å), from Se to the Gln amido N (3.4 Å), and between the Gln and Trp

nitrogens (3.9 Å) were constrained for the first round of energy minimization, after which the constraints were removed for the final minimization of the model. For comparison, a monomer from the bovine GPx structure was minimized similarly but without constraints.

Construction of HIV-1 GPx eukaryotic expression vector. Because the hypothetical HIV-1 GPx protein encoded by *env-fs* is predicted to be expressed by a -1 frameshift (6), the primary gene product would be an *env* gp120-GPx fusion protein, with an expected MW of about 130 kDa. The GPx homology region is the C-terminal 89 amino acids of that predicted fusion product, in which form it might function as a minor *env* glycoprotein isoform on the outer membrane surface of virions or infected cells (10). However, a potential HIV-1 protease site was predicted immediately upstream of the GPx homology region (6,7), so the GPx module may also be cleaved and released as an independent small (9 kDa) protein, functioning inside the cell and possibly in the HIV-1 virion. It is this low MW isoform of the putative HIV-1 GPx protein that we have cloned, incorporating a Met start codon immediately before the Gly residue that is at the N-terminal end of *env-fs* in the GPx alignment shown in Fig. 1. The DNA fragment corresponding to the putative HIV-1 GPx homolog was PCR-amplified from the *env* gp41 coding region of the pBH10 clone of HIV-1, obtained from the NIH AIDS Research and Reference Reagent Program, Rockville, MD. The PCR primers used for the HIV-1 *env*-GPx fragment were: 5' sense, 5'-TTTGCTAGCATGGGAAGCAGCAGGAAGCACTATG-3', containing a NheI site, and 3' reverse complement 5'-GCAAGCTTCTAGCATTCCAAAGCACAGC-3', containing a HindIII site. Note that in the first primer listed above the required ATG start codon has been incorporated just before the start of the coding sequence (GGAAGC...).

The established mechanism of Sec insertion in eukaryotic selenoproteins involves an RNA stem-loop structure called a selenocysteine insertion sequence (SECIS) element in the 3'-untranslated region (3'-UTR) of the mRNA. Via a looping back interaction with the ribosome, the SECIS element facilitates the decoding of in-frame UGA codons in the upstream coding region as Sec (8). Incorporation of a segment of the 3'-UTR spanning the SECIS element of a known eukaryotic selenoprotein has been shown to be sufficient for Sec incorporation in heterologous selenoprotein genes, and in synthetic peptides containing UGA codons (13, 14). Because the mechanisms and location of downstream RNA structure elements that HIV may use for recoding the UGA stop codon as a sense codon for Sec are still unknown (10), the putative HIV-1 GPx

expression construct was designed to include the SECIS element of the rat 5'-deiodinase (5'-DI) gene (13), in order to ensure the translation of the upstream in-frame UGA codon of the HIV-1 *env-fs* peptide as Sec during expression in mammalian cells. A region of the 3'-UTR of the rat type I 5'-DI gene was PCR-amplified from pCDM8-G21, provided by Dr. Marla Berry, Harvard Medical School, Boston, MA. The PCR primers used for the 5'-DI SECIS were: 5' sense, 5'-GCAAGCTTCGAGTAACTCTGTTCCACTG-3', containing a HindIII site, and 3' reverse complement, 5'-CCGGATCCCGGATTATAATCGTTAGC-3', containing a BamHI site.

To generate the eukaryotic expression vector pGD, the HIV-1 *env*-GPx and 5'-DI SECIS fragments were subcloned into the pEGFP-C1 vector (Clontech), using the following cloning sites: NheI, HindIII (GPx) and HindIII, BamHI (SECIS). The sequence of the entire transcribed region of the resultant plasmid pGD was verified by DNA sequencing. Sequencing and oligonucleotide syntheses were performed at the University of Georgia Molecular Genetics Instrumentation Facility. Restriction enzymes were obtained from Promega; other chemicals were obtained from Sigma unless otherwise specified.

Cell culture and transfection. The MCF-7 human breast cell line was obtained from the American Type Culture Collection (Rockville, MD). The MDCK (canine kidney) cell line was a gift from Dr. Fengxiang Gao, Centers for Disease Control and Prevention, Atlanta GA. Both cell types were grown under 5% CO_2 at 37°C, in DMEM/F12 medium supplemented with 2 mM L-glutamine, 5 μg/ml each of insulin, transferrin and gentamycin, and 0.5% newborn calf serum (Atlanta Biologicals). MDCK cells were transiently transfected using lipofectamine (GIBco), on 6-well plates, with each well seeded with 2.5×10^5 cells and 10 μg of plasmid DNA. After transfection, cells were grown in medium containing 20 nM sodium selenite for 72 hours and then harvested for the GPx activity assay. MCF-7 cells were stablely transfected by the same method, with 10 μg plasmid used for 3×10^5 cells. Transfected cells were selected for antibiotic resistance to G418 at 400 μg/ml, by changing G418-containing medium supplemented with 100 nM sodium selenite every 3 days for 21-30 days. Positive clones were pooled and propagated in the same medium until confluence and harvested for the GPx activity assay.

GPx enzyme activity assay. Both transiently transfected MDCK cells and stablely transfected MCF-7 cells were harvested and resuspended in phosphate buffered saline. Cells were lysed by sonication at 4° C at low power. Cell lysates were centrifuged at 25,000G for 25 min. at 4° C.

GPx activity in the supernatant was measured by spectrophotometric determination of NADPH utilization (as described in ref. 15), in the presence of standardized concentration of exogenous glutathione and t-butyl-hydroperoxide as substrates. The specific enzymatic activities are presented as means +/- standard deviation, in units/mg protein. Protein concentration was measured by the Lowry method with bovine serum albumin as the standard.

RESULTS

Alignment and similarity assessment of HIV-1 *env-fs* vs. GPx sequences. As detailed previously, the putative viral GPx module is encoded overlapping the HIV-1 *env* gp41 coding region in the -1 reading frame (6, 7); thus the gene was tentatively named *env-fs*, for *env* frameshift. It contains a single UGA codon (potentially encoding Sec) near the middle of a small but highly conserved novel open reading frame, lacking a start codon but having a conserved -1 frameshift signal near its 5' end. The translated HIV-1 *env-fs* sequence contains a common variant of the GPx active site consensus sequence spanning the catalytic Sec, shown as a U in Fig. 1. A strong similarity between the entire HIV-1-encoded *env-fs* sequence and an aligned set of GPx sequences has been demonstrated: as previously aligned by Taylor et al. (7), the similarity score of this novel HIV sequence vs. an aligned group of GPx sequences is 5 standard deviations (SD) above the average similarity score of randomly shuffled sequences of identical composition. We have examined alternative alignments of the C-terminal region of *env-fs* to GPx in the light of the bovine GPx crystal structure and its active site (11, 12), and identified the viral sequence potentially matching a third GPx active site region with a conserved Trp (W), shown as region 3 (R3) in the revised alignment of Fig. 1. Thus, this alignment is different in the C-terminal region from that published previously (7). Both the revised alignment and the proposed homology are strongly supported by the fact that the Trp (W) codon of *env-fs* aligned with the known active site Trp residue in R3 (Fig. 1) is very highly conserved in HIV-1 sequences. This conservation also provides compelling theoretical evidence that this is a functional gene, because the UGG Trp codon in the -1 frame is in the context of the sequence UCUGGA, which encodes Ser-Gly in the HIV envelope gp41 protein. Due to the degeneracy of the genetic code, Ser could be encoded using any base in the third codon position (i.e. UCN), so the absolute conservation of a U in this position in HIV-1 sequences can only be explained by a functional requirement, such as the need for the UGG to en-

code Trp in the -1 frame (there is no known HIV-1 RNA structural element spanning this UGG codon, which could have provided an alternative explanation for the conservation).

The significance score for the *env-fs* vs. GPx similarity increases to 6.3 SD based on the revised alignment of Fig. 1, which reveals a total of 3 major internal deletions (of 19, 11 and 41 residues) in the HIV-1 sequence relative to the mammalian GPx sequences, as well as a truncation of 30 residues at the C-terminal (not shown). These deletions in *env-fs*, which comprise a total of over 50% of the sequence of the mammalian GPx homologs, lie between or downstream of the three conserved active site regions (YUG, GHQ, and W) in the primary sequence.

The putative HIV-1 GPx is most similar to the plasma GPx sequences, having 23 of 89 residues (26%) identical to aligned plasma GPx residues in Fig. 1, whereas only 19 of 89 (18%) residues are identical to those of the aligned bovine cellular GPx sequence (#P00435 in Fig. 1).

Molecular modeling. To assess the proposed homology in structural terms, a 3-D molecular model of the HIV-1 viral GPx (Fig. 2) was made by homology modeling from the bovine GPx structure (11), based on the alignment of Fig. 1. Despite the presence of four major deletions in the HIV primary sequence relative to known GPx genes (labeled 1-4 in Fig. 2), modeling of the putative HIV GPx homolog is structurally feasible, because these deletions lie outside of the active site domain, and several of the internal deletions, despite their large size (up to 41 residues), are located in places where their excision permits the backbone to be easily reconnected despite the loss of numerous residues. For example, from an examination of deleted region 3 as shown in Fig. 2 it can be seen that this domain begins and ends at approximately the same location (a little above the active site W label in the figure), and thus coils back on itself, so that its deletion permits the resulting structure to be annealed without disruption of the structural core. A similar situation exists for deletion 1, which consists of a helix and part of a β sheet that folds back upon the helix. The deletion labeled 4 in Fig. 2 is the C-terminal region not included in Fig. 1; this deletion involves the loss of an outer strand of the core β sheet, which is thereby reduced from 5 to 4 strands, along with the C-terminal helix seen at the upper center of Fig. 2, which lies upon the β sheet in the bovine GPx structure. Despite these major deletions, the truncated GPx homolog encoded by HIV-1 includes the structural β sheet core of the enzyme, and the entire active site region including the catalytic triad, the residues of which are located in 3 separate regions (R1-R3 in Fig. 1) of the protein chain that come together in 3-D space to form the active site (labeled U, Q

and W in Fig. 2). Significantly, the second and third deletions involve non-catalytic regions whose function in the bovine GPx is in multimerization to form GPx tetramers. The absence of these domains suggests that the HIV-1 GPx homolog probably does not function as a multimer, for which there is a precedent, because the phospholipid hydroperoxide GPx is known to act as a monomeric enzyme (16).

Minimization of the protein model as described in the Methods section was readily accomplished, using a simple distant dependent dielectric model for solvation. In the final unconstrained model, the critical distances between the Se atom and the 2 H-bonding NH donors were still very close to those in the bovine GPx structure (Se to Trp N1, 3.2 vs. 3.5 Å; Se to Gln N, 3.3 vs. 3.4 Å). A comparison of the active site geometry of the fully minimized model vs. the bovine GPx crystal structure was made by means of a root-mean-square (RMS) fit of the three heavy (non-H) atoms of the residues of the catalytic triad involved in H-bonding (Sec Se, Trp N1, Gln amide N). This gave an RMS value of 0.42 Å, suggesting that the required active site geometry is conserved in the HIV-1 GPx homolog despite the extensive deletions and low sequence identity (18%) relative to the bovine sequence.

Energy minimization of the protein model with the Kollman all atom force field led to a substantially negative molecular mechanics "strain" energy of -1409 kcal/mole for 89 residues (-15.8 kcal/mole/residue), which on a per-residue basis is reasonably close to, but not quite as low as, the value obtained by minimizing the bovine GPx crystal structure with the same parameters, -3619 kcal/mole for 185 residues (-19.6 kcal/mole/residue). A closer examination of the non-bonded energy terms (which are the major contributors to the energy) suggests that the volume packing of side chains in the model is not quite as good as for the crystal structure (total van der Waals energy of -2.9 kcal/mole/residue for the model vs. -4.0 kcal/mole/residue for the bovine GPx), but that the electronic stabilization of the structure is almost as good (total electrostatic energy of -16.9 kcal/mole/residue for the model vs. -18.2 kcal/mole/residue for the bovine GPx; the total H bond energy is essentially identical, -0.39 vs. -0.40 kcal/mole/residue). This electrostatic stabilization arises in part because of a favorable distribution of ionizable acidic and basic residues which, in the model, participate in a number of salt bridges between distant residues, which can potentially stabilize the structure, particularly by anchoring the N- and C-terminals of the protein chain. These predicted ion pairs include Arg4-Glu88, Lys5-Glu88, His6-Glu30, Arg9-Glu30, Glu34-

His50, N-term-NH_3^+-Asp72. We are continuing to refine this initial model by molecular dynamics simulations, which is expected to generate more optimal side chain orientations for residues that, when mutated, were placed in somewhat arbitrary computer-generated conformations; this should improve the van der Waals energy contribution.

Cloning, expression and functional assay of the putative HIV-1 GPx homolog. The expression construct pGD was designed to include the SECIS element of the rat 5'-DI gene, in order to ensure the expression of the HIV-1 *env-fs* peptide as a selenoprotein in mammalian cells, such as the MCF7 and MDCK canine kidney cell lines, which contain all the necessary cellular machinery for selenoprotein expression. The question is, does that expressed peptide encode functional GPx enzyme activity, or is it a "nonsense" peptide, the expression of which would lead to a *decline* in cellular GPx activity by competing for limited cellular pools of $tRNA_{Sec}$? In cells transfected with the pGD construct, we observed a consistent and significant increase in GPx activity relative to cells transfected with an "empty" construct, the parent pC1 plasmid lacking the *env-fs* and 5'-DI SECIS inserts. In transiently transfected canine kidney (MDCK) cells, an increase in GPx activity ranging from 21 to 43% relative to controls was observed (avg. 30%, n=9, $p<0.0001$), whereas in stablely transfected MCF7 cells, which have low endogenous GPx activity, a near 100% increase could be demonstrated (avg. 99%, n=3, $p<0.05$). However, the absolute increase in GPx activity was greater in MDCK cells, probably because MCF7 cells have low levels of $tRNA_{Sec}$ and/or other cellular factors required for selenoprotein synthesis, consistent with their low endogenous GPx activity. The concentration of sodium selenite in the medium required for optimal GPx expression was also different in the two cell types, being 100 nM in MCF7 cells, as opposed to only 20 nM in MDCK cells. These results are detailed in Table 1.

DISCUSSION

By means of comparative sequence analysis, molecular modeling, and functional assay of the expressed protein, we have assessed the two-fold hypothesis that a novel HIV-1 gene, *env-fs*, is encoded in an overlapping reading frame of the *env* gene, and that the protein it codes for is a highly truncated Se-dependent GPx module (6, 7). Although the pairwise identity of the putative HIV-1 GPx homolog to individual GPx sequences is very low, by using a sensitive multiple sequence comparison method, we have demonstrated a highly significant sequence similarity, with a

Z score of >6 SD (Fig. 1). Furthermore, the essential sequence elements (residues of the GPx catalytic triad) are highly conserved within HIV-1 subtypes, as discussed previously (7, 10).

The molecular modeling studies strongly support the proposed homology, by demonstrating that:

i) despite several extensive deletions, the truncated GPx homolog encoded by HIV-1 includes the structural core and the complete catalytic center of the enzyme;

ii) the deletions are structurally feasible, because the deleted regions project away from the active site and in several cases consist of non-catalytic domains involved in dimer and tetramer formation;

iii) the geometry of the GPx active site, e.g., distances between critical active site atoms and residues, is not substantially changed in the model relative to the bovine GPx crystal structure;

iv) there is no exceptional molecular mechanics strain energy associated with the HIV-1 GPx model, which appears to have an energetically favorable distribution of charged residues leading to the electronic stabilization of the structure.

Thus, overall, the molecular modeling results suggest that the proposed homology is structurally feasible and consistent with the known structural requirements for Se-dependent GPx catalysis (12), supporting the hypothesis that the hypothetical HIV-1 gene *env-fs* encodes a truncated but potentially functional Se-dependent GPx homolog. Using a standard enzyme assay, we have validated the theoretical results by cloning the putative viral peptide shown as *env-fs* in Fig. 1, and demonstrating that it encodes functional GPx activity when expressed as a selenoprotein in mammalian cells.

The significance of these results can best be understood in the context of current knowledge regarding the biological roles of Se in the immune system and in the regulation of HIV-1 transcription. Se is an essential trace mineral that serves as a potent dietary antioxidant, in addition to other biological functions. Many of its cellular actions, mediated by selenoproteins such as GPx and thioredoxin reductase (TDR), are intimately linked to the redox status of the cell, and to the redox regulation of genes that are important for various immune cell functions (17). Significantly, oxidative stress has been widely documented in AIDS patients (18, 19), and is known to be an activator of HIV-1 replication *in vitro* (20, 21). In light of those observations, and the established redox-related roles of Se and selenoproteins, it is not surprising that Se has been found to be of

critical importance in HIV-1 infection. Se status has consistently been correlated with various indicators of HIV-1 disease progression, and Se deficiency is highly correlated with HIV-related mortality (reviewed in refs. 7 and 22; 23-26).

GPx and TDR are two of the most widely distributed selenoproteins, and both have been demonstrated to be potent regulators of nuclear factor κB (NF-κB), which is the primary cellular factor involved in the regulation of HIV-1 transcription. Remarkably, GPx and TDR appear to have opposed functions, i.e. GPx inhibits NF-κB by reducing oxidant tone (21, 27), whereas TDR is involved in the reductive activation of an essential Cys residue required for the DNA-binding activity of NF-κB (28). Thus, via these known cellular selenoproteins, Se is a potent regulator of both NF-κB activity and HIV-1 transcription. It is therefore reasonable that HIV-1 could have evolved to directly participate in those regulatory processes, by encoding its own selenoprotein.

Finally, it must be noted that our results are inconsistent with the conclusions of a recent study by Gladyshev et al. (29), who examined selenoprotein expression in HIV-1 infected and uninfected T cells, and concluded that there was no evidence of HIV encoded selenoproteins. However, they did note that in HIV-infected cells there was a decline in levels of cellular selenoproteins, and an increase in "low molecular mass" Se compounds. The latter observation is of interest, because several potential HIV selenoproteins are predicted to be of low molecular mass (from 7-9 kDa), consistent with what Gladyshev et al. observe in HIV infected T cells. One such product is the 9 kDa isoform of the HIV GPx homolog that we have cloned and found to be active in our functional assays reported above. Furthermore, the primary observation of Gladyshev et al., that levels of cellular selenoproteins decline in HIV-infected cells, is also highly consistent with the predictions of the HIV selenoprotein theory, being expected as a consequence of HIV selenoprotein expression [22].

In conclusion, we have presented compelling theoretical and functional evidence which strongly suggests that HIV-1 encodes a Se-dependent GPx gene. The fact that this gene remained undetected in the HIV-1 sequence for 10 years shows that the identification of genes, even in known mRNA sequences, is not always a trivial matter. Our ability to predict the function of the encoded protein despite its low similarity and extensive truncation relative to individual GPx sequences shows that, even in cases of very distant homology, it is sometimes possible to predict protein function from sequence. Because of that remote relationship, particularly when combined

with the extensive truncation of the HIV-1 GPx homolog relative to the cellular enzyme, our model for the 3-D structure of the viral homolog is not expected to approximate a high resolution structure, but instead reflects the potential for a similar structural core and active site geometry. Its degree of correspondence to the actual structure must await verification, pending an experimental structure determination.

Most significantly, the existence of an HIV-encoded selenoprotein gene represents the basis for a novel pathogenic mechanism that can help to explain why the effects of HIV infection are exacerbated in individuals who are Se deficient (23-26).

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Table 1.
GPx activity in cells transfected with HIV-1 construct (pGD) vs. controls transfected with "empty" construct (pC1)

Cells/ construct	transfection	$[SeO_3^{-2}]$ (nM)	Activity (units/mg protein)
MDCK (n=9)			
pC1	transient	20	54.3 ± 4.20
pGD	transient	20	70.8 ± 6.13
			p<0.0001
MCF7 (n=3)			
pC1	stable	100	4.96 ± 2.26
pGD	stable	100	9.86 ± 1.30
			p<0.05

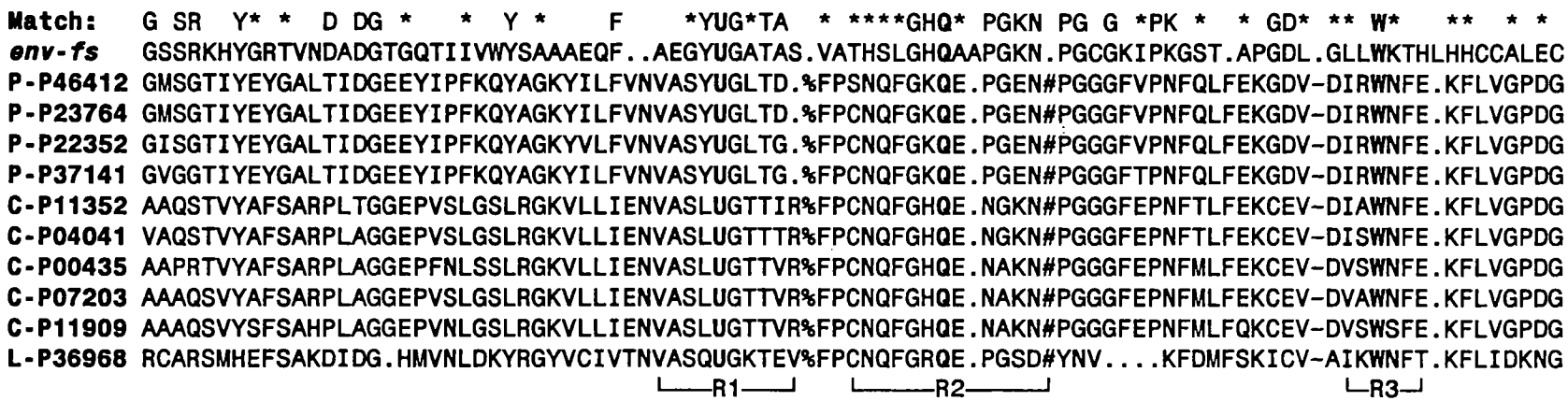

Fig. 1. Sequence alignment of HIV-1 *env-fs* sequence vs. Se-dependent glutathione peroxidases. Sequences are single letter amino acid code, with U as the symbol for selenocysteine, Sec, encoded by the UGA codon in RNA. GPx sequences are listed by Swiss-Prot database accession number, following a prefix of either P- (plasma), C- (cellular) or L- (phospholipid hydroperoxide), identifying three major families of Se-dependent GPx sequences. The N-terminal of mature GPx protein begins at the fourth residue in the alignment. *Env-fs* amino acids identical to one or more of the aligned GPx sequences are shown as letters in the "match" line above the alignment; similar residues are indicated by an asterisk. Three active site regions that are adjacent in 3-D space are shown below the alignment, labeled R1 - R3. All three regions and their essential catalytic amino acids, Sec, Gln and Trp (U, Q and W respectively, shown highlighted in bold), are represented in the truncated *env-fs* sequence. The alignment is similar to that shown as Fig. 1 of Taylor et al. (6) up to the end of R2, but is different beyond R2. There are 3 large internal deletions in *env-fs* relative to the GPx sequences (numbered 1-3 in Fig. 2), at the locations indicated by the symbols %, #, and ~ in the alignment, involving 19, 11 and 41 residues respectively. As computed using the alignment shown, i.e., omitting the 3 internal GPx regions where there are major deletions in the putative HIV-1 GPx homologue, the total similarity score of the *env-fs* sequence to the aligned GPx sequences is 6.3 standard deviations above the average similarity score computed for 100 optimally aligned randomly shuffled sequences of identical composition. This significance score rises to 6.7 SD if the comparison is made only to the plasma GPx sequences, to which the HIV sequence is most similar overall; however, it also has features of the cellular and phospholipid hydroperoxide types of GPx.

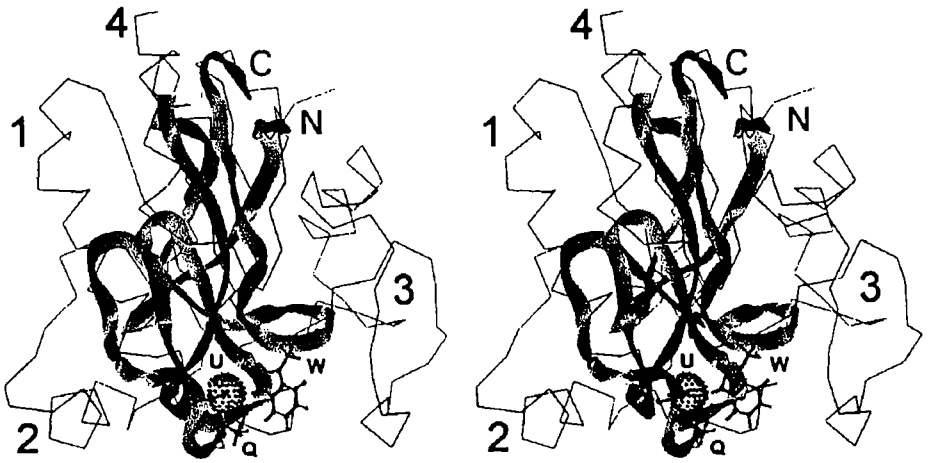

Fig. 2. Stereo view of the aligned protein backbones of the putative HIV-1 glutathione peroxidase (GPx) homology model (ribbon rendition) and the X-ray crystal structure of the bovine GPx monomer (line rendition). The active site is in the lower foreground, with the sidechains of the catalytic triad (Sec, Gln and Trp) shown in a ball and stick rendition and labeled U, Q and W respectively; the Se atom is shown as a dot sphere. The C and N terminals of the HIV-1 peptide are labeled. The regions labeled by numbers 1-3 correspond to the major internal deletions shown as %, # and ~ in the sequence alignment of Fig. 1. The region labeled 4 is a C-terminal deletion, not shown in the alignment. These 4 deletions are domains of the bovine GPx that have no equivalent in the highly truncated HIV-1 GPx homolog. The deleted regions include a helix involved in dimer formation (#2) and another region (#3) that is involved in dimer and tetramer formation. #1 is an internal deletion between the U and the Q. Despite these deletions, the structural and catalytic core of the enzyme is conserved in the truncated HIV-1 homolog.

