NUTRIENTS *and* FOODS *in* AIDS

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chapter three

The thiols glutathione, cysteine, and homocysteine in human immunodeficiency virus (HIV) infection

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Introduction

Infection with human immunodeficiency virus type 1 (hereafter referred to as HIV) results in a progressive impairment of immune function, ultimately leading to opportunistic infections and malignancies of the acquired immunodeficiency syndrome (AIDS). The fundamental immunologic abnormality is a progressive impairment of the number and functions of CD4+ lymphocytes. Because the CD4+ lymphocytes are important immune regulatory cells, various immune functions are affected. In recent years, several reports have suggested that impaired antioxidant defense plays a role in the immunopathogenesis of HIV infection.[1-5] Several investigators have suggested that clinical trials with antioxidants, in particular with glutathione replenishing drugs, should be carried out.[6-9]

Reactive oxygen species

As an essential part of human metabolism, oxygen is required to transform different substrates for the release of energy, oxidize endogenous compounds, and detoxify xenobiotics. In these processes, most of the oxygen acts as a terminal 4-electron acceptor and is completely reduced to water. However, a small amount of oxygen is normally partially reduced, yielding various reactive oxygen species (ROS). ROS include free radicals, i.e., molecular

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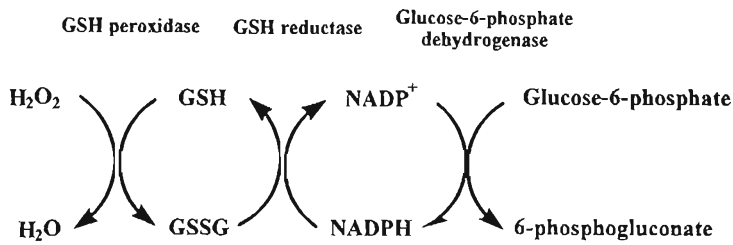

Figure 3.1 Glutathione is the substrate of glutathione peroxidase catalyzing detoxification of hydrogen peroxide and other oxidants while glutathione reductase catalyzes the regeneration of reduced from oxidized glutathione. Glutathione reductase is dependent on NADPH, which in turn is generated from NADP+ by the pentose phosphate pathway.

species containing one or more unpaired electrons, such as the hydroxyl radical and superoxide anion, and species that are not, but may readily be converted to oxygen radicals, e.g., hydrogen peroxide, in the presence of, for example, iron or copper ions.[10,11]

Reactions of free radicals with other biological molecules tend to proceed as chain reactions: one radical begets another and so on[10-12] leading to oxidative damage of proteins, carbohydrates, lipids, and DNA.

Antioxidants

The steady state formation of ROS is normally balanced by a similar rate of consumption by antioxidants that are enzymatic and/or nonenzymatic.[11,13,14] Oxidative stress results from imbalance in this ROS-antioxidant equilibrium in favor of the ROS.[14]

Important scavenger enzymes are superoxide dismutase that catalyzes the conversion of superoxide anion to hydrogen peroxide, catalase that promotes the conversion of hydrogen peroxide to water and oxygen, and glutathione peroxidase which reduces intracellular oxidants, such as hydrogen peroxide, by the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), as shown in Figure 3.1.[10,11,13,14]

There are also many structural defenses, such as compartmentalization of hydrogen peroxide-generating enzymes in peroxisomes, and chelation of free iron or copper ions in transferrin, ferritin, lactoferrin, albumin, or ceruloplasmin, thereby preventing these metal ions from participating in ROS generation.[10-12,15,16]

In addition to the primary defenses (scavenger enzymes and metal-ion sequestration), secondary defenses are also present. Lipid-soluble α-tocopherol, the most effective antioxidant component of vitamin E,[11] and water-soluble ascorbic acid (vitamin C) may function as chain-breaking antioxidants, creating new ROS which are both poorly reactive and can be reconverted to the antioxidant compound.[10,11,15,16]

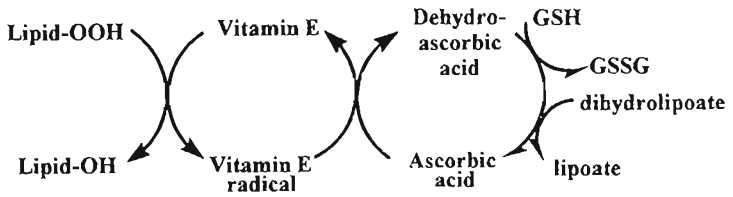

Figure 3.2 The relationship between lipophilic and hydrophilic antioxidants is shown in this figure. Vitamin E is regenerated from its radical by ascorbate which in this process is oxidized to dehydroascorbate. Dehydroascorbate can be recycled by several pathways, such as by GSH and by dihydrolipoate.