10. APPENDIX

to proposal for the creation of a new

Center for Selenobiology and Orthomolecular Medicine

Ethan Will Taylor, Ph.D., Computational Center for Molecular Structure and Design,
College of Pharmacy, The University of Georgia

Items included in appendix:

1. Popular and scientific/medical press items summarizing the basis of my 1994 prediction that Se would prove to be central to AIDS pathogenesis. Several items with varying degrees of technical difficulty are included.

2. Set of title pages with abstracts of scientific papers published since 1994, documenting a progressive Se depletion in HIV infection, and showing that Se status is a strong predictor of mortality in AIDS, as specifically predicted in my 1994 paper, based on a theoretical genomic analysis of HIV-1.

3. Meeting report from *International Antiviral News* detailing recent advances in regard to selenium and other antioxidants in the therapy and prevention of viral diseases, presented at a conference on this topic held in Germany, April, 1996 (see section 5.5). 3 pages.

4. Reprint of a brief manuscript published in Sept. 1997 in the *Journal of AIDS and Human Retrovirology*, entitled: "HIV-1 encodes a sequence overlapping *env* gp41 with highly significant similarity to selenium-dependent glutathione peroxidases", by E.W. Taylor et al. 2 pages, plus an extra figure showing the location of this gene in HIV-1.

5. Abstract of a paper presented in Germany at the 4th Dresden Selenium Symposium, May 15-16, 1999, entitled "HIV-1 encodes a sequence with functional glutathione peroxidase activity: implications for the link between selenium deficiency and AIDS".

6. Several items about decreasing Se intake and decreasing levels of Se in the food chain.

7. Brief CV (biographical sketch) of the author.

Selenium & AIDS

HIV-infected human host cell (CD-4 lymphocyte) magnified 20,000 times. The bumps on the cell's surface are viral particles. (Photo courtesy of Centers for Disease Control and Prevention)

When Will Taylor first predicted the existence of genes that could incorporate the mineral selenium to regulate the production of HIV, the virus that causes AIDS, his work was considered highly theoretical (*Research Reporter*, Summer 1995).

Now, three years later, those theories are panning out in the laboratory, including evidence that an HIV protein incorporates selenium.

In the *Journal of AIDS*, Taylor, a UGA associate professor of pharmacy, recently published a computer analysis showing that one of his predicted HIV genes is a significant match to a gene called glutathione peroxidase (GPx). This gene is an enzyme in the body that uses selenium for defense against free radicals — disease-producing molecules.

Taylor also has found the GPx gene in the hepatitus C virus, a potentially fatal liver infection carried by approximately 4 million people in the United States.

"Hepatitus C is a major problem. In this country, it's five times as prevalent as HIV," he said.

A group at the National Institutes of Health has demonstrated a functional GPx gene in a pox virus. "The idea that viruses can encode a selenium-containing protein is now definitely established," Taylor said.

Taylor also predicted in 1994 that declining selenium levels would help speed the progression of AIDS. His ideas initially met with criticism, but further supporting evidence has emerged. "I'm aware of at least seven papers since '94 that show selenium levels strongly correlate with disease mortality or progression," he said.

Among them was a University of Miami paper which reported that selenium deficiency is associated with a 20-fold increased risk of HIV-related mortality. The Miami research group is planning a five-year clinical trial to see if dietary selenium supplements affect long-term survival of AIDS patients.

It turns out, however, that selenium may be declining in the food chain because of acid rain and other factors.

"A 1997 study showed that the amount of selenium in the typical British diet has declined by half in only 22 years," Taylor said. "This raises serious concerns because selenium can regulate HIV."

Taylor will continue his selenium-HIV research with funding from the National Institute on Drug Abuse.

— *SNK*

Hamburger's Helper

The August 1997 recall of 25 million pounds of ground beef from a Nebraska processing plant set the stage. When Mike Doyle announced he could stop *E. coli* 0157:H7 in its tracks, he stepped into the national spotlight.

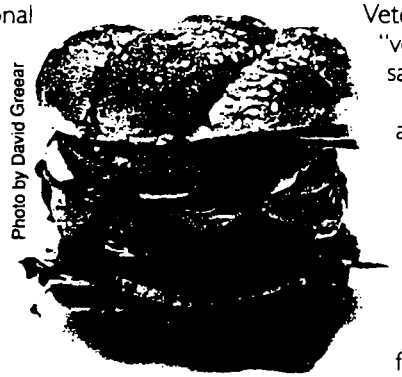

Photo by David Greear

Escherichia coli are bacteria in the intestines of people and animals. Most are harmless. But the strain identified as O157:H7 can make people sick. It can kill some children and elderly people (*Research Reporter*, Fall 1993).

Carried mainly in healthy cattle's forestomachs and intestines, the bacteria contaminate a widening circle of foods, from hamburgers to apple cider, by fecal contact.

But Doyle and other UGA food scientists developed a "probiotic culture," a health-promoting bacterial culture, to stop the deadly *E. coli* in the cow.

"We examined about 1,200 bacteria isolated from cattle that didn't have *E. coli* 0157:H7," said Doyle, who directs the UGA Center for Food Safety and Quality Enhancement in Griffin, Ga. "We looked for beneficial bacteria that prevent the bad bacteria from being carried in the intestinal tract of cattle."

They found 18 strains of the good guys. From these, they produced a culture they fed to cattle, which were then infected with the bad germs.

"We found that in two to three weeks, it virtually eliminated the shedding of *E. coli* 0157:H7," he said. The results were the same when already-infected cattle were fed the good bacteria.

A similar culture Doyle helped develop in 1984 in Wisconsin — to stop campylobacter in poultry — is still unused. But with the *E. coli* problem at center stage, the UGA solution is faring better.

The university has applied for a patent. And scientists evaluating the research for the Food and Drug Administration's Center for Veterinary Medicine were "very excited," Doyle said.

The FDA proposed a fast-track approval process that could be completed in three years. A Dairy Management, Inc., $140,000 grant will fund further research. And several firms are negotiating to pay for continuing research.

It has a way to go yet, Doyle said. But "it looks very promising."

— *Dan P. Rahn*

FOR MORE INFORMATION

www.ovpr.uga.edu/rcd/startrcd1.html

■ SURVIVAL
e-mail Branson Ritchie at britchie@calc.vet.uga.edu
Research Reporter, Winter 1995

■ LIFETIMES
e-mail Leonard Poon at lpoon@omega.geron.uga.edu
or access www.geron.uga.edu/
Research Reporter, Summer 1995

■ SELENIUM
e-mail Will Taylor at wtaylor@rx.uga.edu
or access fafir.rx.uga.edu/~wtaylor/
Research Reporter, Summer 1995

■ HAMBURGER
e-mail Mike Doyle at mdoyle@uga.cc.uga.edu
Research Reporter, Fall 1993

The Observer
SPORTS RESULTS
SUNDAY 2 NOVEMBER 1997
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And for the grown-ups, sexy soup

Pill could slash Aids death rate at 4p a go

Mineral deficiency in bread leaves patients vulnerable to killer viruses

by John Illman
Medical Correspondent

THE disappearance from our diet of a high protein wheat flour used to make bread is hastening the death of patients with HIV and other viral diseases, scientists fear.

The flour is rich in the mineral selenium which plays a key role in maintaining the immune system. The scientists say levies on foreign imports have discouraged US manufacturers from exporting the flour to Britain.

If the scientists are right, a simple treatment costing around £4 for 100 pills could be as vital for prolonging life as HIV drugs costing hundreds of pounds.

The threat to Aids patients was highlighted in a meeting last week at the University of Surrey where a leading US scientist, Dr Will Taylor, spoke of the critical link between selenium and HIV. He said: 'There's great resistance to the idea that complex problems have simple solutions. In the US you can buy a 100 selenium pills for seven dollars — about £4. The "chemoprotective" dose is one 200mg pill a day, though you could probably get significant benefit from half that dose'.

Research reveals that HIV patients with low selenium levels are 20 times times more likely to die from Aids-related causes than those with normal levels. What makes the study especially worrying is that it was carried out in the US where selenium intake is more than twice as high as in Britain. A survey last month by the Ministry of Agriculture, Fisheries and Food revealed that intake in Britain has fallen by nearly half since the mid-Seventies.

Low selenium intake has also been linked to cancer, cardiovascular disease and infertility. But the Aids research — with 125 patients in Miami, Florida — is especially dramatic.

Selenium is also found in kidneys, liver, poultry, fish and cereals, but an editorial in the *British Medical Journal* links its 'substantial decline' to the bread import levies. In addition, the so-called 'bioavailability' of selenium may have fallen because of acid rain or excessive use of artificial fertilisers, which both reduce plant absorption of the mineral.

In the Forties, selenium was mistakenly believed to cause cancer, and in the Seventies, doctors who recommended selenium supplements were regarded with suspicion. But the mineral's immense importance to good health is now increasingly acknowledged and many doctors take supplements themselves — including Dr Taylor, of the University of Georgia, Athens. In his Guildford lecture he suggested that, in a vulnerable individual, HIV 'hijacks' selenium from infected cells, exacerbating the disease. A healthy selenium level has an inhibiting effect on HIV, slowing its replication.

You only

by Richard Brooks
Media and Culture Editor

THE MARCH of the soccerati continues at fever pitch. The latest film from the man behind *Four Weddings and a Funeral* will lift the lid on England's World Cup hopes.

The movie, partly financed by lottery cash, will concentrate on a fictional manager whose travails may appear familiar to former England bosses Bobby Robson, Graham Taylor, Terry Venables and the current incumbent, Glenn Hoddle.

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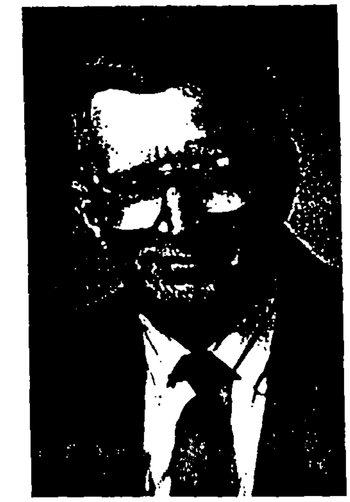
Vitamin Connection

More Exciting Research from Dr. Will Taylor

Selenium Against Viruses



By Richard A. Passwater, Ph.D.

We are witnessing a scientific breakthrough develop from theory to public health practice. In November 1994, Dr. Will Taylor, associate professor in the Department of Medicinal Chemistry at the University of Georgia, explained his hypothesis that opened new inroads into possibly controlling many viruses including AIDS, Ebola and even several "more-routine" viruses.

Last month, Dr. Marianna Baum of the University of Miami discussed her *published* results with selenium and HIV/AIDS. We didn't discuss her latest results because they had not yet been peer-reviewed for publication, but I can tell you that they are very exciting.

Soon, Dr. Orville Levander of the United States Department of Agriculture (USDA) will tell you about his latest findings with selenium and viruses. These three aspects of research with selenium and human viruses received strong interest at an international conference on the subject held in Germany in April.

As one who has conducted laboratory research with selenium and other antioxidants for more than 35 years, I can attest to the scientific and public health importance of this "new direction" in virus research. I don't believe that I have used that terminology to describe completely new concepts since my 1973 publication, "Cancer: New Directions," in which I reported my laboratory research showing that selenium and other antioxidants reduce the incidence of cancers. [*American Laboratory* 5(6) 10-22 (1973)] By the way, an upcoming chat with Dr. Larry Clark will discuss his clinical trial which found that selenium supplements can cut the cancer death rate in half.

In this column, however, we will again speak with Dr. Taylor to see how his new theory has had an effect on AIDS and viral research. It is not necessary to understand the technical aspects of the theory, just that, as his analogy illustrates, selenium can act as a "birth control pill" to some deadly viruses. If you are interested in the details of his theory, please refer to our November 1994 discussion which describes it in detail.

Passwater: Dr. Taylor, it has been only two years since we discussed your exciting new theory about selenium and HIV, but thanks to your new concept, a lot of important and exciting related findings have resulted in that relatively brief time. Not only has the linkup between selenium and AIDS research leaped ahead, but research with selenium on many other viruses—from the rare Ebola to the common flu—has produced dramatic findings.

Are you pleased with the way in which some researchers are comprehending the significance of your research on the role of selenium in limiting the spread of at least some viruses? Or are you disappointed that more scientists have failed to look into this relationship?

Taylor: There certainly are a lot of exciting developments about selenium and viruses, some of which is new work and some of which is research that is only now gaining the attention it deserves, even though it was done a few years back. I am referring to the use of selenium to treat an Ebola-like hemorrhagic fever that broke out in China in the late 1980s. Hemorrhagic fevers can kill up to 90% of those infected, but this study showed that selenium supplementation can reduce that mortality rate dramatically. We can talk about that later.

From my perspective, however, I'd honestly have to say that despite the accumulation of supporting evidence, it has been somewhat frustrating to me that few major virology research groups have made any attempt, let alone a serious effort, to rigorously prove or disprove what I now call the "viral selenoprotein theory."

In essence that hypothesis, first proposed in my 1994 paper, is the idea that certain viruses (initially HIV, other retroviruses, and also some strains of Coxsackie virus) may interact *directly* with selenium in host cells by incorporating selenium into viral proteins.

This would mean that the role of selenium deficiency in some viral diseases might be far more complex than previously thought. Believe me, the potential roles of selenium and other antioxidants in the body's defenses against infectious disease are already very complicated, even without this possibility.

On the positive side, a number of studies have recently come out or are being prepared for publication (Allavena *et al.* 1995, Constans *et al.* 1995, Look *et al.* in press, Baum *et al.* in preparation), confirming that low serum or plasma selenium is a highly significant correlate of HIV disease progression, and a risk factor for mortality.

While this does not prove anything about the *mechanisms* involved, there seems to be more going on here than a simple nutritional effect. These observations are consistent with my 1994 prediction, based on theoretical genomic evidence, that dietary selenium might inhibit HIV replication and slow disease progression.

Of course, based on his studies of the mouse mammary tumor virus (MMTV), a retrovirus relative of HIV, Dr. Gerhard Schrauzer had already predicted more than 10 years ago that selenium would

One-on-one with Will Taylor

Dr. Richard Passwater [illegible]

have an anti-HIV effect. So he has had to be far more patient than I have in waiting to see his idea rigorously tested.

The most encouraging development on the clinical side is the long-overdue initiation of some rigorous clinical studies of selenium supplementation in HIV patients. These include a study by Dr. Marianna Baum in Miami, and a study of African AIDS patients that is being set up by Professor Luc Montagnier of the Pasteur Institute, who recently told me that he thought the data on selenium and HIV are now sufficiently compelling to justify such a study.

I find that very gratifying, particularly since most AIDS patients in impoverished nations in Africa and elsewhere are unlikely to receive any of the expensive new antiviral drugs, like the HIV protease inhibitors. In those countries, all they can realistically hope for is inexpensive ways of slowing down the disease until a vaccine is found—and there is nothing I know of that can do this more cheaply than selenium!

Passwater: Dr. Montagnier is the discoverer of HIV. Our readers may wish to review his research, which was reported in the September 1995 issue. When I visited Dr. Montagnier in his Pasteur Institute Laboratory, I handed him the 1994 article I had done with you, and he became very interested in your theory. Also, I used that article to introduce your theory to Dr. Baum and she too became very interested in your theory, as she commented in last month's column.

Are clinical researchers better understanding the significance of the role of selenium acting directly on viruses themselves, as opposed to protecting the host via such mechanisms as stimulating the immune system?

Taylor: It has been my impression that there has been a lot of interest in my research among practitioners of holistic or alternative medicine, as well as among M.D.s who appreciate the value of prevention and nutritional approaches to therapy. But "mainstream" HIV clinicians are less likely to have heard of it, or indeed to place much hope in any type of nutritional supplementation approach.

Thanks to a story in Dr. Jonathan Wright's excellent newsletter, *Nutrition and Healing*, I have been invited to present these concepts to a large group of M.D.s at a meeting of the American College for Advancement in Medicine (ACAM) in Tampa, FL, next spring. There is no doubt that part of what is catching the attention of these people is the idea that selenium may have a direct effect on some viruses, rather than merely a non-specific immune-boosting effect.

Nevertheless, it's the whole story—putting my findings in the context of the various Chinese selenium studies, the work of Drs. Levander and Beck, Dr. Schrauzer, and so on—that is really intriguing.

Passwater: There is no doubt that you will have an interested audience at the ACAM Conference. These medical practitioners are very familiar with selenium. In addition to the clinical studies which, as you mentioned, link selenium status to HIV disease progression, what are some of the specific new developments that you can point to as support for your "viral selenoprotein theory?"

Taylor: There is not as much as I'd like because in many ways the theory has

hardly been tested. However, we *can* say with considerable confidence that there is now virtually no doubt that *some* viruses can make selenoproteins. It's more a matter of which viruses can do it.

This statement is possible because Dr. Bernard Moss, a scientist at the National Institutes of Health (NIH), recently reported the complete DNA sequence of a common wart virus, the pox virus *Molluscum contagiosum*. This virus appears to encode a gene that is 80% identical to the known mammalian selenoprotein glutathione peroxidase (GPx).

So far he's only done what I have for HIV: show the potential gene is there by theoretical analysis. But with such an unmistakable match to a ***known*** selenoprotein, there's no reason to doubt that this is a real GPx gene.

Similarly, we have now demonstrated GPx-like sequences in Coxsackie B virus, the viral cofactor for Keshan disease, and the subject of the now famous Levander and Beck studies.

If the majority of our readers will bear with me for just a moment, I want to point out to those who are more technically inclined that other developments include a published experimental verification of an RNA "pseudoknot" that we predicted in HIV, ***in vitro*** experimental verification in my lab of a novel frameshift site in HIV associated with a conserved UGA codon, and most recently, from the lab of a collaborating virologist, immunohistochemical evidence in patient samples for some novel HIV protein variants that I predicted.

Finally, in the test tube, selenium has been shown to be a potent inhibitor of HIV reactivation from latently infected cells. In summary: there still is no absolute *direct* proof that a virus can make a selenoprotein, but there is an increasingly strong body of favorable circumstantial evidence.

'In the test tube, selenium has been shown to be a potent inhibitor of HIV reactivation from latently infected cells.'

Passwater: When we discussed the mechanisms you elucidated and presented in your hypothesis, we also included a glossary for the non-virologists. Just so our non-virologists don't have to go back to that article or reach for their scientific dictionaries, a codon is a three-letter code in DNA or RNA that directs insertion of an amino acid into proteins; a frameshift is a shift into a new protein coding region; a pseudoknot is an RNA structure that promotes frameshifts; and UGA stands not only for the University of Georgia but also may signify a "stop" signal for protein synthesis or for selenocysteine insertion.

As I mentioned, I have had the pleasure of introducing your research to several clinical investigators. I am still struggling, however, to get the concept across to many nutritionists and clinical researchers who are not overly familiar with stop codons, frameshifts and pseudoknots.

By the way, if I may inject a personal note, when it comes to complex modern virology, I currently have the advantage of getting help from my youngest son, Michael. You may recall that it was my oldest son, Richard, who was graduated from the University of Georgia, who first led me to your earlier research. Rich and I deal with antioxidants more than viruses.

I find it pleasantly ironic that whereas just a few years ago Michael was asking me, "Hey, Dad, what's DNA?" I now have to ask, "Hey Mike, why do RNA-based viruses mutate more than DNA-based viruses?" Or "Why does the HIV family of viruses have the highest mutation rates among a family (retroviruses) of viruses with high mutation rates?"

Mike has explained to me that the fleets of enzymes which check, double-check and transcribe DNA are at least as important as the DNA itself. RNA is not protected as well. Perhaps it would help clarify your findings if I lead you through the same line of questioning that Mike led me through when we first discussed your research.

Could you briefly explain the significance of UGA codons in your findings on HIV, and how that may relate to the role of the known selenium-containing antioxidant enzyme you mentioned earlier, glutathione peroxidase (GPx)?

Taylor: In essence, the UGA codon is the selenium link because it can direct the insertion of selenocysteine into proteins, an alternative to its more common role as a "stop" signal. We showed that in regions of HIV-1 that were presumed to be inactive or non-coding, UGA codons are "conserved," *i.e.* found in almost all isolates of HIV-1. Along with other structural features we identified, these observations suggested that the virus might encode seleno-

proteins in several such regions.

That was a radical suggestion because apparently no one had ever seriously considered the possibility that viruses might encode selenoproteins, which were believed to be very rare. Only about five mammalian selenoproteins were known at the time, although several more have since been found, and now some leading researchers in this field of research believe that many more probably exist.

GPx is the prototypical selenoprotein. It is an essential antioxidant enzyme in living systems, used to break down harmful peroxides, to maintain cell membrane integrity and to generally reduce the harmful effects of reactive oxygen species.

Here is a deeper question: what would a virus—say *M. contagiosum* or Coxsackie virus—gain by encoding a GPx? There could be many answers to that question. One is that it is now known that the immune system uses free radicals as part of the process of programmed cell death (apoptosis), which also is one of the mechanisms used to kill off cells infected with viruses. Thus, a viral GPx could serve a defensive function for the virus, by countering that process and at the same time keeping the host cell alive—again reminding us

Dr. Will Taylor

that viruses don't necessarily want their host cells to die.

Oxidative stress also is known to activate the replication of many viruses, especially HIV, so increasing the levels of either a host or a viral GPx could act to inhibit viral replication. Thus, a viral GPx could also serve as a repressor of viral replication, similar to what I proposed for one of the hypothetical selenoproteins in HIV, although that one is not a GPx.

Passwater: Regarding the potential role of selenium in viruses such as HIV, Ebola and Coxsackie, would a reduced level of selenium-containing enzymes countering the transcription and/or integration process contribute to the high mutation rate characteristic of these RNA viruses?

Taylor: There are several things going on here. First, as you mentioned, RNA viruses lack the "editing" or error-correcting enzymes characteristic of the DNA-based replication machinery of higher organisms.

Furthermore, RNA is more chemically reactive and unstable than DNA. Thus, RNA viruses are inherently more mutation-prone even than DNA viruses, and far more than their DNA-based hosts. This can be advantageous for a virus because by mutating it can increase its ability to evade the host immune system.

Thus, *anything* that slows down the replication rate of such viruses will reduce their ability to mutate, because mutants are just "sloppy copies." If there are no copies, there are no mutants. That is why in the chemotherapy of AIDS, high drug levels are used, to reduce viral replication almost to zero; otherwise, resistant viral mutants would rapidly emerge, and the drugs wouldn't be able to block them.

As for the potential role of selenoenzymes in this, we do know that selenium somehow boosts the immune system, and cellular immunity in particular; this should help keep viral replication in check. But if you want to know how viral selenoproteins may act, I would have to say this area is so new that we don't have any hard data; all we really have are some "educated guesses" like the repressor hypothesis I mentioned earlier.

Passwater: Wouldn't increasing the selenium concentration in a virally-infected cell cause an increase in host selenoenzymes as well as in viral selenoenzymes?

Taylor: Since the same pool of selenocysteine is involved, one would expect that levels of both host and viral selenoenzymes would increase if more selenium was available. This touches on an aspect of my findings that many people have had difficulty with from the beginning: if the virus uses or "needs" selenium, then why would taking selenium slow viral activity?

Wouldn't that instead "feed" the virus?

The answer is that, first of all, selenium is more essential for us than it is for the virus. So if selenium becomes depleted, we suffer far worse consequences than the virus.

Second, it also depends on *how* the virus uses selenium in its selenoprotein. I explained above how a viral GPx could act to inhibit viral replication. Thus, I have proposed that in some cases a virus might use such a protein in a negative feedback loop, *i.e.* as a repressor. That would permit the virus to respond to conditions of low selenium in the cell—which could be a signal of impending cell death—by replicating at a higher rate, to "escape" from that cell.

For example, under appropriate conditions, HIV is known to remain in cells for long periods of time, either in a latent state or only replicating at a very low level. Selenium-based mechanisms could help regulate that state.

Passwater: You are right that many people ask why "feeding" the virus selenium is a good thing. They wonder if it would not be better to starve the virus.

Nutritionists and clinicians tend to think of selenium in terms of nourishment. But in this case you are not talking about selenium for the nourishment of the virus. Even cancer researchers sometimes miss a similar point when they focus strictly on nutrients and tumor status instead of the more important question of nutrients and immune system status. Your explanation will help more scientists who are not virologists to get the point!

In my lectures, I have used your observation that it's really not in the best interests of viruses to kill their hosts because the viruses also will die. That is, unless they can jump ship (leave the host) by spreading to their next victim.

As you said, but I want to repeat it for emphasis, the important point is that selenium is not really feeding the virus, but is used by the virus to determine the health of the host. If the infected cell (and thus the host) is well-nourished and not in immediate danger of dying there is no urgent need for the virus to invade new cells.

Taylor: Perhaps it would help some of our lay readers if, instead of thinking of selenium as nourishment or food for the virus, they would think of selenium as being sort of a birth control pill for viruses. The viruses don't need selenium for survival so much as for growth regulation.

I already explained how a viral GPx or other selenoprotein can inhibit viral replication by reducing oxidant tone in the cell. Remember that oxidative stress activates HIV. So a very simple analogy would be that a viral selenoprotein could act as a viral birth control pill, inhibiting viral reproduction when selenium is abundant.

Of course, at the same time selenium is boosting the immune system and having other beneficial effects in the host. But when selenium levels are too low, not only do we have a weakened immune system, but the viral birth control is reduced, and the virus replicates at higher levels. This obviously makes sense for the virus, because this is the best time for it to break out—when the immune system is weakened by selenium deficiency.

Thus, by strengthening the immune system with high selenium/antioxidant levels, we force the virus to maintain a low profile. In essence, this analogy explains what a repressor mechanism is, using the "birth control" concept.

Passwater: Does increasing the selenium concentration in the HIV-infected stabilize the HIV genome?

Taylor: Slowing the viral replication rate reduces the opportunity to mutate, which is what is meant by "stabilizing" the viral genome. Since oxidative stress is known to activate HIV transcription, selenium supplementation will reduce HIV replication activity simply as a consequence of increased cellular GPx levels. That has been proved in cell culture studies (Sappey *et al.* 1994).

Furthermore, by protecting against oxidative free radical damage to RNA and DNA, increased dietary selenium would directly reduce the mutation rate. But the possible contributions or roles of viral selenoproteins in these processes still need to be elucidated.

Passwater: If the HIV genome is stabilized, does this give the immune system a steadier target that it can destroy with a "traditional" response?

Taylor: Certainly, if the ability of the virus to mutate is impaired or slowed, it will be easier for the immune system to neutralize it, because it will be less of a "moving target."

Passwater: Does increasing the selenium concentration in HIV-infected cells stimulate the immune system? What happens in uninfected cells?

Taylor: As you know, there is a re-

markably extensive body of literature showing that dietary selenium is critical for a healthy immune system, and that selenium potentiates various aspects of cellular immunity, such as T-cell proliferation responses and the action of the cytokine interleukin 2.

I think only a part of this can be explained by known human selenoproteins like GPx. We really have a lot to learn about *how* selenium produces its immune-stimulating effects. This statement is supported by the fact that, according to an early study by McConnell using radio-labeled selenium in immune cells, only about 20% of the total selenium content was contained in GPx. So selenium probably is doing important things in those cells that we still don't understand.

Passwater: I believe you're referring to a 1959 study by McConnell in which he subcutaneously injected radioactive selenium (75Se) chloride in dogs and measured the amount of selenium incorporated into the leukocytes. This is the first reference to selenium being used in the immune system that I am aware of.

I don't believe that anyone has published figures changing his finding that about 20% of the selenium became incorporated into the protein fraction of the leukocytes, which indeed may be essentially one or both of the glutathione peroxidases. That's a good point for me to check with Dr. Orville Levander. Sorry to interrupt; I hope it didn't make you lose your point.

Taylor: The point that I was getting to is that in HIV-infected individuals, I would expect this role of selenium in immunity to be at least as important as in the uninfected. In addition, since HIV targets the immune system, an important role for selenium in the normal immune response could also help explain why the virus might gain something by getting directly involved in selenium biochemistry, as I have proposed. Mimicry of host proteins and mechanisms is a common viral strategy.

Passwater: Does increasing the selenium level in HIV-infected cells increase glutathione or oxidized glutathione levels?

Taylor: Selenium increases GPx levels, and GPx uses glutathione (GSH) to reduce peroxides, forming GSSG (oxidized glutathione) in the process. So one might expect GSSG to increase when selenium is increased. But another enzyme, glutathione reductase, readily regenerates GSH from GSSG. So the *total* amount of both forms of glutathione is what is really important.

Recently, French researchers showed that, counterintuitively, selenium supplementation actually increases free GSH levels significantly. This is good, because it is the reduced GSH form that is needed for many important detoxification reactions and free radical scavenging in the body. So some complex homeostatic mechanisms must be involved that act to increase total glutathione levels when more selenium and GPx are available.

Passwater: : It was recently noted that Keshan disease seems to have a viral component rather than being strictly a selenium-deficiency disease *per se*. Do you see your research as playing a role in understanding this development?

Taylor: Actually, this link was first noted by the Chinese in research published as far back as 1980. Coxsackie virus, a widespread relative of the common cold virus, was isolated from the hearts of Keshan dis-

ease victims, and was also shown to produce heart damage in selenium-deficient mice that was identical to that seen in human Keshan disease.

I think this is extremely significant in terms of what I am saying about HIV, because Keshan disease is clearly a selenium deficiency disease, apparently with a viral cofactor. Also, I am saying that AIDS is a viral disease with selenium deficiency as a cofactor. And we now have compelling evidence for virally encoded selenoproteins in both HIV and Coxsackie virus.

Passwater: Dr. Melinda Beck of the University of North Carolina, Chapel Hill, made an interesting observation about how a fairly harmless strain of Coxsackie virus mutates within selenium-deficient mice (and presumably in people as well) to become a more harmful virus that can then spread and produce heart damage, even in others who are not selenium-deficient. Was she aware of your research when she made her observation? How does her finding complement your research findings?

Taylor: Dr. Beck's work is an extremely important breakthrough in establishing the selenium-virus link. She and her collaborator, Dr. Orville Levander, were working independently in this area before I was, developing their line of research based on the earlier Chinese observations linking Coxsackie virus to Keshan disease.

When I discovered a potential HIV-selenium link in the spring of 1994, based purely on genomic analysis of HIV, I was unaware of their selenium work because their first paper showing increased virulence of Coxsackie virus in selenium-deficient mice was not yet published (although I had seen an earlier paper they did showing a similar effect with vitamin E).

In a subsequent paper, they showed that, when passed through selenium-deficient animals, the virus actually mutates into a more virulent strain, that retains its virulence in selenium-adequate animals. This has obvious implications in regard to "emerging" viral diseases.

In their published work, Drs. Beck and Levander have focused on known mechanisms to explain their observations along the lines we have already discussed: low selenium leads to weakened antioxidant defenses and reduced immune surveillance, higher viral replication rates, and thus to conditions favoring viral mutation.

However, particularly now that my group has demonstrated unmistakable GPx-related sequences in the Coxsackie B virus strain that they studied, I think they are seriously considering the possibility of a direct virus-selenium link of the type I have proposed for HIV. Obviously, if Coxsackie virus encodes a selenoprotein, it would have to be involved in the mechanism underlying their observations.

Passwater: After publishing your selenium-HIV discovery, you proposed a possible relationship between selenium and the Ebola virus. What did you find, and why did you think to look for this relationship?

Taylor: Coincidentally, I began to study Ebola less than a month before the 1995 outbreak in Kikwit, Zaire that brought this virus so drastically into the public consciousness. I did so because of a poster presentation I had seen that spring in Santa Fe, at a meeting of the International Society for Antiviral Research. A Russian group presented a world map showing the geographic areas where various hemorrhagic viral diseases tended to occur, and I was struck by the fact that the area shown for the filoviruses Ebola and Marburg matched

a region in Africa that I suspected might be a low-selenium region.

What we found was striking: several gene regions in Ebola contained large numbers of UGA codons, up to 17 in one segment. We later published a paper showing that it might be possible for Ebola to synthesize selenoproteins from these gene regions, and we proposed a mechanism whereby this might induce artificial selenium deficiency and contribute to the blood clotting characteristic of Ebola pathology.

During the revisions to the final draft of that paper, we learned of a 1993 paper in a Chinese journal that reported the use of selenium to treat an Ebola-like hemorrhagic fever, with remarkable results. Luckily, the English translation of the abstract was available.

The report indicated that using the very high oral dose of 2 mg selenium per day as sodium selenite, for only nine days, reduced the death rate from 100% (untreated) to 37% (treated) in the very severe cases, and from 22% to zero in the less severe cases. Apparently there were about 80 people involved in this outbreak. Dr. Hou of the Chinese Academy of Medical Sciences, the author of this study, has since told me that he thinks more lives could have been saved if he had been permitted to give the selenite by injection, because in many of the severely affected there was so much organ damage due to internal bleeding that they may have been unable to fully absorb or retain the oral dose of selenium. All in all, this is the closest thing to a curative result in the treatment of hemorrhagic fever that I have ever heard of.

'I think that in a number of viral diseases some degree of direct interaction between the virus and selenium is likely to be involved.'

Passwater: Dr. Hou used selenite because quick and dramatic action was required; the patients were dying in front of him. For normal, long-range protection, organic selenium supplements, such as selenium-rich yeast or selenomethionine, are preferred, as discussed by Dr. Gerhard Schrauzer and by others, as will be discussed later in this series.

How do hemorrhagic fever viruses cause hemorrhaging? Would selenium's effect on blood clotting in the host play a role in such diseases, or is the effect strictly an interaction with the virus itself?

Taylor: The characteristic hemorrhaging produced by various "hemorrhagic fever" viruses involves the production of blood clots that ultimately block small capillary vessels, which rupture under pressure to produce internal and even external bleeding in severe cases. This is known as "disseminated intravascular coagulation," or DIC. Thus, paradoxically, the bleeding is produced by a pro-clotting mechanism, and anticoagulants (which usually promote bleeding) have been used to treat symptoms of the disease.

This may be very significant in regard to selenium involvement, because the biochemical basis for an anti-clotting effect of selenium is very well established. Severe selenium deficiency, usually artificially induced in animals, is known to produce hemorrhagic symptoms. Thus, the idea that hemorrhagic fever viruses might produce a severe selenium depletion would be consistent with the established pro-clotting mechanism of DIC. So there may be an interaction here, where viral activity is having a direct impact on host selenium status over the period of one or two weeks, sufficient to cause serious pathology.

Alternatively, the results obtained in the Chinese study could have been simply due to the anti-clotting effect of selenium, or other mechanisms. Dr. Hou apparently decided to try the selenium treatment because of his own theories about the involvement of selenium in complement activation, another feature of hemorrhagic disease. So additional studies are badly needed, to determine what the predominant mechanism of protection by selenium really is.

Passwater: Then do you see multiple roles for selenium against other viruses?

Taylor: At this point, I'm very optimistic about the potential of dietary selenium as a broad-spectrum chemoprotectant against various viral diseases. A lot of that may be entirely due to the immune-stimulating and antioxidant benefits of selenium, but I think that in a number of viral diseases some degree of direct interaction between the virus and selenium is likely to be involved.

We already have quite a few viral diseases where a clinical correlation or definite selenium benefit has been established:

hepatitis B/liver disease, HIV/AIDS, Coxsackie virus/Keshan disease, hemorrhagic fever, MMTV/cancer, and a number of other animal viral diseases where selenium has been used in veterinary practice. I also strongly suspect that various herpes viruses will prove responsive to selenium therapy, and the strongest case of a selenoprotein in a virus to date is in a pox virus. So the potential scope of this chemoprotection approach is very exciting.

Passwater: Your research is getting dramatic scientific support at least by some researchers. What are you looking into now?

Taylor: After spending much of my efforts over the last two years in trying to extend the scope of our HIV findings in terms of other viruses, and trying to establish some collaborations in order to have the implications experimentally verified, I am now focusing on building up the capabilities in my own laboratory to do some of the necessary experimental research. It's been slow getting started, because we have been hampered by lack of financial resources, and only now is the hard evidence coming in that will enable us to convince Federal funding agencies that this research merits support.

Along with a few other labs, we have already obtained evidence that some of the molecular features we predicted in HIV are real. Our objective is to clone several of the novel genes that we have found by genomic analysis, including several from HIV and the GPx homologue from Coxsackie virus, so that their function can be established. In the meantime we are trying to work with clinical researchers like Dr. Marianna Baum to promote the serious assessment of the potential benefits of selenium as a complementary therapy in HIV disease.

Finally, I've also become very interested in the biochemical roles of selenium in health as well as in cancer and rheumatoid diseases, and so on. My group is now engaged in a search for new selenoprotein genes in the human genome, and we are finding some rather intriguing things.

All that I can say at this point is that I strongly suspect that selenium is playing a role in cell signaling and attachment—very important in the immune system—and that selenium is more than just indirectly involved in gene regulation. So I'm sure I'll be keeping busy well into the next millennium trying to find out if that hunch is really true! Hopefully our readers will be able to say they read it here first.

Passwater: Well, we will all be looking forward to the *Selenium Millennium!*

The information that you have deduced by examining genes is a great help and gives great directions for the biochemists to check out. Without this help we are more or less left to "stumble around" trying to figure out biochemically how selenium does all those things that our laboratory studies show it does.

I am sure that the funding agencies will soon understand the importance of your research. They need time to fully understand its consequences.

You have been on the program of the International Conferences on selenium and human viruses. Now let's see if we can get you on the programs of some of the NIH virus researchers. Remember NIH also stands for Not Invented Here—and if a theory is not invented here (*i.e.* at the National Institutes of Health) it takes longer to get the attention of the establishment funders. Thanks for helping us keep up-to-date on your exciting research. WF

THE TIMES

No. 65,046 TUESDAY AUGUST 30 1994

Research shows that selenium could be the anti-viral agent of the future, reports **Kate Muir**

Mineral deficiency may speed infection

THE MINERAL selenium may play a key role in the speed at which the HIV virus attacks the body and causes full-blown Aids, according to a new report in *The Journal of Medical Chemistry*.

Investigation of the DNA or genetic structure of the human immunodeficiency virus has unearthed four "invisible" genes, three of which need a form of selenium to function.

Selenium deficiency had been noticed for years in Aids patients, but it was assumed to be just another wasting result of the disease, along with the growing inability to process food and vitamins. Dr Will Taylor, an assistant professor at the University of Georgia, theorises in the article that selenium depletion actually triggers growth of the virus, making the symptoms of Aids more devastating. "If this is true, then selenium biochemistry may be the key to understanding the control of the life cycle of HIV and perhaps some of the pathology of Aids," he says.

Selenium is found in seafood, meat and whole grains, but too much of it can be toxic. Previous short-term tests on small numbers of HIV-positive patients have shown that taking selenium supplements causes subjective changes like a decrease in stomach problems, an improvement in appetite, weight maintenance or gain and feelings of healthiness; but fuller research is continuing.

Dr Taylor's computer deconstruction of the DNA of the virus may now explain why selenium is so important. "We have discovered a new group of genes in HIV, and those genes have the potential to use selenium in the proteins they make, so that might explain the mechanism where the virus uses up selenium," Dr Taylor says. "If selenium depletion does speed the progression of HIV infection, then that may be a reason why the virus has such a long latency period in some people, and a short one in others; why some develop full-blown Aids quickly, while others are unaffected for ten or more years." This may also account for the rapacity of Aids among those patients who already have some malnutrition, such as intravenous drug users, the very poor, and sufferers in certain African countries.

HIV-positive survivors have long favoured macrobiotic or vitamin-rich diets, as well as nutritional supplements, as a way to slow the progress of the disease and, inadvertently, they may have been getting high doses of selenium.

Dr Taylor found that selenium was an ingredient of HIV when he did a painstaking computer analysis of the long chain of letters which make up the DNA of the virus. He noticed a group of letters — UGA — previously thought to be a recurring stop sign in the sequence. But the genetic full stop, in fact, was disguising three selenium-using proteins and one other protein encoded in parts of the HIV genetic pattern considered to be inactive.

Clinical research into selenium supplementation carried out by Dr Gerhard Schrauzer at the University of California, San Diego, shows that chronic lack of selenium weakens the immune system. He says the new genetic clues are "very exciting work. They show we must look at all aspects of the virus and treatments that could include simple nutritional agents. It may be that selenium is the anti-viral agent of the future."

DR SCHRAUZER says the selenium research has been slow because scientists have often tended to look at Aids as some separate disease, rather than looking for connections to other retro viruses. Selenium has been seen to have protective effects against viral breast tumours, the bovine leukaemia virus and the hepatitis B virus.

A study on selenium supplementation is presently in progress among 15 HIV-positive men in Frankfurt, Germany, none of whom is taking the drug AZT, which can be toxic in itself. In the first year, selenium produced no adverse side-effects in the patients. They reported better appetite and intestinal functions and their skin conditions cleared up.

Dr Taylor noted that little similar research has been done in America: "No big drug company is going to make money financing research into a mineral that can't be patented." Instead, Dr Taylor said it was acceptable to publish the theory about the four new genes and the role of selenium, even though it was not yet backed by human evidence. "In physics theoreticians come out with theories that sometimes take years to prove — such as Einstein's theory of relativity. So it really shouldn't be so surprising that we're reaching the point where biological theory can be ahead of experiment."

→ See attachments over

→ this prediction has been strongly confirmed by many recent studies

New theory connects HIV disease with selenium levels

Study proposes that HIV replication may be tied to availability of selenium, a trace nutrient whose levels decline significantly in many AIDS patients.

by Jody A. Charnow
Staff Writer

ATHENS, Ga.—Based on theoretical and circumstantial evidence, University of Georgia researchers here have suggested that HIV replication may be tied to availability of selenium, a trace nutrient whose levels decline significantly in many AIDS patients.

The researchers, led by Will Taylor, PhD, theorize that HIV has genes that code for selenium-containing proteins, one of which might bind to HIV DNA and inhibit replication. By producing these selenoproteins, HIV could cause a decline in levels of certain human selenoproteins, including an important antioxidant enzyme.

If selenium levels drop in a host cell, HIV might not have enough selenium to make the inhibitory protein, prompting viral replication. Such a protein could explain why progression of HIV disease varies widely among HIV-infected people and why some people coexist with HIV for more than a decade without developing AIDS, according to the investigators.

Experimental proof that such a DNA-binding protein exists could lead to new approaches to HIV therapy, such as targeting the protein's binding site in the viral DNA.

Taylor and colleagues published their hypotheses in a recent issue of the *Journal of Medicinal Chemistry*.

For years, doctors have observed that dietary supplements of selenium improve the clinical condition of some AIDS patients. The theory offered by Taylor and colleagues could explain why.

Taylor is working with researchers at the Centers for Disease Control and Prevention (CDC) to synthesize peptides from the hypothetical HIV genes. Investigators plan to expose those peptides in a serological assay to HIV-infected blood to see if there is an antibody reaction.

Conceptually, the hypothesis "has some merit" and is easily testable, said Tom Folks, PhD, chief of CDC's Retrovirus Diseases Branch, one of the investigators.

If correct, the investigators would prove the first direct link between a virus and selenium.

The hypotheses advanced by Taylor and colleagues rest in part on a certain "stop" codon present in the RNA of HIV and related retroviruses, as well as many other organisms. A stop codon is a sequence of three nucleotide bases that marks the end of a gene so that ribosomes, which translate messenger RNA (mRNA) code into proteins, know when a protein is complete. Taylor and colleagues theorize that HIV occasionally suppresses that stop codon to produce a different protein.

Taylor and colleagues found that HIV and some closely related primate retroviruses have the same stop codon, uracil-guanine-adenine (UGA), at precisely the same location on their mRNA. UGA stop codons sometimes insert into a protein a rare selenium-containing amino acid called selenocysteine.

The theoretical selenoprotein-producing genes may have eluded detection because in most genes the UGA codon is a stop signal for protein synthesis rather than a signal for selenocysteine insertion, Taylor said.

Because the UGA stop codon also is used for selenocysteine in both bacteria and higher organisms, researchers believe the use of this codon dates back almost 2 billion years. A codon is a set of three adjacent bases on a single strand of RNA or DNA.

Originally, the codon may have coded for selenocysteine much more than it does today. Rising atmospheric oxygen levels may have brought about the change. Selenocysteine is highly oxidizable, and researchers speculate that organisms evolved so that they relied less on this amino acid.

If HIV produces selenoproteins, it probably does so in minute quantities, said Taylor, who is [illegible]ate professor of medicinal ch[illegible]stry.

Taylor does not bel[illegible] that HIV-produced selenoproteins could account for the decline in plasma selenium levels in many AIDS patients. Therefore, much of the decline, or perhaps all, is likely due to impaired gastrointestinal absorption of selenium, the conventional theory.

In Taylor's view, plasma levels are less important than the levels of selenium in host cells. "T[illegible], the viral use of selenocysteine in [illegible] of its proteins could significantly impact the amount of selenium available to make glutathione peroxidase," Taylor told INFECTIOUS D[illegible]ASE NEWS.

This selenoenzyme activates glutathione, an antioxidant that prevents lipid oxidation and thus maintains the integrity of membranes and certain critical biochemical pathways, Taylor said. AIDS patients commonly show significant declines in glutathione levels. Tay-

(Continued on page 22)

→ We now realize that this HIV gene encodes a homologue of NF-kB, a DNA binding protein that is known to regulate HIV-1 as well as many immune-related genes.

See paper to be published in J. AIDS in 1999

HIV may cause a decline in levels of human selenoproteins

(Continued from page 19)

lor and colleagues also cited a study that found that red blood cell glutathione peroxidase levels in AIDS patients averaged 55% of normal controls.

Perhaps more significantly, Taylor said, researchers led by Paul Sandstrom, PhD, reported earlier this year that they found an HIV-associated glutathione peroxidase deficiency in an HIV-infected cell line. Sandstrom and coworkers published their study results earlier this year in the *Journal of Biological Chemistry*.

Taylor also pointed out that AIDS patients often experience declines in levels of thyroid T3 hormone, whose production depends on a selenium-containing enzyme.

Declines in glutathione peroxidase and T3 levels, which Taylor considered circumstantial evidence of a link between HIV and selenium, can produce deficiencies in immune function.

The investigators found regions of HIV RNA capable of forming structures that would ensure that ribosomes could read through the codons and synthesize different proteins, namely, selenium-containing proteins. The hypothetical structures are similar to structures in other organisms that are known to be required for selenocysteine insertion.

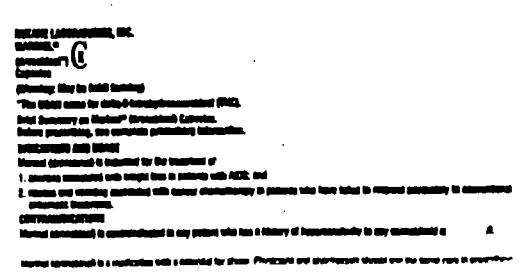
Taylor and colleagues also identified mRNA regions capable of forming structures called pseudoknots that would direct the synthesis of the hypothetical selenoproteins.

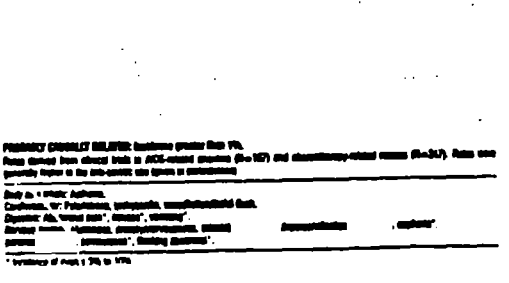
The investigators predicted potential proteins that would result if indeed ribosomes read through UGA stop codons in HIV.

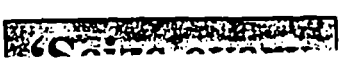
One putative protein would be similar to known DNA-binding proteins that control viral replication. That hypothetical protein would be particularly akin to E2 proteins, a family of viral DNA-binding proteins in papillomaviruses.

Taylor and colleagues calculated that the predominant form of the hypothetical protein could function as an inhibitor of viral replication. ■

For more information, see Taylor EW, Ramanathan CS, Nadimpalli RG, et al. A basis for new approaches to the chemotherapy of AIDS: novel genes in HIV-1 potentially encode selenoproteins expressed by ribosomal frameshifting and termination suppression. *J Med Chem*.1994;91:199-205







FYI - These are some of >20 papers published over the last decade linking Se levels to HIV disease progression.

1995

Constans et al. France

Serum Selenium Predicts Outcome in HIV Infection

To the Editor: Recent studies suggest that serum selenium levels decrease in HIV-positive patients and are lower in patients with advanced disease (1,2). We mea-

DISCUSSION

Serum selenium decreases in several chronic diseases, particularly in HIV-positive patients (1,2). In the present work, we found that selenium decreases like the CD4 cell count. Moreover, serum selenium is related to prognosis, irrespective of CD4 cell count. The functions of selenium

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ELSEVIER SCIENCE IRELAND

Chemico-Biological Interactions 91 (1994) 181–186

New York

Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS)

Brad M. Dworkin

Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology
15:370–374 © 1997 Lippincott-Raven Publishers. Philadelphia

U. Miami - see attached press release

High Risk of HIV-Related Mortality Is Associated With Selenium Deficiency

*Marianna K. Baum, *Gail Shor-Posner, †Shenghan Lai, *Guoyan Zhang, *Hong Lai, †Mary Ann Fletcher, ‡Howerde Sauberlich, and *J. Bryan Page

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Eur J Clin Nutr 51 (4): 266-272 (Apr 1997)

U. Bonn, Germany see attached

Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection.

Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, Sudhop T, Hoch J, Stockinger K, Spengler U, Sauerbruch T

SHORT REPORT

1997

Key words: selenium; oxidative stress; human immunodeficiency virus; muscle; zidovudine

MUSCLE NERVE 20:386–389 1997

France

MUSCLE INVOLVEMENT IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS IS ASSOCIATED WITH MARKED SELENIUM DEFICIENCY

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University of Miami Press Release

STUDY FINDS SELENIUM AFFECTS SURVIVAL IN HIV/AIDS

EMBARGO UNTIL SEPTEMBER 30, 1997

MIAMI, Florida, September 30, 1997 - Scientists at the University of Miami School of Medicine, Center for Disease Prevention, reported today in the Journal of AIDS that deficiency of selenium, an essential trace element for maintaining a healthy immune system, has a profound effect on survival in HIV infected men and women. The researchers, led by Dr. Marianna K. Baum, found that HIV-1 infected patients with selenium deficiency were 19.9 times more likely to die of HIV related causes than those with adequate selenium levels.

The dramatic results of this NIH funded study in 125 HIV infected men and women, published on September 30 in the Journal of Acquired Immune Deficiency Syndrome, demonstrate that selenium plays a critical role in HIV-1 disease. The researchers also found that while other nutrients (vitamins A, B_{12}, and zinc) affect survival, these nutrients produce a substantially lower risk for mortality. In fact, when the nutrients were examined together, SELENIUM had the strongest impact upon mortality.

The authors suggest that the striking relationship between selenium deficiency and mortality could be related to selenium's antioxidant function and/or action in gene regulation which may affect HIV replication. As proposed in a report of Dr. Will Taylor, of the University of Georgia, published in the same issue of the Journal of AIDS, selenium, as part of selenoproteins, could have an important role in regulating HIV expression. He has proposed a novel viral mechanism that contributes to a decline in selenium levels accelerating disease progression, while adequate selenium would be expected to prevent HIV replication, and thus, delay the course of disease progression.

Based on this research, Dr. Baum's team is developing a study to determine whether selenium treatment can slow disease progression and improve survival over time in HIV infected men and women. The study has been approved and is under consideration for funding by NIH Institutes, including the National Institute on Drug Abuse and the Fogarty International Center.