Cells also contain systems which can repair DNA damage after attack by radicals (e.g., a series of glycosylases), degrade protein damage by radicals (e.g., proteases), and metabolize lipid hydroperoxides (e.g., glutathione peroxidase and phospholipase A_2).[10,11,17] Almost all of these defenses appear to be inducible, i.e., the capacity increases in response to damage.[11]

Glutathione: the major intracellular antioxidant

Glutathione is a cysteine containing tripeptide (γ-glutamyl-cysteinyl-glycine) that is found in eukaryotic cells at millimolar concentrations and is regarded as the major intracellular redox buffering principle.[10,11] Glutathione is the substrate of selenium-dependent glutathione peroxidase catalyzing detoxification of hydrogen peroxide and other oxidants while glutathione reductase catalyzes the regeneration of reduced from oxidized glutathione (Figure 3.1). Flavin adenine dinucleotide (FAD), which is synthesized from riboflavin (vitamin B_2), is required as a cofactor for glutathione reductase. Another antioxidant enzyme, glutathione transferase, inactivates reactive electrophilic species[11] and participates in the metabolism of such endogenous compounds as steroids and leukotrienes.[10,11] Moreover, glutathione itself has antioxidant properties.[18] Finally, GSH provides reducing power for the maintenance of other antioxidants, e.g., ascorbic acid (vitamin C), vitamin E, and β-carotene.[10,11]

Ascorbic acid is a powerful antioxidant that reacts with superoxide, peroxide, and hydroxyl radicals causing the formation of dehydroascorbic acid. Dehydroascorbic acid is converted back to the reduced form, ascorbic acid, by GSH.[19] Ascorbic acid and glutathione act together as antioxidants (Figure 3.2), and there is evidence to suggest that GSH can spare ascorbic acid and vice versa.[20,22]

Vitamin E, the major lipophilic antioxidant protecting cell membranes against lipid peroxidation, is coupled to the hydrophilic antioxidants glutathione and ascorbic acid as indicated in Figure 3.2.[23] Also, as shown in Figure 3.2, the alpha-lipoic acid/dihydrolipoic acid couple contributes in the network of interlinked antioxidant systems.[23,24] Dihydrolipoic acid reacts with various ROS in addition to its interaction with ascorbic acid[24] and GSH.[25]

Reduced glutathione not only protects cells against oxidative damage induced by enhanced ROS generation. It is now recognized that glutathione is an important component of the pathway that uses NADPH to provide cells with their reducing equivalents.[20,26] Such reducing power is used for the conversion of ribonucleotides to deoxyribonucleotides and for a variety of thiol-disulfides interconversions.[11,12,26] Glutathione is, therefore, important both for the synthesis and repair of DNA and the folding of newly synthesized proteins, thus influencing the cell cycle regulation and the function of several enzymes. The glutathione redox balance is also of importance for maintenance of the thiol groups of intracellular proteins and other molecules, e.g., cysteine and coenzyme A.[26]

Figure 3.3 summarizes the reactions involved in the synthesis of glutathione. Reduced glutathione is synthesized intracellularly by the consecutive actions of γ-glutamylcysteine synthetase and glutathione synthetase utilizing adenosine triphosphate. The control point in the synthesis is γ-glutamylcysteine synthetase, which is subject to feedback inhibtion by reduced glutathione.[27,28] Breakdown of glutathione is initiated by γ-glutamyl transpeptidase, which catalyses transfer of the γ-glutamyl group of glutathione to acceptors, e.g., amino acids, dipeptides, and H_2O.[11,28] Cystine is the most active amino acid acceptor, but other neutral amino acids are also acceptors (e.g., methionine and glutamate).[10,11] Cysteinyl-glycine, formed in the transpeptidation reaction, is split by dipeptidases to cysteine and glycine, while the γ-glutamyl amino acids are substrates of γ-glutamyl cyclotransferase, which converts them into 5-oxiproline and the corresponding amino acids.[11,29] Conversion of 5-oxiproline to glutamate is catalyzed by 5-oxiprolinase.[11]