Selenium Deficiency Characterizes HIV Infection (April 1997 Reuters report)

WESTPORT, Apr 22 (Reuters) - Selenium deficiency is common among individuals with advanced HIV infection, according to German researchers. Whether this is the result of malnutrition or opportunistic infection, they believe this selenium deficiency needs to be corrected.

Dr. M. P. Look of the University of Bonn and colleagues evaluated the antioxidant defense status of 104 HIV-positive patients. "Evidence...suggests that an imbalance between diminished host antioxidant defenses and increased formation of oxygen radicals and proinflammatory cytokines create a state of 'oxidative stress' in HIV disease, Dr. Look explains.

Because selenium is a key element in the antioxidative enzyme glutathione peroxidase, Dr. Look's group measured the subjects' serum selenium, erythrocyte glutathione peroxidase (GSH-Px) activity, plasma thiol (-SH) and glutathione (GSH) concentrations. In addition, they assessed clinical stage and surrogate markers of HIV disease for each patient.

Overall, they found that selenium levels positively correlated with CD4 cell counts, and inversely correlated with markers that indicated progressing HIV disease. "Serum selenium and GSH-Px activity in hospitalized AIDS patients was significantly lower as compared to asymptomatic patients and healthy subjects, whereas plasma SH and GSH concentrations were lower in both asymptomatic and AIDS patients, than in the controls."

Based on these results, they recommend that controlled studies should be aimed at maintaining maximum levels of micronutrient and vitamin status, to suppress oxidative stress and perhaps also to help suppress HIV replication.

European J Clin Nutrition 1997;51 :267-272.

J Acquir Immune Defic Syndr Hum Retrovirol 1997 Aug 15;15(5):370-4

High risk of HIV-related mortality is associated with selenium deficiency.

Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB

Department of Psychiatry and Behavioral Sciences, University of Miami, School of Medicine, Florida 33136, U.S.A.

To determine the independent contribution of specific immunologic and nutritional factors on survival in HIV-1 disease, CD4 cell count, antiretroviral treatment, plasma levels of vitamins A, E, B6, and B12 and minerals selenium and zinc were considered in relation to relative risk for HIV-related mortality. Immune parameters and nutrients known to affect immune function were evaluated at 6-month intervals in 125 HIV-1-seropositive drug-using men and women in Miami, FL, over 3.5 years. A total of 21 of the HIV-1-infected participants died of HIV-related causes during the 3.5-year longitudinal study. Subclinical malnutrition (i.e., overly low levels of prealbumin, relative risk [RR] = 4.01, $p < 0.007$), deficiency of vitamin A (RR = 3.23, $p < 0.03$), vitamin B12 deficiency (RR = 8.33, $p < 0.009$), zinc deficiency (RR = 2.29.1, $p < 0.04$), and selenium deficiency (RR = 19.9, $p < 0.0001$) over time, but not zidovudine treatment, were shown to each be associated with HIV-1-related mortality independent of CD4 cell counts <200/mm3 at baseline, and CD4 counts over time. When all factors that could affect survival, including CD4 counts <200/mm3 at baseline, CD4 levels over time, and nutrient deficiencies were considered jointly, only CD4 counts over time (RR = 0.69, $p < 0.04$) and selenium deficiency (RR = 10.8, $p < 0.002$) were significantly associated with mortality. These results indicate that selenium deficiency is an independent predictor of survival for those with HIV-1 infection.

PMID: 9342257, UI: 98000304

Eur J Clin Nutr **51** (4): 266-272 (Apr 1997)

Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection.

Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, Sudhop T, Hoch J, Stockinger K, Spengler U, Sauerbruch T

Department of General Internal Medicine, University of Bonn, Germany.

OBJECTIVES: Antioxidant defense status was investigated in HIV-infected patients by measuring serum selenium, erythrocyte glutathione peroxidase (GSH-Px) activity, plasma thiol (-SH) and glutathione (GSH) concentrations along with the assessment of the clinical stage and surrogate markers of HIV-disease. DESIGN, SETTING AND SUBJECTS: Serum selenium levels were determined cross-sectionally in 104 sequentially selected HIV-infected patients (83 outpatients and 21 patients with ongoing AIDS defining events). The patients were classified into three stages of the disease, I, II and III according to the 1993 Centers For Disease Control (CDC) classification system for HIV-infection. GSH-Px activities, plasma SH and plasma GSH concentrations were determined in a subset of 24 patients at stage I and 12 patients at stage III with an active AIDS-defining disease. RESULTS: Mean serum selenium levels were lower in CDC stage II (68.7 +/- 20.9 micrograms/l; $P < 0.01$; $n = 34$) and stage III (51.4 +/- 14.7 micrograms/l; $P < 0.01$; $n = 37$) HIV-infected patients than in healthy subjects (89.2 +/- 20.9 micrograms/l; $n = 72$) and stage I patients (82.3 +/- 20.5; microgram/l; $n = 33$). Serum selenium levels were positively correlated with CD4-count ($r = 0.42$; $P < 0.001$; $n = 104$) and inversely with levels of soluble tumor necrosis factor receptors type II ($r = -0.58$; $P < 0.01$; $n = 35$), neopterin ($r = -0.5$; $P < 0.001$; $n = 80$) and beta 2-microglobulin ($r = -0.4$; $P < 0.001$; $n = 94$). Hepatitis C virus (HCV) and HIV-coinfected patients at CDC stages I and II showed markedly lower selenium concentrations compared to HIV-infected patients without concomitant HCV-infection. Serum selenium and GSH-Px activity in hospitalized AIDS patients was significantly lower as compared to asymptomatic patients and healthy subjects, whereas plasma SH and GSH concentrations were lower in both, asymptomatic -and AIDS-patients, than in the controls. CONCLUSION: The results show that stages I-III of HIV-disease are characterized by significant impairments of antioxidative defenses provided by selenium, GSH-Px, SH-groups and GSH.

MeSH Terms:

- Adult
- Aged
- Antioxidants/metabolism
- Antioxidants/analysis
- Comparative Study
- Erythrocytes/enzymology*
- Female
- Glutathione/blood*
- Glutathione Peroxidase/blood*

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J Acquir Immune Defic Syndr Hum Retrovirol 1999 Apr 15;20(5):508-13

Mortality risk in selenium-deficient HIV-positive children.

Campa A, Shor-Posner G, Indacochea F, Zhang G, Lai H, Asthana D, Scott GB, Baum MK

Center for Disease Prevention, Department of Psychiatry and Behavioral Sciences, University of Miami School of Medicine, Florida 33136, USA.

OBJECTIVE: To determine the independent contribution of specific nutritional factors on disease progression and survival in HIV-1-infected children. POPULATION: HIV-infected children (N = 24), who were perinatally exposed to the virus and symptomatic, were recruited between October and December of 1990 from the Jackson Memorial Pediatric Immunology Clinic, Miami, Florida, and observed for 5 years. METHODS: Immune status was measured by CD4 cell count; nutritional status was determined using serum albumin and plasma trace elements including iron, zinc, and selenium. Cox proportional hazards regression models were used to evaluate the relationship of these parameters to survival. Use of antiretroviral treatment was considered in the statistical model, and age at death was considered a parameter of disease progression. RESULTS: Over the course of the study, 12 children died of HIV-related causes. The final Cox multivariate analysis indicated that, of the variables evaluated, only CD4 cell count below 200 (risk ratio [RR] = 7.05; 95% confidence interval [CI], 1.87-26.5); p = .004], and low levels of plasma selenium (RR = 5.96; 95% CI, 1.32-26.81; p = .02) were significantly and independently related to mortality. Among the children who died, those with low selenium levels (< or =85 microg/L), died at a younger age, suggesting more rapid disease progression. CONCLUSIONS: In pediatric HIV-infection, low plasma level of selenium is an independent predictor of mortality, and appears to be associated with faster disease progression.

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Nutr Rev 1998 Jan;56(1 Pt 2):S135-9

Micronutrient status in relationship to mortality in HIV-1 disease.

Baum MK, Shor-Posner G

University of Miami School of Medicine, USA.

Selenium deficiency has been demonstrated to be a significant predictor of HIV-related mortality, independent of CD4 over time, CD4 < 200 at baseline, and antiretroviral treatment. Although selenium deficiency in healthy humans is relatively rare (Cohen et al. 1989, Lockitch, 1989), a number of studies have documented a decline in plasma selenium levels and decreased glutathione peroxidase activity in individuals with HIV/AIDS (Dworkin et al. 1988, Cirelli et al. 1991, Mantero-Atienza et al. 1991, Staal et al. 1992, Allavena et al. 1995). These findings are of particular concern in light of selenium's influence on immune function, viral replication, and survival. As recent investigations (Delmas-Beauvieux et al. 1996) indicate that supplementation with selenium may help to increase the enzymatic defense systems in HIV-infected patients, further studies to determine possible mechanisms and clinical trials to evaluate the effect of selenium supplementation on HIV disease progression are essential.

Publication Types:

- Review
- Review, tutorial

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The Distribution of Selenium and Mortality Owing to Acquired Immune Deficiency Syndrome in the Continental United States

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ABSTRACT

A hypothesis has been proposed that Selenium (Se) concentration in the environment as measured by its uptake by alfalfa, which sorbs Se from the soil in proportion to what is present, exerted an apparent effect on incidence of (acquired immune deficiency syndrome) AIDS such that AIDS' mortality within the conterminous United States was lower where the Se quantity in the soil was high than where the amount was low. The object of this study was to test this hypothesis for statistical significance and to discover whether the apparent pattern of AIDS mortality in relation to Se distribution holds true with respect to all ages, both races (Black and White), and both genders. The statistical analysis employed was analysis of variance. Age-specific data as well as age-adjusted data were subject to statistical analysis. Ages where AIDS mortality rates per 100,000 were greatest were in the range from 25–54 yr for low-, medium-, and high-Se areas of the US. Black mortality owing to AIDS showed highly statistically significant results for the three Se regions, both genders, and six age groups, whereas White mortality was not as significantly affected by Se. A hypothesis is proposed that the Black population during the last decade or so has been less migratory than the White population. Thus, their food supply and hence its Se content have been more stable than that of the White population, which is more prone to consume imported foods of unknown Se content and be more migratory. A second hypothesis is advanced that suggests that medical care is not equally available to the poor and especially poor Blacks. Black men and women die at a greater death rate than do Whites. This implies that a lack of medical care is the true cause. This article suggests that a pattern exists between the geographical distribution of Se using alfalfa as a dietary guide and AIDS' mortality such that an inverse relationship persists between Se quantity in an area and AIDS' mortality in the same area.

SHORT REPORT
1997

Key words: selenium; oxidative stress; human immunodeficiency virus; muscle; zidovudine

MUSCLE NERVE 20:386–389 1997

MUSCLE INVOLVEMENT IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS IS ASSOCIATED WITH MARKED SELENIUM DEFICIENCY

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Muscular disorders in human immunodeficiency virus (HIV)-infected patients mainly include HIV-associated myopathy, a myopathy that often meets criteria for polymyositis, and zidovudine myopathy, a mitochondrial myopathy induced by long-term zidovudine therapy (see Refs. 5 and 11 for review). However, a causal relationship between the type of myopathy and its putative causal agent may be difficult to establish in some cases. HIV itself has been rarely isolated from muscle cells in patients with the HIV-associated polymyositis.[17,24] The mitochondrial toxicity of zidovudine has been well established,[1,8,18] but experimental studies did not succeed completely in reproducing zidovudine myopathy, possibly because cofactors related to HIV infection itself could be needed to induce the myopathy.[21]

Oxidative stress occurs in tissues of HIV-infected patients.[19] Micronutrient deficiencies have long been recognized in HIV infection and involve vitamins and trace elements such as zinc, iron, and selenium.[2,9] Selenium is a component of glutathione peroxidase, an enzyme having a major function in the removal of oxygen active species from cells and tissues.[22] Selenium deficiency, alone or in association with a deficiency in vitamin E, another antioxidant, may arise under conditions of low alimentary intake in areas of selenium-deficient zones, in chronic malnutrition, and after total parenteral nutrition.[3,10,14,16,27,28] Selenium-deficient patients can develop a congestive cardiomyopathy[10,14] or skeletal muscle involvement manifesting by pain and proximal weakness.[3,16,27,28]

To determine the possible implication of selenium and vitamin E deficiencies in the occurrence of muscle involvement in HIV infection, we retrospectively evaluated selenium and vitamin E status in HIV-infected patients who had muscular symptoms and compared it to that of HIV-infected patients who had no muscular symptoms.

METHODS

We selected 20 HIV-infected patients (blood CD4 cell count: 4–400/mm^3, mean: 153) with muscular symptoms, defined as proximal weakness associated with myalgias, and 20 HIV-infected patients matched for CD4 count who had no muscular symptoms. Of the patients with muscle involvement, 8 had zidovudine (AZT) myopathy, and 12 had other HIV-related myopathies, including HIV polymyositis (6 patients), wasting syndrome (2 patients), and myopathies of unknown origin (4 patients). Patients with clinical

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[Dilated cardiomyopathy and selenium deficiency in AIDS. Apropos of a case].

[Article in French]

Constans J, Sire S, Sergeant C, Simonoff M, Ragnaud JM

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Cardiac-related death of HIV-positive patients is not rare. The etiology of AIDS-associated dilated cardiomyopathies often remains unknown, even at autopsy. We report an observation associated to a severe deficit in selenium. The patient had been diagnosed as HIV-positive 2 years before. He presented Pneumocystis carinii pneumonia then Cryptococcus meningitis. Two months later he was hospitalized for pancreatitis and cachexia. He presented global heart failure that lead to death. No microorganism was found in myocardium at autopsy but plasma selenium was dramatically decreased (24 micrograms/L). The deficit in selenium has been associated to a dilated cardiomyopathy in non-AIDS patients. HIV-positive patients have an early decrease in plasma selenium, this concentration is dramatically decreased in malnourished patients. Selenium deficit might be the cause of some of the AIDS-related dilated cardiomyopathies and selenium supplementation might be useful in these patients.

PMID: 9365739, UI: 98032443

FYI - These are some of >20 papers published over the last decade linking Se levels to HIV disease progression.

Serum Selenium Predicts Outcome in HIV Infection

To the Editor: Recent studies suggest that serum selenium levels decrease in HIV-positive patients and are lower in patients with advanced disease (1,2). We measured serum selenium in 95 HIV-positive patients and established their outcome a year later according to their clinical and immunological status. The relationship between serum selenium and outcome was evaluated in comparison with classic progression markers.

PATIENTS AND METHODS

Ninety-five patients (28 women, 67 men; mean age, 36 years ± 10) had a determination of serum selenium by proton-induced X-ray emission (3) and CD4 cell count (flow cytometry on a FacScan, Becton Dickinson, San Jose, CA, U.S.A.) between June and August 1992. β2 microglobulin (nephelemetry) was also measured in 63 patients, and p24 antigenemia was measured (enzyme-linked immunosorbent assay) in 39. The patients had the following modes of contamination: i.v. drug use in 32, homosexuality in 38, blood transfusions in 10, heterosexuality in 14, and unknown in one. Sixty-four patients were taking zidovudine, one was taking didanosine.

An evaluation of outcome was carried out after 12 months ± 7. Two endpoints were analyzed: death and occurrence of AIDS-defining opportunistic infection (OI). Correlations were calculated between serum selenium, CD4 cell count, p24 antigenemia, β2 microglobulin, and outcome endpoints by the Spearman correlation test and stepwise multivariate analysis.

RESULTS

The 95 patients were divided into four groups according to CD4 cell count (<50/mm^3, 50–200/mm^3, 200–400/mm^3, >400/mm^3). Their serum selenium levels were 55 ng/ml (SD 14), 66 ng/ml (SD 17), 70 ng/ml (SD 16), and 72 ng/ml (SD 9), respectively. Serum selenium correlated with CD4 cell count ($r = 0.447$, $p < 0.0001$) and with p24 antigenemia ($r = 0.601$, $p < 0.0001$). During follow-up, 34 patients died, and 47 had an AIDS-defining OI. The correlation study showed that death was correlated with CD4 cells ($r = -0.709$, $p < 0.0001$), p24 antigenemia ($r = 0.594$, $p < 0.0001$), and serum selenium ($r = -0.416$, $p < 0.0001$) but not with β2 microglobulin. Occurrence of OI correlated with CD4 cells ($r = -0.735$, $p < 0.0001$), p24 antigenemia (0.662, $p < 0.0001$), β2 microglobulin ($r = 0.372$, $p = 0.002$), and serum selenium ($r = -0.416$, $p < 0.0001$). Because we found that serum selenium was correlated with CD4 cells and p24 antigenemia, we carried out a multivariate analysis of correlations between these parameters and death or OI. Death and OI correlated with CD4 cell count ($p = 0.006$ and 0.001, respectively) and serum selenium ($p = 0.01$ and 0.008, respectively). Finally, p24 antigenemia was no longer correlated with death or OI, and only CD4 cells and serum selenium correlated with outcome.

DISCUSSION

Serum selenium decreases in several chronic diseases, particularly in HIV-positive patients (1,2). In the present work, we found that selenium decreases like the CD4 cell count. Moreover, serum selenium is related to prognosis, irrespective of CD4 cell count. The functions of selenium are poorly known, but selenium is required for immune functions: lymphocyte proliferation, macrophage function, and natural killer activity. Benign human Coxsackie enterovirus becomes virulent in selenium-deficient animals, and infections by several viruses are associated with oxidative stress. Selenium acts as an antioxidant through the selenodependent glutathione peroxidase, which catalyzes the degradation of H_2O_2 or hydroperoxides at the expense of reduced glutathione and may decrease the effects of the oxidative stress found in HIV-positive patients (4). A negative effect of oxidative stress is possible in HIV infection: Free radicals play a role in lymphocyte activation and in HIV replication (5). Our results suggest that a selenium supplement, which is able to increase serum selenium levels (1), should be evaluated in large-scale trials as an adjunctive therapy in AIDS.

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1995

Relationship of Trace Element, Immunological Markers, and HIV$_1$ Infection Progression

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ABSTRACT

Trace elements (selenium, zinc, copper), β_2 microglobulin levels, CD_4, and CD_8 cell counts have been determined in 80 HIV$_1$ seropositive patients. The study group consisted of 19 females and 61 males with age mean of 35 ± 10 yr, at stage IV of infection (CDC—Atlanta classification) and treated by AZT. No severe renal or liver diseases or hypoalbuminemia were observed in this group.

Se values were significantly lower than in normal adults, 48.3 ± 17 µg/L vs 71 ± 12 µg/L; Zn was moderately diminished, 1 ± 0.2 mg/L vs 1.2 ± 0.2 mg/L, whereas copper values were in the normal range, 1.2 ± 0.3 mg/L vs 1.1 ± 0.5 mg/L. Se or Zn deficiency was found in 60 and 30 subjects, respectively. Blood Se and Zn decreases were associated in 23 patients. Moreover, all patients showed higher β_2 microglobulin values than the upper normal limit of 2.4 mg/L. Negative correlations were found between Zn and β_2 microglobulin ($p < 0.005$) and between Se and β_2 microglobulin ($p < 0.05$). Moreover, there was a positive correlation between Se and Zn values ($p < 0.05$).

Nineteen subjects died 1 yr later (group I), and 61 remained alive (group II). With respect to the clinical evolution, a significant difference between both groups was found in Se and β_2 microglobulin levels as well as in CD_4 cell counts. The correlations previously observed persisted in group II, whereas no correlation was noted in group I. In addition, the patients of group I had significantly lower Se values, which were below 30 µg/L in 10 cases.

These results confirm the prevalence of abnormalities in Se and Zn levels and their relationships with nonspecific markers of immune system activity in more advanced HIV disease. Impairment of trace element status and mainly Se status appeared, at least partially, to reflect the disease activity/progression and subsequently the immune dysregulation.

Index Entries: AIDS; HIV$_1$ seropositive; serum selenium level; plasma zinc level; serum copper level; trace elements and HIV$_1$ disease activity/progression.

FROM CONCLUSIONS:

In conclusion, the measurement of trace elements, especially Se, may be a useful marker to predict the HIV infection progression. Better understanding of the mechanisms of trace element abnormalities and the strategies to maintain optimal trace element balance could, thereby, improve the immune function and life quality of these patients.

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Serum Selenium Versus Lymphocyte Subsets and Markers of Disease Progression and Inflammatory Response in Human Immunodeficiency Virus-1 Infection

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ABSTRACT

Serum selenium levels were determined cross-sectionally in 57 HIV-infected patients who were classified according to the Centers for Disease Control (CDC) 1993 classification system. Mean serum selenium levels were lower in CDC stage II (58.7 ± 12.2 μg/L; $p < 0.01$; $n = 18$) and stage III (47.6 ± 11.3 μg/L; $p < 0.01$; $n = 19$) HIV-infected patients, than in healthy subjects (80.6 ± 9.6 μg/L; $n = 48$) and stage I patients (73.6 ± 16.5 μg/L; $n = 20$). Serum selenium levels were positively correlated with CD4 count, CD4/8 ratio, hematocrit, and serum albumin ($r = 0.42$; $r = 0.39$; $r = 0.48$; and $r = 0.45$; $p < 0.01$, respectively) and inversely with serum levels of thymidine kinase ($r = -0.49$; $p < 0.01$; $n = 49$) and β2-microglobulin ($r = -0.46$; $p < 0.001$; $n = 49$). In addition, serum selenium levels in 20 randomly selected AIDS-free individuals (CDC I: $n = 10$; CDC II: $n = 10$) were inversely correlated with serum concentrations of interleukin-8 (IL-8) and soluble tumor necrosis factor receptors (sTNFR) types I and II. There was no correlation with serum immuneglobulin A and total serum protein levels. The results show that the progressive deprivation of serum selenium in HIV-infection is associated with loss of CD4+-cells and with increased levels of markers of disease progression and inflammatory response.

Index entries: AIDS; HIV-infection; serum selenium; thymidine kinase; β2-microglobulin; interleukin-8; soluble tumor necrosis factor receptors I and II.

INTRODUCTION

In HIV-disease the balance between pro-oxidants, antioxidants, and cytokines is disturbed toward a chronic immune activation state *(1–8)*.

Trace Elements and Electrolytes, Vol. 11 No. 3 – 1994 (130–134)

Trace elements in patients with HIV-1 infection

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Abstract. Urine and serum zinc, selenium, copper, iron, magnesium and manganese concentrations were determined in 19 patients with AIDS compared to 25 individuals with asymptomatic HIV-1 infection and 18 healthy controls. No significant differences between groups were found in the urinary levels of the trace elements. There were no significant differences in the serum zinc, magnesium and iron concentrations between control subjects and patients with AIDS or asymptomatic HIV-1 individuals, whereas there was a significant decrease in the serum selenium levels in both groups of HIV-1 infected patients vs controls. Serum manganese concentrations in patients with AIDS were significantly diminished in comparison with controls and HIV- 1 asymptomatic subjects, whereas the serum copper levels were decreased for HIV-1 asymptomatic subjects compared to patients with AIDS. Although the HIV-1 infected patients were selenium-deficient, supplementation of this element might not be advisable.

Key words: trace elements – serum – urine – HIV-1 infected patients

Introduction

In recent years, a considerable amount of information on the spectrum of clinical consequences of infection by human immunodeficiency virus (HIV-1) and on the underlying immunologic abnormalities has been accumulated [Levy 1989, Seligmann et al. 1987]. However, as yet, there is no vaccine and only palliative antiviral chemotherapy is available. Consequently, HIV-1 infected subjects frequently seek alternative means of optimizing immune function to prevent disease progression [Mantero-Atienza et al. 1991a, 1991b].

Among the clinical abnormalities of the acquired immunodeficiency syndrome, a significant degree of malnutrition and wasting is characteristic in the later stages of AIDS [Kotler et al. 1985]. Such malnutrition involves both changes in overall body composition as well as deficiencies of specific nutrients [Mantero-Atienza et al. 1989, 1991a, 1991b]. Since deficiencies of multiple nutrients can impair immunologic host defense mechanisms, it would be possible that malnutrition contributes to the morbidity or mortality of some AIDS patients [Dworkin et al. 1988].

Decreased levels of serum zinc in human immunodeficiency virus-infected individuals have been reported [Allavena et al. 1993, Caselli and Bicocchi 1986, Fabris et al. 1988, Weiner 1984]. It has been found that a number of immunological abnormalities exhibited by patients with AIDS [Seligmann et al. 1987] are similar to the defects observed in both experimental animals and humans with zinc deficiency [Walter et al. 1990]. Moreover, it has been recently demonstrated that zinc copurifies with mature HIV-1 particles, which raises the possibility that zinc may be associated with the nucleocapsid protein in virus particles, in addition to functioning in retroviral gene recognition at the gag protein level [Summers et al. 1990, South et al. 1990]. This finding may have significant consequences in the development of vaccines for the prevention of AIDS [Summers et al. 1990, South et al. 1990].

In contrast to zinc, increased plasma copper concentrations were found in patients with AIDS [Walter et al. 1990], whereas selenium status in HIV-1 infection is also of particular interest, in light of studies documenting alterations in plasma selenium levels in HIV-1 infected patients [Allavena et al. 1993, Dworkin et al. 1988, Mantero-Atienza et al. 1991a and 1991b].

In view of the alterations in zinc and selenium turnover reported in patients with AIDS, and taking into account the interest that zinc and selenium supplementation might have for HIV-1 infected subjects, the purpose of the present study was to provide a detailed characterization of zinc and selenium status in individuals with HIV-1 asymptomatic infection and in patients with AIDS. In order to

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Plasma antioxidant status (selenium, retinol and α-tocopherol) in HIV infection.

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Summary

Plasma antioxidant status can be evaluated by selenium, retinol and a-tocopherol. The present study concerns 89 HIV-1 positive patients. The plasma level of selenium was determined by PIXE (proton-induced X-ray emission analysis) and by measurement of levels of vitamins A and E by HPLC. The data were compared to controls; among the HIV-1 patients, significant differences were found for plasma selenium between groups selected on the basis of the CD4 lymphocyte count. Differences were also observed for plasma retinol between the HIV-1 patients and the control subjects. Correlations with other nutritional and immunological parameters were shown.

Introduction

Alterations in nutritional status are widespread among the HIV I population and are known to involve vitamins and trace elements. It has also been shown that deficiencies in selenium, zinc, vitamins A, E and B6 contribute significantly to the pathogenesis accompanying severe immunosuppression (Meydani et al., 1992). Among natural antioxidants occurring in cells under physiological conditions, vitamins A and E and selenium protect membranes and cytoplasm respectively (Packer 1991, Spallholtz 1990).

The aim of the present work was to investigate the antioxidant status with respect to selenium and vitamins A and E in the plasma of these HIV patients and in a control group. Selenium was measured by Proton-Induced X-Ray Emission (PIXE) after chemical preconcentration (Simonoff 1988) at the Centre d'Etudes Nucléaires de Bordeaux-Gradignan. Retinol and α-tocopherol were extracted chemically and quantified by High Performance Liquid Chromatography (HPLC) (Thurnham 1988) on a C18 μBondapack column, using a Waters HPLC system. Triglycerids, Cholesterol (Ch) and HDL-Cholesterol (HDL-C) were measured on a multiparameter automate Paramete (Baxter France). Apoprotein apo A1 and B and Lp(a) were determined by nephelometry using a BNA instrument (Behring France). CD4 and CD8 lymphocytes were counted by flow cytometry. P24 antigenemia was determined in 39 patients (Abbott ELISA).

From a conference in France in 1993

ELSEVIER SCIENCE IRELAND

Chemico-Biological Interactions

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Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS)

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Abstract

Selenium is required for activity of the enzyme glutathione peroxidase, and selenium deficiency may be associated with myopathy, cardiomyopathy and immune dysfunction including oral candidiasis, impaired phagocytic function and decreased CD4 T-cells. We assessed selenium status in 12 patients with AIDS compared to normals and found significantly low plasma and red blood cell levels. Plasma selenium in AIDS was 0.043 ± 0.01 μg/ml vs 0.095 ± 0.016 in controls ($P < 0.001$). Selenium status correlated with serum albumin ($r = 0.77$; $P < 0.001$) and 60% had documented GI malabsorption as determined by abnormal D-Xylose tests. In a subsequent study blood selenium and glutathione peroxidase were diminished in 12 AIDS and 8 ARC patients compared with normals (all $P < 0.001$). For glutathione peroxidase the mean levels were decreased by 45% in AIDS and 27% in ARC versus controls ($P < 0.001$). Both plasma selenium and glutathione peroxidase significantly correlated with total lymphocyte counts ($r = 0.65$; $P < 0.001$; glutathione peroxidase and lymphocyte counts). This occurred in both homosexuals and drug users with AIDS and irrespective of the presence or absence of diarrhea or GI malabsorption. To determine if tissue levels of selenium were also depleted we studied cardiac selenium levels in autopsy AIDS hearts compared to age and sex matched controls. Cardiac selenium in AIDS was 0.327 ± 0.082 μg/g dry weight versus 0.534 ± 0.184 in controls ($P < 0.01$). Two cases had histologic cardiomyopathy pathologically consistent with the cardiomyopathy described in Keshan disease associated with low selenium blood levels. To further assess mechanisms of nutrient and selenium deficiency in AIDS we studied dietary intake in outpatients and inpatients with various stages of HIV infection. Inadequate selenium intake based on a computer (Nutritionist 3) analysis of 72 h diet records was present in only 17% of clinically stable HIV positive outpatients and 71% of inpatients with AIDS. Conclusions: Selenium deficiency is common in HIV positive patients as documented by low plasma and red blood cell levels of selenium, diminished activity of glutathione peroxidase, and low cardiac selenium levels in AIDS hearts. Patients with AIDS tend to have more severe deficits than those with earlier stages of HIV infection. The selenium deficit in blood does correlate with serum albumin levels and total lymphocyte counts. Poor dietary intake and malabsorption could lead to this condition which has important implications for both cardiac and immune functions in HIV positive patients.

!! sounds an awful lot like AIDS...

i.e. Se depletion correlates with stage of HIV disease (T-cell loss) in a way that cannot be completely ascribed to GI malabsorption

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First International Symposium on Human Viral Diseases: Selenium, Antioxidants and Other Emerging Strategies of Therapy and Prevention

19–21 April 1996, Nonnweiler, Germany

BACKGROUND

Why selenium and viral diseases?

Several decades ago selenium (Se) was shown to be an essential trace mineral in mammals. It is a component of the important antioxidant enzyme glutathione peroxidase (GPx), which is critical for defence against lipid peroxidation and other types of oxidative and free radical damage.

A possible link to viral diseases became apparent almost 20 years ago, first with Schrauzer's demonstration that Se is chemoprotective against virally-induced mammary tumours in mice and shortly thereafter with the implication of Coxsackievirus as a probable cofactor in Keshan disease, the classical Se-deficiency disease of humans. This cardiomyopathy was once endemic among peasants in low-Se regions of China, and was essentially eradicated by Se supplementation programmes. In fact, Se has now been shown to have a significant chemoprotective effect against a number of human and animal viruses, including hepatitis B infection linked to liver disease and cancer (1), Coxsackievirus–Keshan disease (2), viral haemorrhagic fever (3) and the mouse mammary tumour virus (4). A link between Se and HIV is still unclear, although low plasma Se is a correlate of disease progression and Se has been shown to inhibit HIV activation *in vitro* (5).

In humans, Se appears to be important not only for antioxidant defences via GPx, but also for thyroid function, reproduction, cellular immunity and aspects of gene regulation. Se acts via 'selenoproteins' that contain the rare amino acid selenocysteine (SeC), which is encoded by the UGA codon when specialized RNA structures are present; otherwise, UGA will direct termination of protein synthesis. In bacteria, SeC is also encoded by UGA, suggesting that this is a very ancient codon usage; thus, it has been proposed that selenoproteins may have been more common early in evolution, before atmospheric oxygen levels increased, and that UGA evolved into a stop codon owing to decreasing bioavailability of SeC. If there is any merit to the concept of RNA viruses as 'molecular fossils' of the RNA world, then one might expect to see some vestigial traces of ancient selenoproteins in such viruses. Intriguingly, it was recently shown that RNA viruses typically have an up to threefold higher incidence than expected of UGA codons in the -1 reading frame overlapping their known genes (6).

Although there is overwhelming evidence of a role for oxidative stress in AIDS pathogenesis, and antioxidants show promise as complementary therapies, the mechanisms involved are poorly understood. HIV infection apparently induces an antioxidant defect in the host (7), and can even produce a deficiency of the mammalian selenoprotein GPx in HIV-infected cells (8). Such data are consistent with the possibility that viral selenoproteins might play a role in the stimulation of HIV replication by oxidative stress or Se deficiency (9,10).

The fact that selenium deficiency has been linked to the incidence, disease progression or virulence associated with a number of viral infections provides ample incentive for a deeper look at these phenomena, and was the basis for convening the 1996 symposium on Se and antioxidants in human viral diseases.

An interdisciplinary group of basic and clinical researchers gathered in Nonnweiler, Germany to review the current status and potential of Se and antioxidants as chemoprotective and therapeutic agents in human viral diseases. In this regard, probably the most striking report of the meeting was that of Jian-Cun Hou (Chinese Academy of Medical Sciences, China). In an outbreak of viral haemorrhagic fever (HF) involving 80 patients, he used oral sodium selenite at 2 mg Se per day for 9 days to achieve a marked reduction in mortality, which in the 'fulminant' category fell from 100 per cent (untreated) to 37 per cent (treated), and from 22 per cent to 0 per cent in the less severe cases. The mechanism remains obscure, but the researchers showed that complement activation was

reduced by the Se treatment. Alternatively, Chandra Ramanathan (University of Georgia, Athens, USA) pointed out that extreme Se deficiency has a pro-clotting and even haemorrhagic effect in animals. Since reading frames with a very high content of UGA codons can be found overlapping the coding regions of various HF viruses, including Ebola, it is possible that the extremely high viral loads seen in HF might cause host Se depletion, either by programmed selenoprotein synthesis, or by low frequency SeC insertion at UGA codons due to random slippage errors during viral transcription and translation.

Another exciting story involves new findings relevant to the Coxsackievirus-Keshan disease link. Orville Levander (US Department of Agriculture, Beltsville, USA) and Melinda Beck (University of North Carolina, Chapel Hill, USA) reviewed their recent work, showing how the cofactor role of Coxsackie B virus (CVB) is strongly supported by their demonstrations that Se deficiency can trigger cardiomyopathy in Coxsackie-infected mice, and that even a 'benign' strain of CVB3 becomes virulent in Se-deficient animals, where it mutates into a more virulent strain that can produce myocarditis even in Se-adequate mice. Similar results were obtained with vitamin E, which has a sparing effect on Se.

These findings have profound implications in regard to the possible role of nutritional status in emerging viral diseases. The underlying mechanisms in both the host and virus may be multifaceted, and so provoked much discussion. Esteban Domingo (Universidad Autónoma de Madrid, Spain) reviewed mechanisms of RNA virus evolution, including hyper-mutability and the viral quasispecies concept, which can explain the rapid mutation of CVB into a more virulent form when the immune system is weakened by nutritional deficiency, unleashing viral replication and permitting the selection of more virulent strains. Ernst Peterhans (University of Berne, Switzerland) amplified this mechanism in terms of the role of reactive oxygen species in cellular responses to viral infections, a recurring theme of the meeting. Luc Montagnier (Pasteur Institute, Paris, France) elaborated extensive evidence for the role of oxidative stress in activating HIV. The involvement of cellular transcription factors like NF-κB in this process was described in detail by Jean-Louis Virelizier (Pasteur Institute).

Alain Favier (UFR des Sciences, La Tronche, France) described multiple antioxidant abnormalities in persons living with HIV/AIDS (PWAs) (including low beta-carotene, zinc and Se), and *in vitro* studies of the roles of trace elements, e.g. showing that Se (as selenite) decreases activation of NF-κB as well as inhibiting the reactivation of HIV-1 by hydrogen peroxide and tumour necrosis factor. Multiple nutrient abnormalities in PWAs, including Se, were also discussed by Marianna Baum (University of Miami, USA), whose most striking finding was that low plasma Se was the highest single risk factor for mortality in the HIV cohort she has been studying, being substantially more significant than low CD4 count (defined as CD4 <200 cells/mm^3) as a risk factor.

MP Look (Medizinische Universitaetsklinik, Bonn, Germany) reported a brief clinical trial of supplementation with Se and *N*-acetyl cysteine (a glutathione precursor) in HIV infected individuals. While a consistent increase in CD4 counts was not seen, favourable shifts in some survival indicators such as T-cell subsets were observed.

Ursulla Cowgill (Department of EPO Biology Carbondale, USA) analysed AIDS death rates in the USA by race and sex relative to Se levels in forage plants in different counties and states. Data for blacks showed a correlation between low Se regions and AIDS mortality, but as might be expected, due to extensive food distribution networks, data for whites generally did not correlate. The hypothesis was offered that since AIDS in blacks is strongly associated with socio-economic factors like poverty, the black HIV population might be more likely to partake of locally grown foods in their diets.

Shu-Yu Yu (Chinese Academy of National Sciences, China) presented data from several extensive studies of human Se supplementation in regions of China where hepatitis B and primary liver cancer are endemic, showing significant reduction in disease incidence even with small daily doses. The mechanistic basis of this effect has yet to be determined.

A classical demonstration of the chemoprotective effects of Se is the work of Gerhard Schrauzer (Biological Trace Element Research Institute, San Diego, USA) with the mouse mammary tumour virus (MMTV). The incidence of mammary tumours consequent to infection with this retrovirus is reduced by increasing dietary Se. The relevance of this animal model to human disease is considerable; aside from the obvious implications for HIV and other retroviral diseases, the implications for human cancer are now quite inescapable. Isabelle Pelisson (Mt Sanai Medical Center, New York, USA) reported the remarkable discovery of sequences highly homologous to the MMTV envelope gene in 38 per cent of human breast cancer specimens. The investigators ruled out any known endogenous retrovirus, and showed a specific localization only to cancerous human breast tissues. This provides a new incentive for research into the role of viruses in carcinogenesis.

My own presentation involved an update on the 'viral selenoprotein theory' as a potential factor in some of the observations documented above. An analysis of the genomic structure of HIV-1 shows the potential for several novel frameshift sites, and shows that certain UGA codons in overlapping reading frames are well conserved in known HIV-1 isolates. Several genomic features required for the expression of such potential selenoprotein genes in HIV-1 have now been experimentally confirmed, such as an RNA pseudoknot in the reverse transcriptase coding region. We now have firm in vitro evidence of activity at a novel frameshift site in the HIV-1 *nef* coding region. Since the -1 reading frame has a highly conserved UGA codon, the possibility that this region encodes a viral selenoprotein must be considered. The most compelling data, highly distinctive GPx-related sequences in several strains of CBV, consistent with a virally encoded GPx enzyme, may relate to the results of Levander and Beck on CVB3. This suggests that a direct utilization of Se by the virus may contribute to observations on CVB3.

This unique meeting was organized by Dr Gerhard Schrauzer, and sponsored by Biosymposia and the International Association of Bioinorganic Scientists. The proceedings will be published in a special issue of *Biological Trace Element Research*, to be co-edited by Dr Schrauzer and Professor Luc Montagnier. Hopefully these proceedings will stimulate the development of new antiviral agents that go beyond simple Se supplementation.

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The Clinical Evaluation of Antiviral Drugs

28 February 1996, London, UK

Introduction

At the end of 1993 the British Society for Antimicrobial Chemotherapy and the Society of Pharmaceutical Medicine agreed to establish a joint working party, to decide upon guidelines and recommendations for the clinical development of new antiviral agents. An inaugural meeting was held on 14 April 1994 at the offices of The Ciba Foundation in London. The working party was chaired by Hugh McDade (Glaxo) and membership included experts in the field from academia, clinical practice and the pharmaceutical industry; sub-committees were established to concentrate on specific topics (regulatory aspects, preclinical considerations, laboratory evaluations in clinical research, and clinical pharmacology) and disease areas (HIV infection, herpesvirus infections, viral hepatitis and respiratory tract infections). Over the following 18 months a series of meetings was held, and draft recommendations were produced by each of the sub-committees and circulated for comment both within the group and to outside experts. A comprehensive document is now in the final stages of preparation.

The meeting, organized by the Society of Pharmaceutical Medicine and held at The Scientific Societies' lecture theatre in London, presented the draft recommendations of the working party to approximately 30 participants from the UK and Europe. It was structured as an interactive discussion meeting, where feedback from the delegates could be incorporated during the preparation of the final recommendations.

Antiviral clinical development

Regulatory aspects of antiviral clinical development were discussed by the sub-committee chair, Dr Flic Gabbay (Gabbay Group Ltd). Potent antiviral agents tend to be toxic, so the risks and benefits to the patient need to be carefully assessed and balanced. 'Fast-track' registration for life-threatening diseases such as AIDS may be possible. Increasingly, pharmaco-economic evaluation of potent, expensive new therapies will be necessary. Companies may well be subjected to substantial pressures to release unlicensed drugs on a 'compassionate-use' basis for people living with HIV/AIDS, which may raise regulatory issues.

Preclinical considerations reviewed by Dr Charles Penn (Glaxo Wellcome) indicated that a greater understanding of the properties and mode of action of antiviral agents is now expected before clinical studies can proceed. Studies should include demonstration of an antiviral effect *in vitro*, assessment of potential interactions, investigations into the mechanism of action and potential for the development of viral resistance, evaluation of possible mechanisms of toxicity and determination of specific toxicity.

Dr Jane Grundy (Royal Free Hospital) considered the question of laboratory evaluations during clinical development. For multicentre studies it is preferable to select a single laboratory to process and report on the specimens. An appropriately qualified member of the laboratory staff should be nominated to take day-to-day responsibility for the processing of trial samples to "Good Laboratory Practice" standards, and in compliance with the study protocol. The choice of clinical sample and timing of collection should be relevant to the natural history of the virus under investigation; high concentrations of the antiviral agent at the time of sampling may interfere with subsequent laboratory investigations.

Initial investigation in humans

Dr Michael Barry (University of Liverpool) addressed the issue of initial investigation in humans. Phase I studies with agents that have the potential to cause serious toxic effects may be performed in seriously ill patients rather

HIV-1 Encodes a Sequence Overlapping *env* gp41 With Highly Significant Similarity to Selenium-Dependent Glutathione Peroxidases

To the Editor: A progressive decline in plasma or serum selenium levels paralleling the loss of $CD4^+$ T cells has been widely documented in HIV-1 infections. As reviewed (1), such findings have been reported in more than 20 papers published during the last decade originating from independent laboratories in at least six different countries and have been observed for asymptomatic and symptomatic HIV patients, for children and adults (2–7).

The observations of several investigators suggest that this HIV-associated decline in selenium levels cannot be explained simply by malnutrition or nutrient malabsorption. According to Dworkin (3), levels of plasma selenium, cardiac selenium (at autopsy), and the antioxidant selenoprotein glutathione peroxidase in patients with AIDS-related complex and AIDS are "significantly correlated with total lymphocyte counts," but selenium depletion can occur "irrespective of the presence or absence of diarrhea or gastrointestinal malabsorption" (3). This suggests that the decline in selenium levels that parallels the progression of HIV disease cannot be attributed entirely to malabsorption. Similarly, other researchers have pointed out "a surprisingly high prevalence of low levels of selenium in early stages of the disease" (2) well before wasting is usually observed and found that ". . . a low selenium intake seems unlikely [as a cause of low plasma selenium], because urinary excretion, which closely reflects the actual selenium intake, was similar in HIV-1 infected patients and controls" (5). Allavena et al. (6) exclude malabsorption as the underlying cause, correlate selenium levels with survival prognosis, and conclude that "the measurement of trace elements, especially selenium, may be a useful marker to predict the HIV infection progression." Similarly, in a 12-month study involving 95 patients, Constans et al. (7) show that serum selenium is a statistically significant predictor of outcome in HIV infection.

The dramatic results of the study by Baum et al. (8), a 3.5-year longitudinal study of 125 HIV-positive intravenous drug users, show that selenium deficiency is an exceptionally high risk factor for HIV-associated mortality. The relative risk (RR) for mortality associated with selenium deficiency (RR = 10.8, $p < 0.002$) is far higher than that for any other nutrient deficiency and 15 times higher than that associated with a low CD4 count (defined as <200 cells/mm^3; RR = 0.69, $p < 0.04$).

In light of this body of clinical evidence, we would like to report an observation of potential significance for the molecular mechanisms underlying any interaction between HIV and dietary selenium. Taylor et al. (9) previously pointed out several regions of HIV-1 with the potential to encode selenoproteins due to the existence of potential frameshift sequences and conserved UGA codons (potentially encoding selenocysteine) in several locations overlapping known HIV genes. One of these overlaps the HIV-1 *env* gp41 coding region in the −1 reading frame and contains a single UGA codon near the middle of a highly conserved novel open reading frame (ORF) of about 120 amino acids, following a conserved −1 frameshift signal (A AAA), consistent with the established "P-site" slippage mechanism.

We have identified the sequence encoded in this ORF, which we call *env-fs*, as a homologue of glutathione peroxidase (GPx), the prototypical eukaryotic selenoprotein. The database search method we used in 1994 was not sensitive enough to pick up this similarity, in part probably because of several deletions in the active site region and the high mutation rate of retroviral sequences, leading to a low overall similarity to any single GPx sequence.

Nonetheless, the presence in this HIV-1 genomic region of a common variant of the GPx active site consensus sequence, spanning the catalytic selenocysteine, is apparent on examination of a multiple alignment of GPx sequences (Fig. 1: YUGAT in HIV-1 versus YUGLT in GPx, where U = selenocysteine). This similarity occurs precisely at the location of the single conserved UGA codon in the −1 reading frame of the gp41 coding region of HIV-1. This UGA codon is highly conserved in the predominant North American–European HIV-1 strains (subtype B), but other HIV-1 subtypes and most HIV-2 subtypes have a conserved arginine codon (AGA) at this position. This substitution is also highly consistent with peroxidase activity, because arginine is the active site residue of many peroxidases (e.g., horseradish peroxidase), and arginine variants of the GPx active site motif (e.g., SLRG rather than SLUG or SYUG) are common in non–selenium-dependent peroxidases.

Using more sensitive sequence comparison methods, a highly significant similarity between this HIV-encoded sequence and GPx can be demonstrated. For example, we have assessed the similarity of *env-fs* to the aligned set of GPx sequences by a variation on the standard method for comparing two sequences, which we implemented in a computer program developed in our laboratories. In brief, the program produces an optimal alignment of the probe sequence (*env-fs*) compared with a fixed multiple alignment (GPx) by optimizing a scoring function that correctly takes into account linear gap penalties in the multiple alignment. The score of the optimal alignment of the probe sequence is the best possible value of the sum of the scores of the pairwise alignments induced between the probe sequence and the rows of the input multiple alignment. Then the probe sequence is randomly shuffled (50 times in this case) and realigned by the same algorithm. The average alignment score and the standard deviation (SD) for random sequences of identical composition are then calculated, and the *significance* of the actual score is expressed as a Z score (i.e. the number of SD from the "expected" random score).

Using a truncated GPx alignment as shown in Fig. 1, the blosum62 similarity matrix, gap creation penalty of 5, and gap extension penalty of 2, the *env-fs* sequence is aligned essentially as shown, with a significance score of 5.0 SD. Using the full GPx alignment (including the region shown as % and extending at the C terminus), with slightly different gap parameters, a Z score of 4.9 SD is obtained, producing an alignment that is somewhat looser in the C-terminal region but otherwise identical to that in Fig. 1 up to the YUGLT active site region.

The possibility of a virally encoded selenium-dependent GPx gene is not unprecedented. A GPx homologue was reported in the molluscum contagiosum virus (10). We have also identified GPx-related sequences in coxsackie B virus, the cofactor in Keshan disease, a classic selenium-deficiency disease (1).

Because a GPx with a UGA codon at the active site is a selenoprotein, the existence of this sequence in HIV-1 strongly suggests that a conserved UGA codon in HIV may encode selenocysteine, rather than a conventional amino acid, which can also occur. This possibility merits serious consideration in

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Match: G SR  Y  TV DADG *   *  Y * A  F**  *YUG*TAS *A  S  *GHQ* PGKN       KGS  PG  G*L K  L** C LEC
env-fs GSSRKHYGRTVNDADGTGQTIIVWYSAAAEQFAE..GYUGATAS.VATHS..LGHQAAPGKNPGCGKIPKGSTAPGDLGLLWKTHLHHCCALEC
S05317 AAQSTVYAFSARPLTGGEPVSLGSLRGKVLLIENVASLUGTTIR%LGFPCNQFGHQV.YGARW....VALGSRLPRA.GHRLLSPLV..LQLEC
A53395 RCARSMHEFSAKDIDG.HMVNLDKYRGYVCIVTNVASQUGKTEV%LAFPCNQFGRQE.PGSDAEI....KE...FAA.GYNVKFDMFSKICVNG
S20501 SKPQSIYDFTVKDAKG.NDVDLSIYKGKVLIIVNVASQCGLTNS%LAFPCNQFGGQE.PGSIEEIQN.MVCTR......FKAEYPIFDKVDVNG
Jq0476 GISGTIYEYGALTIDGEEYIPFKQYAGKYVLFVNVASYUGLTG.%LGFPCNQFGKQE.PGENSEILPTLKYVR.PGG.GFVPNFQLFEKGDVNG
A55086 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%LGFPSNQFGKQE.PGENSEILPSLKYVR.PGG.GFVPNFQLFEKGDVNG
Jx0176 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%LGFPCNQFGKQE.PGENSEILPSLKYVR.PGG.GFVPNFQLFEKGDVNG
Jx0280 GVGGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTG.%LGFPCNQFGKQE.PGENSEILATLKYVR.PGG.GFTPNFQLFEKGDVNG
S18912 DEKGTIYDYEAIALNKNEYVPFKQYVGKHILFVNVATYCGLTA.%LGFPCNQFGKQE.PGDNKEILPGLKYVR.PGG.GFVPNFQLFEKGDVNG
A47367 DVKGTIYDYEALSLNGKEDIPFKQYRGKHVLFVNVATYCGLTI.%LGFPCNEFGKQE.PGDNLEILPGLKYVR.PGK.GFLPNFQLFAKGDVNG
B40464 GVAGTVYEYGANTLDGGEYVQFQQYAGKHILFVNVASFCGLTA.%LGFPCNQFGKQE.PGKNSEILLGLKYVR.PGG.GFVPNFQLFEKGDVNG
A45207 FIAKSFYDLSAISLDG.EKVDFNTFRGRAVLIENVASLUGTTTR%LGFPCNQFGHQE.NCQNEEILNSLKYVR.PGG.GYQPTFTLVQKCEVNG
S21119 VAQSTVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTR%LGFPCNQFGHQE.NGKNEEILNSLKYVR.PGG.GFEPNFTLFEKCEVNG
S06304 AAAQSVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTVR%LGFPCNQFGHQE.NAKNEEILNSLKYVR.PGG.GFEPNFMLFEKCEVNG
S03723 AAAQSVYSFSAHPLAGGEPVNLGSLRGKVLLIENVASLUGTTVR%LGFPCNQFGHQE.NAKNEEILNSLKYVR.PGG.GFEPNFMLFQKCEVNG
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FIG. 1. Sequence alignment of hypothetical HIV-1 *env-fs* sequence compared with various selenium-dependent glutathione peroxidases. The HIV-1 sequence shown as *env-fs* is as given in Table 1 of Taylor et al. (9), beginning right after a predicted putative protease cleavage site. It is aligned with a set of eukaryotic glutathione peroxidases (GPx) from various sources (single letter amino acid code, with U as the symbol for selenocysteine [Sec], encoded by the UGA codon in RNA). A match to one or more of the aligned GPx sequences is shown as a letter in the match line above the alignment; similar residues are indicated by an asterisk. Active site regions of GPx are indicated by ++++++ below the alignment. Compare the matches in the region of the conserved catalytic selenocysteine, with *eniv-fs* residues YUGATA, with YUGLTG in GPx sequence Jq0476 (here, UGA means Sec-Gly-Ala, not the UGA codon). The most common variant in the predominant North American–European HIV-1 subtype B is SYUGAT instead of GYUGAT, an even better match to many of the GPx; the active site motif usually is SYUGLT or SLUGTT. After a 19 residue deletion in the HIV sequence (shown as % in the alignment), a serine (S) in *env-fs* aligns with a conserved cysteine (also one GPx has S rather than C), followed by GHQ . . . PGKN, which are exact matches to several GPx sequences. The deletion (at %) in *env-fs* between these two conserved regions (YUG and GHQ) is not part of the GPx active site and thus is not a problem structurally. The N terminus of the mature GPx protein begins at the fourth residue in the alignment, and one human GPx variant (S05317) ends just beyond the end of the alignment; thus, a known GPx variant is truncated almost identically as this putative HIV GPx homologue. The total similarity score of the *env-fs* sequence compared with the aligned GPx sequences is 5 standard deviations above the average similarity score of randomly shuffled sequences of identical composition, each optimally aligned by the same algorithm. Assuming a normal distribution of random scores, this significance level (5 SD) means that the probability of obtaining this degree of similarity purely by chance is less than 1 in 1 million.

light of the high statistical significance of the *env-fs* similarity to the GPx sequence and the clinical data strongly linking HIV disease progression to the host's selenium status.