The relative levels of oxidized and reduced glutathione is normally regulated by a series of enzymes, which include the glutathione peroxidase and glutathione reductase (Figure 3.3).[11,27] The latter is dependent on NADPH, which is resupplied by a reduction of NADP+ via the pentose-phosphate pathway.[28] Normally, almost all (>99%) of the intracellular glutathione is in the reduced form.[27] However, GSSG can accumulate under certain circumstances such as rapid GSSG production, reduced glutathione reductase activity, or impaired transport of GSSG out of the cell.[27]

It is notable that γ-glutamyl transpeptidase is mainly extracellularly located, whereas glutathione is found principally within cells. It seems that many cells normally export glutathione, which then interacts with γ-glutamyl transpeptidase and dipeptidases bound to the outside of the cell membrane.[30,31]

Substrates (glutamate, cysteine, and glycine) for the synthesis of glutathione are provided by transport of either amino acids or γ-glutamyl amino acids into the cells.[26,27] It seems that cysteine availability is rate-limiting for glutathione synthesis.[26-28]

Liver is an important source of extracellular glutathione.[31] Plasma glutathione is used by many tissues which have high levels of γ-glutamyl transpeptidase, e.g., kidney, lung, and brain.[30] Glutathione itself is not transported into

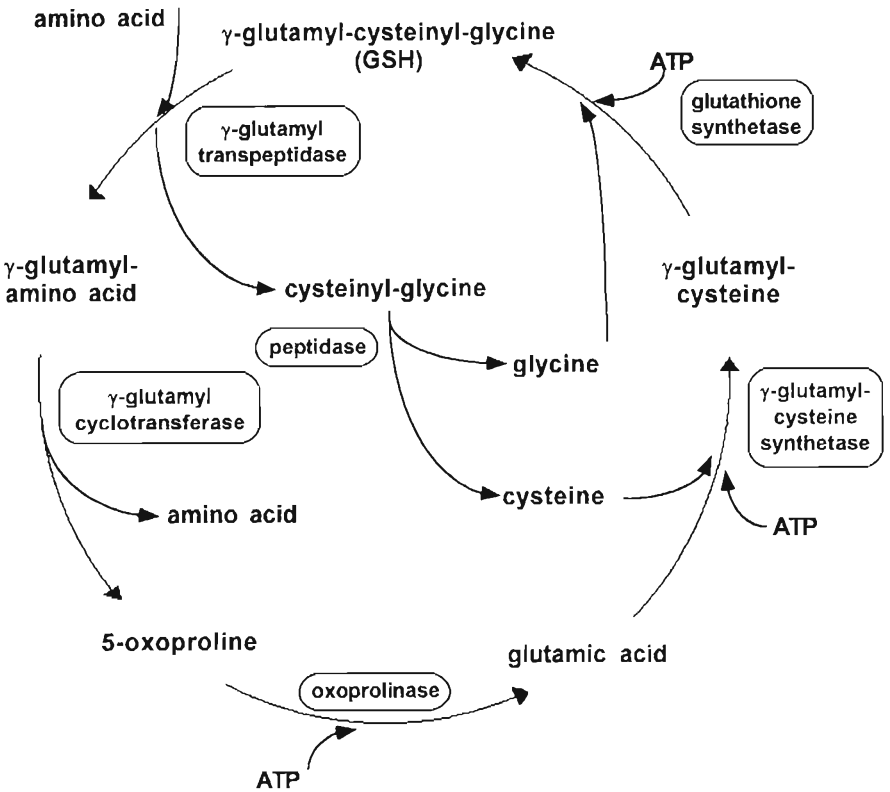

Figure 3.3 The synthesis of glutathione.

most of the cells of these tissues, but is broken down by the membrane-bound γ-glutamyl transpeptidase and dipeptidases, and the breakdown products are transported and utilized for glutathione synthesis. This is an important pathway of glutathione metabolism.[26,28,30] Thus, γ-glutamyl transpeptidase is not only important in the breakdown of glutathione, but also in preventing loss of thiol moieties and in the transport of glutathione precursors into cells.