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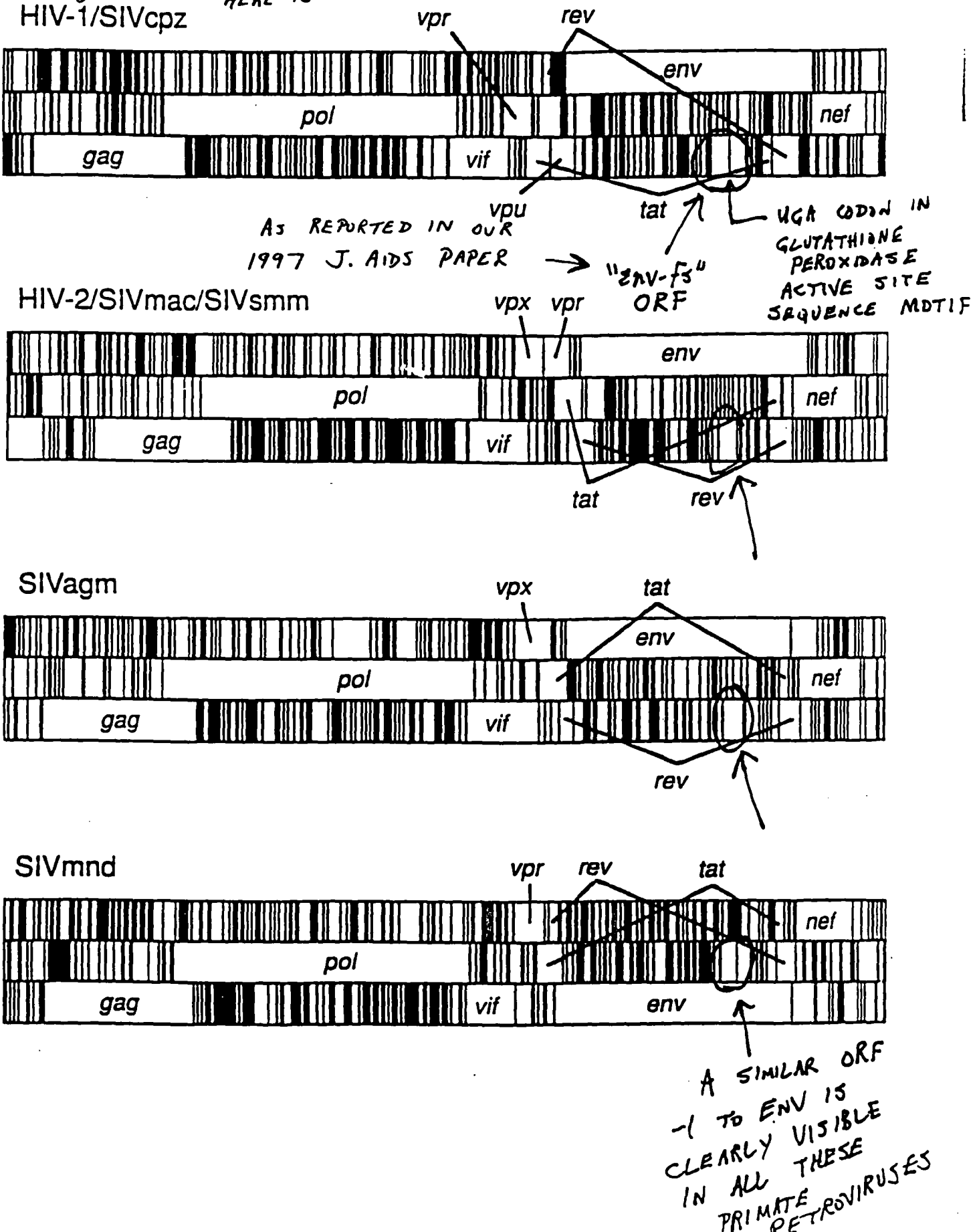
Fig. FROM GIBBS & DESROSIERS, IN HUMAN RETROVIRUSES, ED. CULLEN, 1993

Fig. SHOWS KNOWN HIV GENES.
HERE IS ONE THEY MISSED!



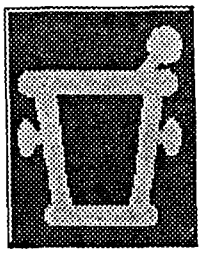
HIV-1 encodes a sequence with functional glutathione peroxidase activity: implications for the link between selenium deficiency and AIDS.

Presented at the **4th Dresden Selenium Symposium**, Dresden, Germany
May 15/16, 1999. Submitted for publication as a full paper, Aug. 1999.
L. Zhao, J. Ruzicka, A.G. Cox, E.W. Taylor

Abstract

Since the mid-1980s, evidence linking the selenium status of HIV-infected individuals with various indicators of disease progression and mortality has continued to accumulate, at ever higher levels of statistical significance. Low plasma selenium has been linked not only to increased risk of disease progression and mortality, but also with specific symptoms such as myopathy of both heart and skeletal muscle. Despite this body of evidence, the significance of these observations and the nature of the mechanisms involved remain highly controversial. In 1994, based on theoretical evidence, Taylor et al. proposed that HIV-1 might encode several selenoprotein modules. Subsequently, in 1997, one of the proposed HIV selenoprotein genes was demonstrated to have highly significant sequence similarity to the mammalian selenoprotein glutathione peroxidase (GPx). We report here for the first time the cloning of that hypothetical HIV-1 gene, and the finding that it encodes functional GPx activity when expressed as a selenoprotein in mammalian cells. In stably transfected canine kidney cells, GPx activity can be increased from 21 to 43% relative to controls (avg. 30%, $n=9$, $p<0.0001$), whereas in MCF7 cells, which have low endogenous GPx activity, a close to 100% increase can be demonstrated (avg. 99%, $n=3$, $p<0.05$). These observations are inconsistent with the conclusions of a recent study of selenoprotein expression in HIV-1 infected and uninfected T cells (Gladyshev et al., 1999), whose authors concluded that there was no evidence of HIV encoded selenoproteins. They did note that in HIV-infected cells there was a decline in levels of cellular selenoproteins, and an increase in "low molecular mass" selenium compounds. The latter observation is of interest, because several potential HIV selenoproteins are predicted to be of low molecular mass (from 7-9 kD), consistent with what they observe in HIV infected T cells. One such product is the 9 kD isoform of the HIV GPx homologue that is active in our functional assays reported above. Furthermore, the primary observation of Gladyshev et al., that levels of cellular selenoproteins decline in HIV-infected cells, is also highly consistent with the predictions of the HIV selenoprotein theory, being expected as a consequence of HIV selenoprotein expression. This novel pathogenic mechanism could help to explain why the effects of HIV infection are exacerbated in individuals who are selenium deficient. The role of selenium in AIDS, and the rationale for why HIV might benefit from encoding a selenoprotein, can now be better understood in the light of recent demonstrations of the involvement of selenoproteins like GPx and thioredoxin reductase in redox regulation of the transcription factor NF-kappaB, which is the primary cellular factor involved in the activation of HIV transcription.

British researcher urges increased selenium intake



It is time that we begin to take the declining intake of the trace mineral selenium seriously, says the British researcher Dr. Margaret P. Rayman, in an editorial in the February 1997 issue of the British Medical Journal. Among the problems that she sees associated with the low selenium levels in blood are infertility, cancer and heart disease.

"Declining sperm quality" and "increased incidence of cancer" are headlines that are becoming more and more common, and they are among the problems that the British university researcher Dr. Margaret P. Rayman says can be explained by reference to the drastic fall in the public's intake of the trace mineral selenium. In England alone, where Dr. Rayman lives and works, the average selenium intake has declined by half in the course of the past 22 years. Today it lies under the recommendation established by British public health authorities.

Somewhat the same situation prevails in the Scandinavian countries and in the rest of Europe. Dr.Rayman sees the falling selenium intakes as a threat to public health, because selenium is one of the most important elements in the body's defences against cancer and heart disease. The rare trace mineral is also decisive for human reproduction. This relationship was made clear when the Scottish researcher Dr. Alan MacPherson showed some years ago in a double-blind, placebo-controlled trial that selenium supplementation could increase infertile men's sperm quality by 100 percent. [*MacPherson A et al. 1993*] It has been demonstrated that "relatively harmless viruses can become virulent by passing through a selenium deficient host" which raises new questions about the prevention of viral diseases in a period in which HIV and new influenza types are flourishing globally.

"It is time to act"

As Dr. Rayman sees it, we must act now. Selenium deserves more attention than it has formerly received. Dr. Rayman wonders why it is that British farmers have been giving their animals selenium supplements for years in order to prevent the occurrence of disease but no one has introduced a similar policy for humans.

"Perhaps it is time to consider measures such as those adopted in Finland" in 1984, she says in her editorial. (In Finland, a program for selenium enrichment of the soil through selenium-enhanced fertilizers has been in operation for some years.) Alternatively, she suggests that we could fortify various foodstuffs with selenium, eat Brazil nuts (the best food source of selenium) or take selenium as a supplement.

Pharma Nord 24 February 1997

See also: BMJ Press Releases for the 8 February 1997 issue

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in Selenium in Biology - Medicine, Part A AVI Van Nostrand, NY 1987 Combs et al, Eds.

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Why the Level of Selenium in the Food Chain Appears to Be Decreasing

Douglas V. Frost

Evidence that plant uptake of selenium is being reduced has accumulated over the past quarter century. The fact that sulfur dioxide is passed through a solution of selenite to reduce the Se^{4+} to Se^{0}, which would then settle out, became known to me through members of the Selenium-Tellurium Development Association in the early 1960s. Whereas sulfite is reducing, selenite is oxidizing. This fact, together with a series of observations by others, led me to advance the hypothesis that Se deficiencies represent growing worldwide problems. It is offered here as a learning experience for confirmation or denial.

DEVELOPMENT OF THE RATIONALE

Having challenged the reality of "selenium cancer" in 1960 (*1*) and sought its reevaluation beginning in 1961 (*2*), I have pursued the truth about Se for years. Prior to 1960 I followed the work of Klaus Schwarz closely, along with that of others such as J. E. Oldfield and Milton Scott, working in the field of the role of Se in animal nutrition. In 1962, we twice visited New Zealand in connection with the Twelfth World's Poultry Congress in Sydney. Through correspondence with J. M. Barnes of the British Medical Research Council, I learned that the legally enforced removal of arsenic (As) from superphosphate fertilizers for New Zealand had also removed the Se. This led directly to the

sudden epidemic of Se deficiencies in sheep and cattle in New Zealand. As Barnes, now deceased, wrote me, this reflected the nonsense about possible hazard from As that need not have occurred. I wrote then of the involvement of both As and Se in the British beer poisoning of 1900 (*3*) and noted the apparent increased loss of available Se. Under a heading, Se and the Soil, I wrote:

> How soils are gradually depleted of available Se thus remains to be answered in all its ramifications. . . . Whether to correct a deficiency in the soil, in the feed, or directly in the animal poses problems in basic agriculture, in biochemistry, and now in jurisprudence. . . . If the transition of Se excreted by animals back to inorganic Se available to plants is not rapid enough, depletion of available Se from the soil is certain to occur. Strange as it may seem, man may need to conserve Se as a valued soil constituent.

When the above was written, I knew of Shrift's Se cycle (*4*) and of Butler and Peterson's finding that the Se in sheep feces was unavailable to grass (*5*). These were cited, along with Cousins and Cairney's finding that ruminants excrete elemental Se^{0} (*6*), in my initial picturization of the Se cycle as one in which its bioavailability is partially lost (*7*). This was revised later (*8–11*) to include what I believe occurs to make the Se emitted during fossil fuel burning unavailable to plants. The evidence that some of the Se in fly ash is available for plant uptake (*12–14*) represents a possible flaw in the overall hypothesis. The alkalinity of soils in which such tests were made and other factors should be taken into consideration in assessing this question. Certainly, evidence seems clear that the acidification of soils via acid rain, plus deposition of massive amounts of S and N serve to decrease the uptake of Se by plants. In a compilation of many papers dealing with plant uptake of Se from soils and the influence of fertilizers and individual elements, Gissel-Nielsen (*15*) summarized work up to 1977. Gissel-Nielsen has published much since then and wrote me that he fully agrees that Se deficits are increasing.

Early work by P. J. Peterson and his associates (*16,17*), plus recent communication with him, aided in the development of my hypothesis. Thus, in my initial representation of the loss of Se availability (*7*), I cited work of Butler and Peterson (*16*) suggesting that hydrogen selenide, a likely product of ruminant digestion, might be autooxidized to Se^{0}. This would account for the fecal excretion of Se^{0} by ruminants (*6*) and the apparent reduction of Se bioavailability from intensively grazed grasslands. It was also noted (*7*) that "At acid pH, insoluble metal selenides may also contribute to unavailability." Over the years, with the growing knowledge of acid rain and the emission of metals

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Ethan Will Taylor	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Manitoba	B.Mus	1974	Music, Mathematics
University of Winnipeg	B.Sc.	1981	Chemistry
University of Arizona	Ph.D.	1985	Pharmacology
University of Arizona	Postdoc.	1986-1987	Medicinal Chemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

1984-1985: Pharmaceutical Manufacturers Association Foundation "Advanced Predoctoral Fellow" in the lab of Dr. D.L. Nelson, Dept. of Pharmacology & Toxicology, Univ. of Arizona.

1986-1987: NIH Postdoctoral Research Associate with Dr. Arnold Martin, Head, Dept. of Pharmaceutical Sciences, University of Arizona.

1988-1992: Assistant Professor of Medicinal Chemistry, University of Georgia, College of Pharmacy.

1992-current Member of Computational Center for Molecular Structure and Design, Univ. of Georgia.

1993-current Associate Professor, University of Georgia (since 1998, in Department of Pharmaceutical and Biomedical Sciences).

Awards, Honors and Scholarships: Student of Highest Distinction, Univ. of Winnipeg, 1980 and 1981; Graduate Academic Scholarship, Univ. of Arizona, 1981-82 and 1982-83; PMAF Predoctoral Fellow, Univ. of Arizona, 1984-85. N.E. GA Section of American Chemical Society "Research Chemist of the Year", 1996.

Reviewer for: Life Sciences, Journal of Pharmacology and Experimental Therapeutics, Antiviral Chemistry and Chemotherapy, Journal of Pharmaceutical Sciences, Journal of Computational Chemistry, Genetica, Biological Trace Element Research, Journal of Medicinal Chemistry, Journal of the American Chemical Society.

NIH Study Sections: NIDA-1 (ad hoc); Infect. Disease and Microbiol., AIDS and Related Study Section.

Patents: "Selenoproteins, coding sequences and methods", pending U.S. patent appl. #08/679,493 filed 7/96.

Research Projects Ongoing or Completed During the Last 3 Years:

1. "Novel frameshift sites and additional coding potential in HIV-1." (Involved preliminary molecular biology studies related to potential new genes in HIV-1, e.g. *in vitro* demonstration of novel HIV-1 frameshift sites).
   PI: Ethan Will Taylor, Ph.D.
   Agency: University of Georgia Biotechnology Grant Program
   Type: University seed grant program. Period: 7/1/96-6/31/98.
2. "Selenium and outcome in HIV+ IV drug users: role of viral selenoproteins". (Investigates the potential role of an HIV-1 encoded glutathione peroxidase homolog and a novel HIV protease transframe protein in the clinical outcome related to Se status in the U. Miami cohort. Does not include *nef*-related studies).
   PI: Ethan Will Taylor, Ph.D.
   Agency: National Institutes of Health, NIDA
   Type: Adminstrative supplement to RO1 (subcontract to Univ. of Miami) Period: 6/1/98-5/31/02.

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Zhang W., Cox A.G., and Taylor E.W. *Hepatitis C virus encodes a selenium-dependent glutathione peroxidase gene: implications for oxidative stress as a risk factor in progression to hepatocellular carcinoma.* Med. Klinik., in press.

Zhao, L., Cox, A.G., Ruzicka, J., Bhat, A.A., Zhang, W. and Taylor E.W. *Molecular modeling studies and in vitro activity of the expressed protein are consistent with the hypothesis that HIV-1 encodes a functional glutathione peroxidase gene.* Submitted for publication Aug. 1999.

Relevant abstract not yet published as a full article:

Taylor, E.W., Nadimpalli, R.G., Ramanathan, C.S., Dean, R.G., Hamilton, J.A., Thakur, A. and Blumberg, B.M. (1997) *Novel isoforms of HIV-1 nef are expressed by frameshifting and selenium-dependent suppression of UGA termination codons.* Antiviral Res., 34: A23.

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Why nutrition is critical for surviving HIV

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In the last issue of *Choices*, (July-August 1999, Vol. 3, No. 4) Dr. Alawode Oladele discussed the great importance of nutrition in the treatment of HIV infection.In this brief review, I will elaborate on some of the known and potential mechanisms involved.

Numerous studies have demonstrated that the intake or blood levels of various micronutrients are significantly correlated with disease progression or outcome (i.e., survival vs. mortality) in HIV-1 infection. These nutrients include vitamins B6 and B12, beta carotene, zinc and selenium (Se); similar correlations exist for nutrition-dependent biomolecules like the antioxidant glutathione, which requires the amino acid cysteine. Deficiencies of these nutrients can have serious to profound effects on resistance to secondary infections, quality of life, and survival time.

Certainly, these correlations seem easily understandable in the light of our ever-expanding knowledge of the role of nutrition (particularly antioxidants and trace minerals) in supporting our natural immunity. For example, in protein malnutrition, even the formation of antibodies (which are proteins) is compromised. And correlations between some of the above-mentioned nutrients and general immunity have been widely documented in the general population in the past.

But is there something more profound going on in HIV infection, that goes beyond this general effect of nutrients on immunity? My answer is a resounding *yes*, and it relates to the fact that, of all the micronutrients, the most widely documented and statistically significant correlation with HIV disease progression and outcome has been demonstrated for Se status. This is of particular interest, because Se, although classified as a mineral, is a potent dietary antioxidant, and its cellular actions are intimately linked to the oxidative status of the cell, and thereby to the regulation of genes that are important for various immune cell functions. Because an extensive body of evidence shows that "oxidative stress" (increased free radical activity) and antioxidant defects like glutathione deficiency are hallmarks of AIDS, and that oxidative stimuli can activate HIV-1 replication in the test tube, it is not really surprising that Se should prove to be of critical importance in HIV-1 infection. But the core of the matter goes even deeper than that.

In 1994, based on a detailed analysis of the viral genome using "computational biology" techniques, my research group predicted the existence of several novel genes in HIV-1 (Taylor et al., *J. Med. Chem.* 37: 2637-54). The possibility that several of these genes might encode Se-containing proteins ("selenoproteins") also led us to predict that dietary Se levels may play a *unique* role in HIV pathogenesis and disease progression in AIDS, a prediction that has been strongly supported by several recent papers, e.g. Constans et al., *J. AIDS*, 10:392, 1995, entitled **"Serum selenium predicts outcome in HIV infection"** and Baum et al., *J. AIDS*, 15: 370-376,

1997, "**High risk of HIV-related mortality is associated with selenium deficiency**". In fact, over 20 papers have been published over the last decade documenting Se depletion in HIV/AIDS (reviewed in *Biol. Trace Elem. Res.* **56:** 63-91, 1997). Furthermore, Se (as sodium selenite) has been shown to inhibit HIV replication by at least two independent labs. We have now shown that one of the potential selenoprotein genes predicted in HIV in 1994 is a virally-encoded homolog of glutathione peroxidase (GPx), the prototypical mammalian selenoprotein (*J. AIDS*, 15: 393-4, 1997). This gene has now been cloned and shown to have functional GPx enzyme activity when expressed as a selenoprotein in human cells, suggesting a novel molecular mechanism whereby HIV infection can contribute to the depletion of Se from infected cells, and thus exacerbate the effects of Se deficiency. Even more relevant to the pathogenesis of AIDS is the existence of HIV-1 *nef* gene associated selenoprotein coding potential, the essential features of which are highly conserved, in over 99% of worldwide HIV-1 isolates in the gene databases. The *nef* gene is considered to be the major source of HIV pathogenic effects; in fact, deletion of *nef* is the primary feature of experimental "live attenuated" HIV vaccines. A transgenic mouse incorporating *the HIV-1 nef gene alone* developed AIDS-like symptoms even though the rest of the virus was absent. Thus, I strongly suspect that *nef*-mediated Se-depletion in infected cells may be one of the primary effects of HIV that leads to immunodeficiency, especially because Se is known to be critical for cellular immunity. (McKenzie et al., **Selenium: an essential element for immune function**, *Immunology Today*, 19: 342-5, 1998).

The discovery of selenoprotein genes in HIV also provides a basis for understanding the role of many oxidative stress-inducing cofactors that are known to accelerate the progression of AIDS. HIV infection can be aggravated by cofactors such as malnutrition, coinfection with other microorganisms, and the use of various oxidant drugs, such as nitrites or "poppers", which has been strongly correlated with the incidence of Kaposi's sarcoma in HIV-infected individuals. Because the immune system generates free radicals to combat certain bacterial infections, and microorganisms like mycoplasma themselves generate free radicals, it is plausible that the common element in these cofactors is oxidative stress caused by antioxidant deficiency (in malnutrition) or oxidative stimuli (drug abuse and coinfections). Similarly, in hepatitis C virus (HCV) infections, progression to serious liver disease is associated with oxidative stressors (e.g. alcoholism and iron overload), which is significant because we have identified the same selenoprotein gene (GPx) in HCV. And in both HIV and HCV infection, there has always been a significant fraction of infected people who either never develop symptoms, or do so extremely slowly; it seems likely that nutritional status is one of various factors that may be involved in these cases.

The potential significance of the role of Se in AIDS can best be understood by analogy to another disease called Keshan disease, which is a classical Se deficiency disease, named after a region in China where outbreaks occurred because of the very low Se levels in soils of the region. Women and young children seem particularly susceptible to the disease, of which the primary symptom is a non-obstructive cardiomyopathy, leading to death by heart failure in severe cases. 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 from the hearts of disease victims a virus called coxsackievirus, a relative of the

common cold virus. The role of coxsackievirus in Keshan disease is strongly supported by demonstrations that a deficiency of Se can trigger a similar cardiomyopathy in coxsackievirus-infected mice, called "viral myocarditis". What is particularly intriguing (and frightening) is the finding by Dr. Melinda Beck and coworkers (Beck et al., *Nature Med.* **1:** 433-436, 1995) that even a "benign" strain of coxsackievirus becomes virulent in Se-deficient mice, where it mutates into a more virulent strain that can produce myocarditis even in normal mice that are *not* Se-deficient.

Because Keshan disease was thought to be simply a Se deficiency disease, but proved to have a viral cofactor, *it would not be unprecedented if AIDS and other chronic viral diseases like hepatitis B and C ultimately prove to be viral diseases with selenium deficiency as a major cofactor*. There is also reason to believe that, like many other trace minerals, Se is being depleted from the food chain due to bad agricultural practices, and environmental changes like acid rain, which, in light of the above, could be a factor in "emerging" viral diseases.

A caution must be sounded that toxicity is a concern with Se supplementation; however, 200 micrograms (mcg) per day for adults is generally considered totally safe; that dose was used in a long term cancer prevention trial in the U.S. (Clark et al., *JAMA*, **276:** 1957-1963, 1996). No adverse effects are expected for long-term uptakes of up to 400 mcg daily, or even up to twice that dose, according to some experts. However, because Se levels are tightly controlled by excretion, there is very unlikely to be any benefit in taking over 200 mcg unless GI malabsorption is present; as this is not uncommon even in asymptomatic HIV positives, the best option in that case is to have your blood levels tested. Above all, remember that Se is likely to be most beneficial along with other antioxidants and vitamins, as part of a comprehensive nutritional program, which can be of benefit even to those on antiviral drug regimens. If antivirals are not an option (as for many people in developing nations), the nutritional approach to HIV treatment may be one of the most promising and low cost interventions available.

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Nutrition, HIV and drug abuse: the molecular basis of a unique role for selenium.

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Summary: HIV-infected injection drug users (IDUs) often suffer from serious nutritional deficiencies. This is a concern because plasma levels of micronutrients such as vitamin B_{12}, zinc and selenium have been correlated with mortality risk in HIV+ populations. Injection drug use also increases lipid peroxidation and other indicators of oxidative stress, which, combined with antioxidant deficiencies, can stimulate HIV-1 replication via activation of NF-κB transcription factors, while weakening immune defenses. As detailed herein, these pro-oxidant stimuli can also increase the pathogenic effects of HIV-1 by another mechanism, involving viral selenoproteins. Overlapping the envelope coding region, HIV-1 encodes a truncated glutathione peroxidase (GPx) gene (Taylor et al., *J. AIDS Human Retrovirol.*, 15: 393-4, 1997). Sequence analysis and molecular modeling show that this viral GPx (vGPx) module has highly significant structural similarity to the known mammalian GPx, with conservation of the catalytic triad of selenocysteine (Sec), glutamine and tryptophan. In addition to other functions, the HIV-1 vGPx may serve as a negative regulator of proviral transcription, by acting as an NF-κB inhibitor (a known property of cellular GPx). Another potential selenoprotein coding function of HIV-1 is associated with the 3' end of the *nef* gene, which terminates in a conserved UGA (potential Sec) codon in the context of a sequence (Cys-Sec) identical to the C-terminal redox center of thioredoxin reductase, another cellular regulator of NF-κB. Thus, in combination with known cellular mechanisms involving Se, viral selenoproteins may represent a unique mechanism by which HIV-1 monitors and exploits an essential micronutrient to optimize its replication relative to the host.
Key Words: Glutathione Peroxidase - HIV - NFkappaB - Selenium - Selenoprotein.

A number of recent studies have demonstrated that the intake or blood levels of various micronutrients are significantly correlated with survival in HIV-1 infection, both in HIV-infected injection drug users (IDUs), and in general HIV+ populations (1-3). Of all the micronutrients, the most widely documented and statistically significant correlation with HIV disease outcome has been demonstrated for selenium (Se) status, which has been correlated with various indicators of disease progression, and is an independent predictor of mortality in HIV infection (3-5), as reviewed previously (6). This is of particular interest, because Se, although classified as a mineral, is a potent dietary antioxidant, and its cellular actions are intimately linked to the redox status of the cell, and to the redox regulation of genes that are important for various immune cell functions (7). Thus, given an accumulating body of evidence that oxidative stress is a hallmark of AIDS (8,9), and an activator of HIV-1 replication *in vitro* (10,11), it is not surprising that Se should prove to be of critical importance in HIV-1 infection.

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Selenium and cellular immunity

In addition to nonspecific incorporation as selenomethionine, Se can be specifically incorporated into proteins as the rare amino acid selenocysteine (Sec), the Se analog of cysteine. In mammals, Se has many critical functions, including antioxidant defense, thyroid action, reproductive function, and immune function (12). These biological functions of Se are generally associated with specific selenoproteins, e.g., Se is critical for antioxidant defenses largely because it is an essential component of Se-dependent glutathione peroxidases (GPx).

It is now well established that adequate levels of Se are necessary for the immune system, and cellular immunity in particular, to function properly (7,13). Se supplementation increases the cytotoxicity of killer cells as well as their proliferation in response to mitogens and antigens (e.g. 14), whereas Se deficiency has the opposite effect, and is commonly associated with impaired immune function (7). Correlations between CD4+ T cell counts and plasma Se levels have been widely documented, most significantly in HIV-infected patients (6). Se also potentiates the action of the cytokine interleukin 2, by upregulating the IL-2 receptor (15).

Significantly, the mRNA for a selenoprotein gene called Sps2, involved in biosynthesis of selenophosphate, the activated Se species required for formation of selenocysteine, is up-regulated upon activation of T lymphocytes (16); this suggests that selenoprotein synthesis is required for some aspect of T cell function. Note that, because cellular immunity is our primary defense against both cancer and viruses, a role of Se in cellular immune function represents a common factor in the known anticancer (17) and antiviral benefits of dietary Se (reviewed in 18).

Drug abuse, malnutrition and immune function

Injection drug users (IDUs) are a population known to be exceptionally prone to malnutrition, for several reasons. An addict's prime concern is to obtain the next drug dose; obtaining food is secondary. Furthermore, the majority of IDUs are socially marginalized and impoverished, in part due to the cost of supporting their drug habit, so that the quality of their diet is often inadequate. These nutritional deficiencies associated with drug addiction have been linked to compromised immune function even in HIV- drug users (19), which is not surprising in light of the importance of nutrition for maintaining optimal immune function (20).

Of particular significance for the role of antioxidants in HIV+ IDUs is the fact that *injection drug use exerts an exceptional oxidant stress on the body and the immune system*. In part because of tissue damage and cellular injury, drug abusers show increased lipid peroxidation products in blood, liver and urine (21-23), and low serum thiol levels indicative of increased oxidative stress are predictive of outcome in HIV+ IDUs (24). This effect of drug abuse leads to an increased requirement for nutritional antioxidants, which is very unlikely to be met in typical malnourished drug users. A study of heroin users showed significantly low urinary Se levels (25), which suggests that dietary Se intake is low and/or that absorption is impaired in IDUs. This would exacerbate the oxidative stress that is a direct consequence of injection drug use.

The now well-established correlation between Se status and HIV-1 disease progression (3-6) should be particularly apparent in HIV+ IDUs, in the light of the facts reviewed above. To summarize: 1) Se is essential for cellular immunity, as well as for antioxidant defenses; 2) malnutrition is common in drug addicts; 3) injection drug use can be associated with increased oxidative stress and lipid peroxidation; and 4) HIV-1 transcription is activated by oxidative stress, via the transcription factor nuclear factor κB (NF-κB). In essence, the multiple risk factors of increased oxidative stress and inadequate (dietary) antioxidant intake can activate HIV-1 replication via known mechanisms, while weakening immune defenses. The logic of this argument is supported by the fact that the most significant correlation to date between Se status and HIV-1 disease outcome was observed in a cohort of HIV+ IDUs, in which low plasma Se was associated with a 20-fold increased risk of AIDS-related mortality (3).

The cellular selenoproteins GPx and thioredoxin reductase regulate transcription factor NF-κB

In the cell nucleus, by binding to a specific DNA consensus sequence, the active form of NF-κB acts as a transcriptional activator of a number of immune-related genes, including various cytokines, adhesion molecules, and genes that regulate cell proliferation and apoptosis (26-28). By incorporating DNA sequences to which NF-κB can bind into its promoter region, HIV has evolved so that it can be activated by the same cellular factor that stimulates these various immune-related functions.

The activity of NF-κB is regulated in a complex manner by cellular location and redox status (26). Initial activation in the cytosol occurs via separation of the inhibitory kappa B subunit I-κB, which exposes a nuclear translocation signal on NF-κB, so that active NF-κB dimers can enter the nucleus and bind to their cognate DNA sites. This cytosolic activation is stimulated by oxidative stress (e.g. peroxides), inflammatory cytokines (e.g. tumor necrosis factor-α) and various

```
Match: G SR  Y* *  D DG *   *  Y *    F    *YUG*TA  * ****GHQ* PGKN PG G *PK *  * GD* ** W*   **  * *
env-fs GSSRKHYGRTVNDADGTGQTIIVWYSAAAEQF..AEGYUGATAS.VATHSLGHQAAPGKN.PGCGKIPKGST.APGDL.GLLWKTHLHHCCALEC
P46412 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%FPSNQFGKQE.PGEN#PGGGFVPNFQLFEKGDV-DIRWNFE.KFLVGPDG
P23764 GMSGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTD.%FPCNQFGKQE.PGEN#PGGGFVPNFQLFEKGDV-DIRWNFE.KFLVGPDG
P22352 GISGTIYEYGALTIDGEEYIPFKQYAGKYVLFVNVASYUGLTG.%FPCNQFGKQE.PGEN#PGGGFVPNFQLFEKGDV-DIRWNFE.KFLVGPDG
P37141 GVGGTIYEYGALTIDGEEYIPFKQYAGKYILFVNVASYUGLTG.%FPCNQFGKQE.PGEN#PGGGFTPNFQLFEKGDV-DIRWNFE.KFLVGPDG
P11352 AAQSTVYAFSARPLTGGEPVSLGSLRGKVLLIENVASLUGTTIR%FPCNQFGHQE.NGKN#PGGGFEPNFTLFEKCEV-DIAWNFE.KFLVGPDG
P04041 VAQSTVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTR%FPCNQFGHQE.NGKN#PGGGFEPNFTLFEKCEV-DISWNFE.KFLVGPDG
P00435 AAPRTVYAFSARPLAGGEPFNLSSLRGKVLLIENVASLUGTTVR%FPCNQFGHQE.NAKN#PGGGFEPNFMLFEKCEV-DVSWNFE.KFLVGPDG
P07203 AAAQSVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTVR%FPCNQFGHQE.NAKN#PGGGFEPNFMLFEKCEV-DVAWNFE.KFLVGPDG
P11909 AAAQSVYSFSAHPLAGGEPVNLGSLRGKVLLIENVASLUGTTVR%FPCNQFGHQE.NAKN#PGGGFEPNFMLFQKCEV-DVSWSFE.KFLVGPDG
JN0608 RCARSMHEFSAKDIDG.HMVNLDKYRGYVCIVTNVASQUGKTEV%FPCNQFGRQE.PGSD#YNV....KFDMFSKICV-AIKWNFT.KFLIDKNG
                                          └──R1──┘   └──────R2──────┘                  └R3─┘
```

Fig. 1. Sequence alignment of HIV-1 *env-fs* sequence vs. Se-dependent glutathione peroxidases. Sequences are single letter amino acid code, with U as the symbol for selenocysteine, Sec, encoded by the UGA codon in RNA. GPx sequences are listed by PIR accession number. *Env-fs* amino acids identical to one or more of the aligned GPx sequences are shown as letters in the "match" line above the alignment; similar residues are indicated by an asterisk. Three active site regions that are adjacent in 3-D space are shown below the alignment, labeled R1 - R3. All three regions and their essential catalytic amino acids, Sec, Gln and Trp (U, Q and W respectively, shown highlighted in bold), are represented in the truncated *env-fs* sequence. The alignment is similar to that shown as Fig. 1 of Taylor et al. (6) up to the end of R2, but is different beyond R2. There are 3 large deletions in *env-fs* relative to the GPx sequences, at the locations indicated by the symbols %, #, and ~ in the alignment, involving 19, 11 and 41 residues respectively. As computed using the alignment shown, i.e., omitting the 3 internal GPx regions where there are major deletions in the truncated HIV-1 vGPx homologue, the total similarity score of the *env-fs* sequence to the aligned GPx sequences is 6.3 standard deviations above the average similarity score computed for 100 optimally aligned randomly shuffled sequences of identical composition (see experimental section).

noxious stimuli including UV light and radiation, in all of which the formation of reactive oxygen species is a common factor. Thus, oxidative stress is a well-documented activator of NF-κB (26-28), and of HIV transcription via NF-κB activation (8-11).

It is therefore not surprising that in cell culture studies Se, as a component of the selenoprotein GPx, has been consistently found to be an inhibitor of NF-κB activation, presumably due to the reduction of peroxide levels by GPx (11,28,29). However, although those and many other cell culture studies have demonstrated that, in general, oxidants stimulate whereas antioxidants inhibit NF-κB activation and HIV-1 transcription, the activation of NF-κB does not involve exclusively oxidative stimuli. Despite the important role of oxidative stress in the *cytosolic* activation of NF-κB, in order to bind DNA in the nucleus, the thiol group of an active site cysteine residue of NF-κB must be in a *reduced* state (26). Thus, although oxidative stress appears to be a primary activator of NF-κB, this must be balanced by the action of selective cellular redox systems that, in the midst of this oxidative environment, can provide reducing power to maintain NF-κB in its active, reduced state. This reductive activation of NF-κB is one of the major functions of the selenoprotein thioredoxin reductase (TDR) and its substrate thioredoxin (26,30).

GPx and TDR are two of the most widely distributed selenoproteins, and it is remarkable that both have been demonstrated to be potent regulators of NF-κB activity, and all the more so that they appear to have opposed functions, i.e. GPx inhibits (11,29,30) whereas TDR activates (26,30) NF-κB activity. It is difficult to say which of these two may predominate, because the well documented inhibition of NF-κB by selenite in cell culture may be due in part to the direct formation of inactive Se adducts at relatively high concentrations (>5μM), and thus, rather than being mediated entirely via selenoproteins, a portion of this inhibition may represent a unique effect of selenite ion (27). Nonetheless, there is compelling evidence that, under normal physiological conditions, the selenoproteins GPx and TDR are major regulators of NF-κB activity. This is of considerable significance in the light of evidence presented below that HIV-1 encodes selenoprotein modules having distinct similarities to both GPx and TDR.

An HIV-1-encoded Se-dependent GPx gene

An *env* gene variant associated with one of the potential -1 frameshift sites predicted in HIV-1 by Taylor et al. in 1994 (31) was subsequently identified as a virally-encoded homologue of GPx, the prototypical mammalian selenoprotein (6). This viral GPx (vGPx) module is encoded overlapping the HIV-1 *env* gp41 coding region in the -1 reading frame; thus the gene was tentatively named *env-fs*, for *env* frameshift. It contains a single UGA codon (potentially encoding Sec) near the middle of a small but highly conserved novel open reading frame, with a conserved -1 frameshift signal near its 5' end. The translated HIV-1 *env-fs* sequence contains a common variant of the GPx active site consensus sequence spanning the catalytic Sec (U in Fig. 1).

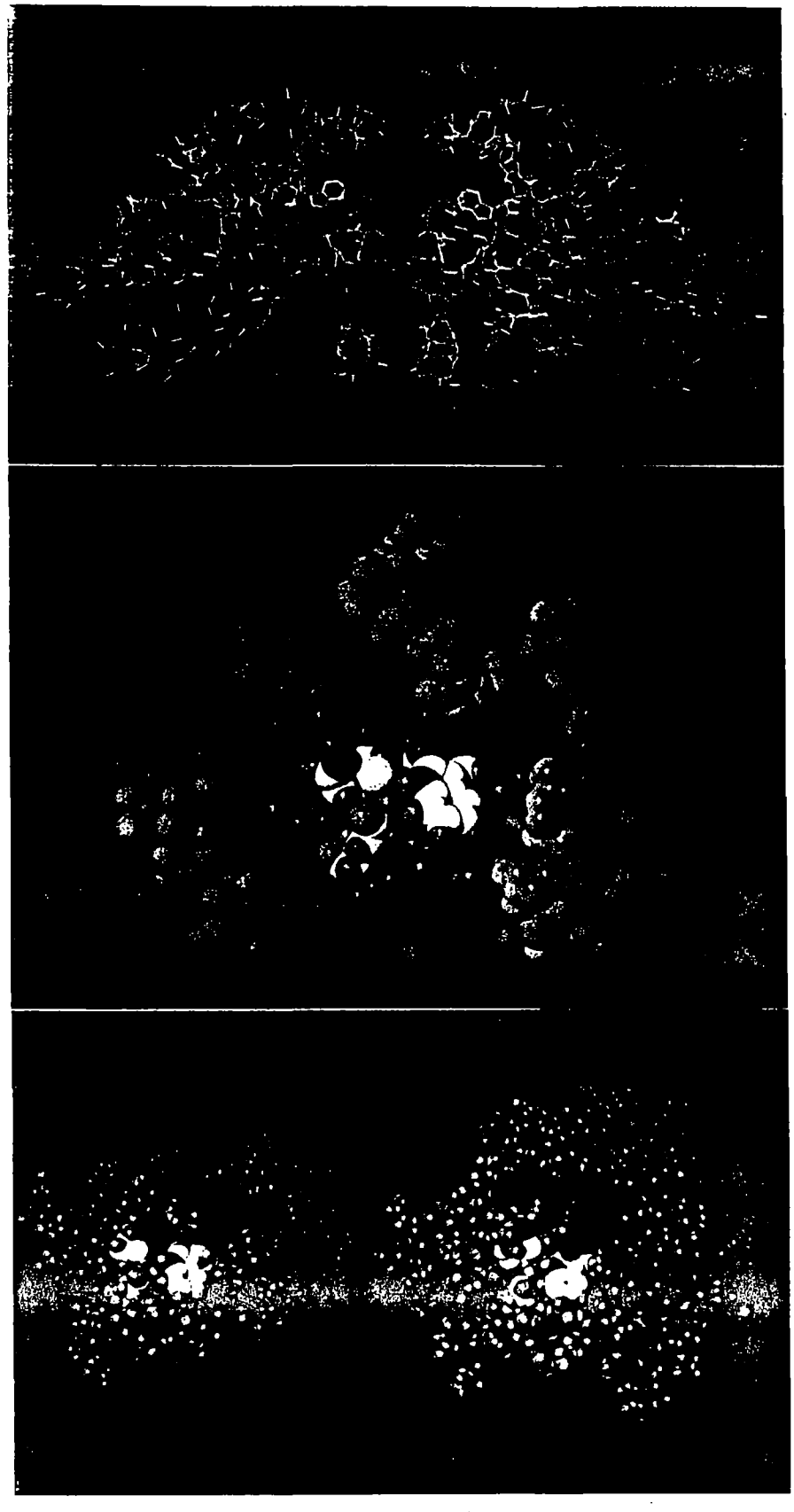

Fig. 2. Molecular modeling studies of the putative HIV-1 glutathione peroxidase (GPx) module. **A.** View of the protein backbone of the X-ray crystal structure of the bovine GPx dimer (1GP1.pdb) with C-terminal and internal regions that are deleted from the HIV-1 GPx homologue shown in red, and the regions retained in HIV-1 (*env-fs* in the alignment of Fig. 1) shown in green, with three conserved active site residues (Sec, Gln and Trp) highlighted in magenta, with sidechains. The deleted regions (red) include a helix involved in dimer formation (bottom, center) and another region (top) that is involved in dimer and tetramer formation (tetramer not shown). The catalytic core of the enzyme is conserved in the truncated HIV-1 homologue. **B.** A spacefill view of the protein backbone of the GPx monomer, colored as in **A**, except that the catalytic triad of Sec, Gln and Trp are shown in CPK colors. The deleted regions (red) all lie outside of the catalytic core domain. **C. Left:** An all-atom homology model of the putative HIV-1 GPx with the deleted (red) regions shown in **B** removed and the resulting gaps reconnected by loop remodeling, giving a continuous peptide backbone matching the HIV-1 sequence (*env-fs* in Fig. 1). The active site triad is shown in CPK colors, with the Se atom in yellow. A cluster of basic residues (one Lys and several His) is shown in blue. **Right:** A similarly oriented and rendered all-atom view of the bovine GPx structure. The basic residues (blue) are all Arg, at least one of which is involved in binding glutathione. **Conclusion:** the HIV-1 GPx homologue, although highly truncated, includes the complete catalytic core of the enzyme, but is likely to function as a monomer due to the deletion of regions involved in tetramer formation in the cellular GPx enzyme. The alignment of Fig. 1 is structurally feasible, because the locations of the internal deletions permit the rest of the core structure to be maintained.

Using a sensitive multiple sequence comparison method, a strong similarity between the entire HIV-1-encoded *env-fs* sequence and the aligned set of GPx sequences was demonstrated. As previously aligned by Taylor et al. (6), the similarity score of this novel HIV sequence vs. an aligned group of GPx sequences is 5 standard deviations (SD) above the average similarity score of randomized sequences of identical composition. That significance score increases to 6.3 SD based on the revised alignment shown in Fig. 1.

We examined alternative alignments of the C-terminal region of *env-fs* to GPx in the light of the bovine GPx crystal structure and its active site (see Fig. 2), and identified the viral sequence matching a third GPx active site region with a conserved Trp (W), shown as region 3 (R3) in the alignment of Fig. 1. This reveals a total of 3 significant internal deletions in the HIV-1 sequence relative to the mammalian GPx sequences. These deletions in *env-fs*, involving 19, 11 and 41 residues respectively, lie between the three conserved active site regions (YUG, GHQ, and W) and project away from the active site in 3-D space (Fig. 2A and 2B).

To assess this proposed homology in structural terms, a 3-D molecular model of the HIV-1 vGPX (Fig. 2) was made by homology modeling, based on the alignment of Fig. 1. This showed that all three deletions are structurally feasible, although the first and third deletions would involve loss of domains that participate in formation of the tetrameric GPx enzyme. This suggests that the HIV-1 GPx homologue probably does not function as a multimer, which would make it similar to the phospholipid hydroperoxide GPx, which acts as a monomer (12). The proposed homology is also strongly supported by the fact that the Trp (W) active site residue in R3 (Fig. 1) is very highly conserved in HIV-1 sequences. This conservation also provides compelling theoretical evidence that this is a functional gene, because the UGG Trp codon in the -1 frame is in the context of the sequence UCUGGA, which encodes Ser-Gly in the HIV envelope protein. Due to the degeneracy of the genetic code, Ser could be encoded using any base in the third codon position (i.e. UCN), so the conservation of a U in this position in HIV-1 sequences can only be explained by a functional requirement, such as the need for the UGG in the -1 frame (there is no known RNA structural element spanning this UGG codon). This putative HIV-1 vGPx gene has now been cloned and, when expressed as a selenoprotein in transfected mammalian cells, was found to yield as much as a 100% increase in GPx activity relative to controls (32). Furthermore, the Cys homolog (Sec to Cys mutant) of the vGPx has been expressed in bacteria, and purified to homogeneity; this purified 9kDa protein has significant GPx activity (Zhao, Ruzicka and Taylor, unpublished results).

The 3' terminal of HIV-1 *nef* is a UGA codon in a motif identical to the TDR C-terminal redox center.

As detailed previously (18), the 3'-terminal UGA codon of *nef* is highly conserved in the known group M HIV-1 isolates, which is a unique feature of HIV-1 as compared to HIV-2 and SIV *nef* genes, in which UAA and UAG stop codons are also found at the 3' end of *nef* in various strains and subtypes, with no particular bias. Conservation of this 3'-terminal UGA codon is perhaps the most striking difference between the *nef* gene of the more pathogenic HIV-1 vs. that of the other primate retrovirus. A selenoprotein coding function is a simple and appealing hypothesis, particularly in light of the fact that HIV-1 encodes a vGPx gene upstream in the *env* region (6). Preliminary evidence suggests that readthrough suppression of the *nef* 3' UGA codon can occur *in vivo*, and that *in vitro* this effect is Se-dependent (33). Furthermore, additional results consistent with ^{75}Se incorporation in a *nef* isoform during *in vitro* translation of a patient-derived *nef* isolate have been obtained (Benjamin M. Blumberg, personal communication).

Because *nef* is highly expressed as an early gene, and no frameshift is required to encounter this in-frame 3'-terminal UGA codon during *nef* protein synthesis, we previously suggested that readthrough of this UGA codon, leading to sequestration of Sec in a *nef* selenoprotein isoform, might contribute to *nef*-associated pathogenicity of HIV-1 (31,18). However, until recently, there was little basis for developing a hypothesis as to the possible function of this *nef* isoform, other than a potential pathogenic role in intracellular Se depletion by the above mechanism.

The discovery that TDR is a selenoprotein presents an intriguing possibility. Another feature of the *nef* 3' terminal is a conserved Cys residue immediately preceding the UGA codon. Thus, this HIV-1 encoded sequence is identical to that of the redox center of TDR, where a geminal Cys-Sec pair at the protein C-terminal has been shown to be essential for the redox activity of TDR (30). In addition, a sequence downstream of the UGA codon in *nef*, RRGLGGT(X_7)CCI, is quite similar to the sequence of a *second* disulfide redox center located near the N-terminal (# 52-65) of TDR, RWGLGGTC(X_4)CI.

It must be remembered that *nef*, along with *tat* and *rev*, is expressed early in the cell infection cycle, at a point when HIV needs to activate its transcription. If this hypothetical selenoprotein isoform of *nef* can, like TDR itself, contribute to activation of NF-κB, while

reducing cellular GPx levels via Se sequestration (thereby producing oxidative activation of NF-κB), that would be perfectly consistent with the viral agenda at that point in the infection cycle, as exemplified by the transactivating effect of the HIV *tat* gene that is expressed simultaneously with *nef*. Thus, in the case of *nef*, we predict that HIV-1 is using a viral selenoprotein for NF-κB activation, whereas the HIV-1 vGPx – a late gene – would be expected to deactivate NF-κB by decreasing oxidant tone. Notably, another role of TDR is in the synthesis of deoxyribonucleotides, via ribonucleotide reductase (30). DNA synthesis is certainly another function that could help explain why HIV-1 might encode a module with TDR-like activity. This would be consistent with the enhancement of infectivity and stimulation of proviral DNA synthesis activities that have been demonstrated for the HIV-1 *nef* gene (34).

An HIV-1-encoded NF-κB homologue?

Taylor et al. (31) noted the existence of a potential HIV-1 selenoprotein module potentially expressed by a -1 frameshift from the protease coding region, which was thus later named *pro-fs* (18). This was predicted to be a very basic, positively charged protein (pI=11), suggesting a nucleic acid binding protein; database searches produced matches to various DNA binding proteins. In particular, Taylor et al. suggested a similarity to the papillomavirus E2 DNA binding protein, which has a conserved Cys residue that aligns with the highly conserved UGA codon of *pro-fs* (31).

This hypothesis can be refined in light of data contained in a paper that was in press (26) at the time our 1994 paper (31) was submitted. Significantly, like the E2 proteins, the NF-κB family of transcription factors also contain an essential Cys residue in their DNA-binding domain; this Cys is in fact the amino acid that TDR maintains in a reduced (thiol) state in order for NF-κB to bind to DNA (26,30). Comparison of the alignment of *pro-fs* vs. the E2 proteins in Fig. 9 of Taylor et al. (31) with the alignment vs. the DNA-binding "NRD" domain of the NF-κB family (Fig. 3), it is evident that the alignment of *pro-fs* to the NRD domain gives a tighter alignment, with no gaps in the primary DNA contact region. The statistical significance of this similarity relative to randomly shuffled sequences is 4.5 SD for the region shown in Figure 3.

The protein sequence encoded in this overlapping gene region of protease is very highly conserved in HIV-1 sequences (e.g. 100% for the UGA codon), which cannot be explained by any known coding or functional requirement of HIV. Furthermore, using a standard assay, we have established that the predicted -1 frameshift

```
Match:     IKI  Q KG  IR*R *C    *  *     T KN*R
pro-fs   VTIKIGGQLKGSSIRYRSRCYSIRRNEFARKMET.KNDR
                                /
DIF-D    PHLRIVEEPTSNIIRFRYKCEGRTAGSIPGMNSSSETGK
DORS-D   PYVKITEQPAGKALRFRYECEGRSAGSIPGVNSTPEN.K
KBF1-C   PYLQIIEQPKQRGFRFRYVCEGPSHGGLPGASSE.KNKK
KBF1-H   PYLQILEQPKQRGFRFRYVCEGPSHGGLPGASSE.KNKK
KBF1-M   PYLQILEQPKQRGFRFRYVCEGPSHGGLPGASSE.KNKK
KBF2-C   PYLVIIEQPKQRGFRFRYVCEGPSHGGLPGASSE.KGHK
KBF2-H   PYLVIVEQPKQRGFRFRYGCEGPSHGGLPGASSE.KGRK
KBF3-H   PYLVIVEQPKQRGFRFRYGCEGPSHGGLPGASSE.KGRK
RELB-H   PHLVITEQPKQRGMPFRYECEGRSAGSILGESST.EASK
RELB-M   PYLVITEQPKQRGMRFRYECEGRSAGSILGESST.EASK
RELB-X   PELNITEQPKQRGMRFRYQCEGRSTGSILGEKST.EHNK
REL-A    PYIEIFEQPRQRGTRFRYKCEGRSAGSIPGEHST.DNNK
REL-C    PYIEIFEQPRQRGMRFRYKCEGRSAGSIPGEHST.DNNK
REL-H    PYIEIIEQPRQRGMRFRYKCEGRSAGSIPGEHST.DNNR
REL-M    PYVEIIEQPRQRGMRFRYKCEGRSAGSIPGERST.DNNR
TF65-C   PFVEILEQPKQRGMRFRYKCEGRSAGSIPGEHST.DSAR
TF65-H   PYVEIIEQPKQRGMRFRYKCEGRSAGSIPGERST.DTTK
TF65-M   PYVEIIEQPKQRGMRFRYKCEGRSAGSIPGERST.DTTK
TF65-X   PPVEIIEQPKQRGMRFRYKCEGRSAGSIPGERST.DTSK
DNA recognition:     ************
```

Fig. 3. The HIV-1 *pro-fs* sequence (31) compared to a multiple alignment of the DNA binding domain of the NF-κB/Rel/Dorsal (NRD) family of transcription factors; the region involved in sequence-specific DNA recognition is indicated. The following amino acid similarities are highlighted in bold: Basic: R, K, H (Arg, Lys, His); Aromatic: Y, F (Tyr, Phe); Hydrophobic: L, I, V, F (Leu, Ile, Val, Phe); Acid/amide: D, E, N, Q (Asp, Glu, Asn, Gln). *Pro-fs* amino acids identical to one or more of the aligned NRD sequences are shown as letters in the "match" line above the alignment; similar residues are indicated by an asterisk. The underlined C in *pro-fs*, aligned with the conserved C of the NRD domain, is a potential Sec residue, encoded by a highly conserved UGA codon in HIV-1. Statistical significance of the sequence similarity is 4.5 SD relative to that expected for random sequences of identical composition. The region shown above corresponds to positions 8-46 of the alignment for Domain #1239 at the ProDom web site (www.toulouse.inra.fr/prodom/doc/prodom.html), from which the full names of the NRD sequences shown can be obtained.

site in the HIV-1 protease gene (31) is active *in vitro* (Fig. 4). This frameshift occurs just upstream of *the pro-fs* sequence (RYRSRC) that matches the "signature" of the NRD domain (RFRYXC). Given the combination ofan active frameshift into this novel genomic region of HIV-1, and a highly conserved sequence in the overlapping -1 frame, there is little reason to doubt that this sequence is expressed *in vivo*.

From the gene alignment, it is clear that the protease processed form of *pro-fs* could be at the most a minimal DNA-binding domain, and lacks the transcriptional activating (TA) domain of NF-κB. By analogy to the E2 proteins (31), a DNA-binding domain alone could function as a repressor by preventing the complete protein (DNA-binding plus TA domain) from binding, or by forming inactive heterodimers. Thus, *pro-fs* may function as a transcriptional silencer or repressor, which could serve to maintain latency in an infected cell, i.e., keep the provirus from being overexpressed, perhaps to

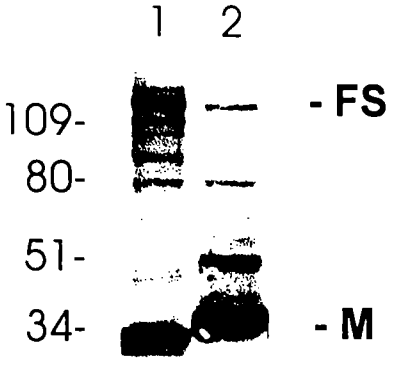

Fig. 4. *In vitro* translation (IVT) assay showing -1 frameshift activity induced by a fragment of the HIV-1 protease gene (31) when inserted into the RW-201 vector. In addition to a major low molecular weight "zero frame" translation product (about 40 kDa, shown as **M**), if the insert can induce -1 ribosomal frameshifting, higher molecular weight minor products (full length product at about 150 kDa, shown as **FS**) are observed. **Lane 1:** IVT of RW-201 with an insert from a high efficiency (20%) -1 frameshift sequence from the mouse mammary tumor virus, used as a positive control. **Lane 2:** IVT of RW-201 with a *pro-fs* insert, with the UGA stop codon in the -1 frame of the insert mutated to a sense codon to permit readthrough in the -1 frame. Note the **FS** band similar to lane 1 but less intense. No bands were visible in control lanes with no DNA added (not shown). See experimental section for details.

evade immune surveillance. In essence, processed *pro-fs* could function as an antagonist to NF-κB, which would be consistent with the clinical data on Se status and HIV-1 disease progression and outcome (3-6).

Conclusions

Not only does HIV-1 appear to encode homologues or catalytic motifs of selenoproteins known to *regulate* NF-kB, i.e. its own GPx, and a module in *nef* that is identical to the thioredoxin reductase "redox center", but it actually may encode its own NF-κB homologue (*pro-fs*), that has Se instead of S in its DNA-binding domain.

Although the precise mechanisms by which HIV-1 may recode specific UGA codons as sense codons for Sec have not yet been established, the sequence similarities to known selenoproteins, high degree of conservation of certain UGA codons, and demonstrated enzyme activity in the case of the HIV-1 GPx (32), strongly suggest that HIV-1 is able to do so. Potential RNA structures that may be involved in Sec insertion have been identified (including pseudoknots associated with UGA codons), and preliminary evidence of Se-dependent readthrough suppression of the 3' UGA codon of *nef* is promising (33). More research on this question is obviously needed.

By our interpretation, the results of ^{75}Se labeling of HIV-infected cells reported by Gladyshev et al. (35), contrary to those authors' conclusions, are actually highly consistent with the possibility of *in vitro* expression of one or more of the predicted HIV-1 selenoproteins. In HIV-infected cells, Gladyshev et al. noted a distinct decline in levels of cellular selenoproteins – which is exactly what the HIV selenoprotein theory predicts (18, 31) - and an increase in "low molecular mass" Se-labeled material. Although they did not explicitly identify the actual mass range in question, it is evident from their data (ref. 35, Fig. 1B) that much of this novel ^{75}Se-labeled material lies immediately *above* the 6 kDa band that they identify (p. 836) as the lowest ^{75}Se-labeled band in uninfected cells. Hence, their conclusion that this result disproves the possibility of HIV-encoded selenoproteins seems premature in light of the fact that the predicted masses of the protease processed *pro-fs* and *env-fs* (vGPx) are in the range of 7-9 kDa. It is precisely this 9 kDa form of *env-fs* that has been shown by Zhao et al. to possess GPx enzyme activity (32).

Because NF-κB is a central factor in HIV-1 gene regulation, and is also regulated by cellular selenoproteins and redox status, it is not really surprising that HIV-1 would have evolved to directly participate in those regulatory processes, by encoding its own selenoprotein modules. To fully understand the interactions between HIV-1 and risk factors like malnutrition, Se status, and oxidative stress associated with drug abuse, this novel Se-based mechanism of HIV-1 gene regulation and pathogenesis will have to be fully elucidated.

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Experimental Section

Sequence analysis: Statistical significance of similarity between a query sequence (i.e., *env-fs* or *pro-fs*) and a pre-aligned protein family (GPx or NRD domain, respectively) was assessed using the BlockAlign program (Zhang, Kececioglu and Taylor, unpublished). This program produces an optimal, gapped alignment of a probe sequence against an existing sequence alignment. By random shuffling of the query sequence, the average similarity score for optimally aligned random sequences of identical composition can be calculated. Significance is calculated as the distance of the

actual score from the average random score in standard deviations. The blosum62 amino acid similarity matrix was used, with gap creation and gap extension penalties of 6, 2 for Fig. 1 and 10, 2 for Fig. 3. Sec residues were treated as Cys for the purpose of these calculations.

Molecular modeling: The SYBYL program (Tripos Assoc., St. Louis, MO) was used to build the putative HIV-1 vGPx homology model, starting from the bovine cellular GPx X-ray crystal structure, file 1GP1 from the Brookhaven Protein Data Base. The model was constructed based on the alignment of Fig. 1, using the biopolymer protein loop option to reconnect regions where deletions were predicted from the alignment. The final model was minimized using the Kollman united atom and all-atom force fields, with parameters for Sec estimated by analogy to Cys and from *ab initio* molecular orbital calculations on methyl selenol using the Gaussian 94 program (Gaussian, Inc., Pittsburgh, PA).

***In vitro* -1 frameshift assay (Figure 4)**: A region of the HIV-1 *pol* gene, encompassing about 60 nucleotides spanning the potential frameshift site (31) was PCR amplified from a lab strain of HIV-1, pBH-10 (vector obtained from the NIH AIDS Research and Reference Reagent Program, Ogden BioServices Corp., Rockville, MD), with incorporation of HindIII and ApaI restriction sites in upstream and downstream oligonucleotide primers, respectively. The resulting protease sequence fragment was cloned into HindIII and ApaI-digested pRW201 vector and upon ligation with T4 DNA ligase, the recombinant plasmids (pro-fs-RW201) were transformed into *E. coli* K-12 SU1675. The positive clones were confirmed and junctions were verified by sequencing the inserts in both directions. Coupled transcription and translation reactions were performed using TNT Coupled reticulocyte lysate (Promega, USA) with 1 μg of circular pro-fs-RW201 plasmid. Briefly, multiple 25 μl *in vitro* reactions were set up with and without RW201 plasmid DNA added to 20 μl rabbit reticulocyte lysate mix, which includes T7 RNA polymerase and charged aminoacyl tRNAs. ^{35}S-Met label and water were added to a final vol. of 25 μL and incubated at 30°C for 90 min. After adding the SDS-PAGE sample loading buffer the samples were denatured at 100°C for 3 min and then separated on 10% SDS-PAGE gels for 60-90 min at 90V. The translation reaction products were electroblotted onto a neutrally charged nylon membrane for 35 min at 23V using the Semi-Phor system (Pharmacia, USA). Finally the membranes were exposed to X-OMAT Kodak X-ray film and developed. The film was then scanned using the IS-1000 Digital Imaging System (Alpha Innotech Corp., San Leandro, CA).

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AIDS Virus Predicted To Have Up To Four Unexpected New Genes

■ ***Theoretical study also suggests selenium may play key role in AIDS, but experimental verification is needed***

Stu Borman, C&EN Washington

Photo by Rick O'Quinn, University of Georgia Public Information

Taylor with RNA pseudoknot structures and University of Georgia (UGA) license plate. In retroviral genomes such as that of HIV, UGA usually functions as a transcription stop signal but sometimes encodes the amino acid selenocysteine.

In findings that suggest possible new approaches to the fight against AIDS, researchers have discovered theoretical evidence for several previously unnoticed genes in the AIDS virus and propose that selenium may play an important role in the disease. However, experimental verification is needed before any conclusions can be drawn about the study's implications for AIDS therapy.

In last week's *Journal of Medicinal Chemistry* [37, 2637 (1994)], E. Will Taylor, Chandra S. Ramanathan, Ravi K. Jalluri, and Ram G. Nadimpalli of the Computational Center for Molecular Structure & Design and the department of medicinal chemistry of the University of Georgia report detailed theoretical evidence that human immunodeficiency virus (HIV) contains heretofore unknown genes, some of which potentially encode selenium-containing proteins.

"I have to strongly emphasize that all we really have here is a theory," says Taylor. "I don't want to create unrealistic expectations. But it's a fairly powerful theory, and I'm really very confident we have discovered a group of new genes in HIV."

Analysis suggests that one of the proposed new genes encodes a DNA-binding protein. Based on sequence similarities to a known activator-repressor gene in papillomaviruses, the authors suggest that one form of the proposed DNA-binding protein could act as a repressor of HIV transcription.

"That means the gene has the potential to turn off the expression of HIV," says Taylor. "That could have significant implications for genetic approaches to the treatment of AIDS because it opens up the possibility of creating artificial repressors or boosting the synthesis of the natural repressor."

The amino acid selenocysteine is a component of the proposed repressor protein. Hence, the researchers speculate that adequate levels of dietary selenium could facilitate production of the repressor protein, inhibit HIV transcription, and thus delay the onset of AIDS symptoms in infected individuals. They believe this could also potentially explain the long-term survivor phenomenon—the tendency of a small percentage of HIV-positive individuals to remain asymptomatic.

The selenium findings are consistent with experimental and clinical data on the effects of selenium on AIDS patients. The researchers point out that selenium is toxic at levels significantly exceeding those commonly used for nutritional supplementation.

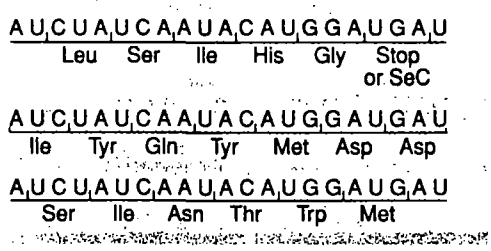
The prediction of the new genes arises from a discovery by Taylor and coworkers of potential new pseudoknots (interlocking RNA structures) in HIV's RNA-based genome. One of the few well-established functions of pseudoknots is that they can cause frameshifting, an extension of transcription from one gene to a second gene located in an alternate reading frame.

A reading frame is a genetic frame of reference used by a cell's protein-making machinery to determine which triplets of nucleotide bases are interpreted as codons for subsequent translation into amino acids. Each piece of RNA has three reading frames because there are three ways to arrange a linear string of RNA bases into a set of codon triplets.

For frameshifting to occur, two genes must be present on the same piece of RNA, but in different reading frames. Proteins produced by frameshifting are called fusion proteins because they are hybrids of two different genes.

When Taylor and coworkers first discovered new pseudoknots in the HIV genome, they examined the possibility that potential fusion proteins might be expressed by frameshifting.

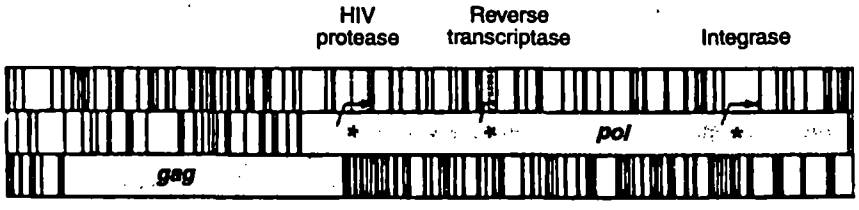
A genome has three reading frames



RNA bases: A = adenine, C = cytosine, G = guanine, U = uracil. Encoded amino acids: Asn = asparagine, Asp = aspartic acid, Gln = glutamine, Gly = glycine, His = histidine, Ile = isoleucine, Leu = leucine, Met = methionine, SeC = selenocysteine, Ser = serine, Stop = transcription termination signal, Thr = threonine, Trp = tryptophan, Tyr = tyrosine.

Sequence of nucleotide bases in *pol* gene of HIV can be arranged into three unique sets of codons, or reading frames. Each reading frame encodes a different set of amino acids. Middle reading frame is previously known encoding region for reverse transcriptase. Top reading frame is predicted by Taylor and coworkers to encode part of a fusion protein. The researchers believe the transcription machinery of the cell is able to read through the UGA stop signal in the presence of selenocysteine.

New protein-encoding regions predicted in HIV



Key: * Pseudoknot (interlocking RNA structure that can cause frameshifting)

Selected UGA (uracil-guanine-adenine) stop codons

Stop codons (shown as thick bars where densely packed)

Frameshift (extension of transcription activity from one reading frame to another)

Schematic of three reading frames of partial HIV genome shows three newly predicted pseudoknots in *pol* gene and associated frameshifts (transcriptional shifts to alternate reading frame). *Pol* encodes several proteins, including HIV protease, reverse transcriptase, and integrase. Taylor and coworkers initially discovered two new pseudoknots, in the protease and reverse transcriptase regions of the gene. They realized that UGA stop codons located near the pseudoknots in the top reading frame could potentially encode selenocysteine, making it possible for two new fusion proteins to be expressed by frameshifting. Then they noticed two other possible encoding regions in supposedly nonencoding areas of the genome—one in the top reading frame in the integrase region of *pol*, and a second (not shown) in the region of the *env* gene. They hypothesized that frameshifting might also be possible at those locations, assuming pseudoknots were present. "Sure enough," says Taylor, "I looked in both of those places and there were pseudoknots." This and other evidence led Taylor and coworkers to propose that HIV may have as many as four previously unknown genes.

This seemed unlikely because there were UGA (uracil-guanine-adenine) stop codons (transcription termination signals) in the alternate reading frame near the frameshift sites.

On the basis of a supposition that the stop signals could potentially be suppressed in some way, the researchers determined some possible peptide sequences encoded in the frameshift regions and found some intriguing matches with sequences of known peptides and proteins. This prompted a detailed literature search for possible mechanisms by which a cell's transcription machinery might be able to read through some of the stop codons.

The literature search revealed that UGA codons have recently been shown to be unique in the genetic code owing to their ability to encode selenocysteine, in addition to their more common function as stop codons. This led to the major finding of the study: that three selenocysteine-containing proteins and one other protein are possibly encoded by regions of the HIV genome formerly considered to be inactive.

Several of the UGA stop codons in HIV are highly conserved in a series of primate immunodeficiency viruses—that is, the stop codons are almost always found in corresponding genetic sites in such viruses. "The only way we could have this kind of conservation of UGA codons is if a gene was being expressed," says Taylor.

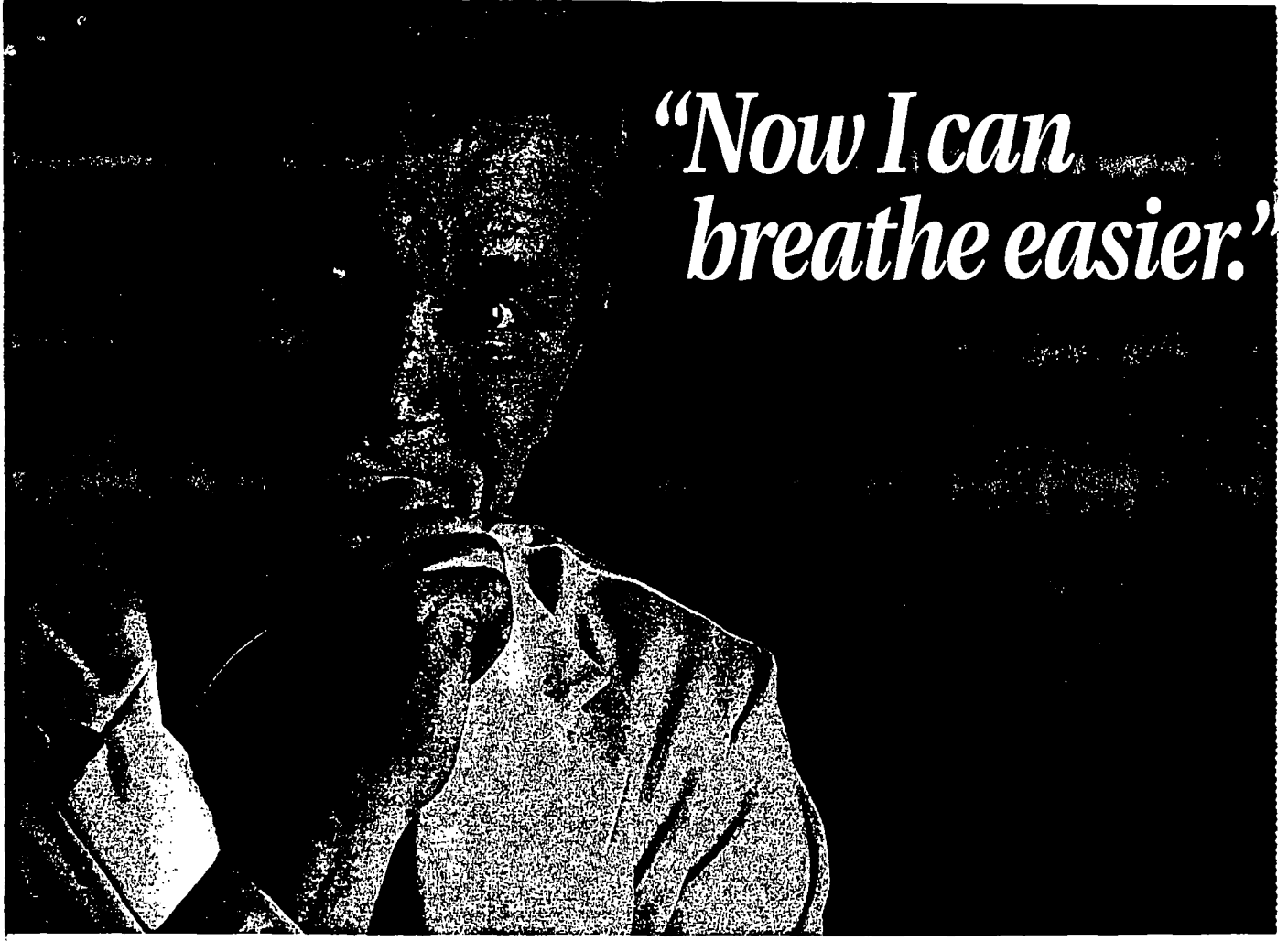
"If a stop codon was needed it could be one of the other ones—UAA or UAG," he says. "Why would it always have to be UGA? In fact, why should there be a conservation of a stop codon at all if it's just in a junk reading frame? Sometimes it could be a stop codon and sometimes it could be something else, because the message in the other reading frame is the one that's supposed to be important. ... Evolutionary selection must be causing the conservation of those UGA codons. This is a compelling argument that the proposed genes are real."

If the genes are real and they encode selenium-containing proteins, "then selenium biochemistry may be the key to understanding the control of the life cycle of HIV and perhaps some of the pathology of AIDS," says Taylor. Links between selenium and AIDS have previously been established. For example, professor Gerhard N. Schrauzer of the department of chemistry and biochemistry at the University of California, San Diego, and coworkers have found that selenium supplementation causes symptomatic improvement in AIDS patients and possibly slows the course of the disease.

Selenium levels are also known to decline in the blood of AIDS patients. Taylor points out that this is consistent with the view that selenium may become depleted, at least in part, because of its incorporation into HIV-encoded selenoproteins.

Taylor says professional colleagues had warned him that he would have trouble getting his paper published and that his theoretical results would not be accepted unless he first obtained some experimental data to back them up. However, he disagreed.

"It's perfectly accepted in physics that



theoreticians come out with theories that sometimes take years to prove—such as the existence of quarks or Einstein's theory of relativity," says Taylor. "And quantum chemistry is often used to make predictions in advance of experimental verification. So it really shouldn't be so surprising that we're now reaching the point where biological theory can be ahead of experiment."

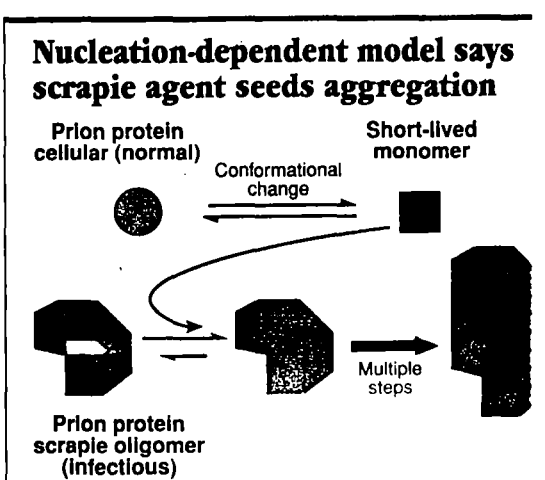
A group led by Thomas M. Folks, chief of the retrovirus diseases branch of the Centers for Disease Control & Prevention, Atlanta, is currently conducting experiments to determine if HIV-positive individuals harbor antibodies to synthetic peptides based on the proposed fusion proteins.

"The preliminary results are encouraging," says Folks. "We have seen some seroreactivity [antibody response to the synthetic peptides] in patient sera. But we don't know yet if it is truly an immune response directed at a gene product made by HIV, or if the antibody is cross-reacting to something else. It could be a staph or strep antigen, or almost anything."

Taylor hopes the publication of his paper will spark additional efforts to test the findings experimentally. □

Prion study shows that proteins can 'replicate

A new study shows that a naturally occurring protein can convert to a potentially pathogenic form in a cell-free system—providing evidence favoring the controversial hypothesis that scrapie and related neurological diseases are caused by infective proteins called prions, rather than by viruses or other traditional infectious agents.



Prions have been controversial because they appear to infect animals, replicate, and cause disease like microorganisms do, but apparently lack the specific DNA or RNA normally believed to be necessary for replication. Now, associate professor of chemistry Peter T. Lansbury Jr. of Massachusetts Institute of Technology, Byron Caughey of the National Institutes of Health's Rocky Mountain Laboratories (Hamilton, Mont.), and coworkers, have developed an in vitro system demonstrating "reproduction" of on-associated protein. The work reported recently in *Nature* [370, (1994)].

The proposed infectious form o on protein is insoluble in aqueous tion and protease-resistant, but a of the same protein that occurs nat ly in animals is soluble and suscep to proteases. Lansbury, Caughey,