Homocysteine

Among the plasma thiols, homocystine has recently received considerable attention, especially because hyperhomocysteinemia is a risk factor for early-onset cardiovascular disease[32,33] and is a useful marker of impaired function of cobalamin or folate.[34] Furthermore, ROS formation has been demonstrated during autooxidation of homocysteine.[35,36] Depending on parameters such as pH, concentration, and the presence of metal ions, reduced homocysteine may function either as an antioxidant or as a pro-oxidant.[37,38] In cardiovascular patients with elevated plasma homocysteine, reduced homocysteine

has been suggested as a possible atherogenic agent due to its pro-oxidant effect.[38]

Homocysteine is a product of transmethylation and is either remethylated to methionine or converted to cystathionine. The former reaction is in most tissues catalyzed by cobalamin-dependent methionine synthase, which requires 5-methyltetrahydrofolate as co-substrate.[34] Cystathionine is metabolized to cysteine, which is a precursor of glutathione.[31,39] Glutathione in turn is degraded to cysteinylglycine which is further cleaved to cysteine and glycine.[31] Thus, these thiols are metabolically related.

Oxidative stress in disease processes

Oxidative stress has been implicated in the pathogenesis of several noninfectious clinical disorders including heart and brain ischemic diseases, several lung disorders (e.g., asthma, emphysema, and bleomycin toxicity), and seems to be involved in carcinogenesis.[40-43] Oxidative stress may also play a pathogenic role in several autoimmune and inflammatory disorders such as glomerulonephritis, rheumatoid arthritis, drug-induced vasculitis, Crohn's disease, and adult respiratory distress syndrome.[40,41] It has also been suggested that oxidative stress is a major contributor to degenerative diseases of aging such as brain dysfunction and cataract.[14,17]

In addition to HIV infection (see later), increased oxidative stress may also contribute to the pathogenesis of other chronic infections, e.g., chronic hepatitis B and C virus infection and chronic parasitic infections such as schistosomiasis and *Clonorchis sinensis* infection.[17]

The mechanisms by which oxidative stress causes disease include several factors in which induction of glutathione redox disturbances and oxidizing of thiol groups are of major importance and will be discussed below.

Other factors in part related to disturbed glutathione metabolism seem also to be involved in the pathogenesis of various diseases. At least two important transcription factors, nuclear factor (NF)-κB and activator protein (AP)-1, are regulated by the intracellular redox state.[44] DNA binding sites for these factors are located in the promoter region of many genes that are involved in the pathogenesis of various diseases.[44]

A major feature of enhanced oxidative stress is increased DNA strand breakage, and these oxidative DNA lesions may play an important role in the pathogenesis of aging and cancer.[12,17,41]

Oxidative stress is also characterized by a loss of intracellular NADPH, probably related to increased poly (adenosine diphosphate-ribose) polymerase activity, which uses NADPH as substrate.[12,41] This enzyme is activated under conditions of DNA strand breakage.[45] Rise in intracellular Ca^{2+} with secondary activation of Ca^{2+}-dependent enzymes such as proteases and phospholipases leading to worsening of oxidative damage appears also to be a consequence of oxidative stress.[12,18] This rise in intracellular Ca^{2+} may in part be mediated by impaired Ca^{2+}-adenosine triphosphatase and activated mitochondrial pores.[12,46]

Finally, lipid peroxidation caused by oxidative stress gives rise to mutagenic and toxic lipid epoxides, lipid hydroperoxides, lipid alkoxyl, peroxyl radicals, and enals.[17,47]

Formation of nitric oxide, which is a highly reactive free radical, may also contribute to the cytotoxic effects of increased oxidative stress.[48]

Thiol status in patients with HIV infection

In recent years, several reports have suggested that impaired antioxidant defense plays a role in the immunopathogenesis of HIV infection. With regard to glutathione homeostasis, several reports have demonstrated abnormalities *in vivo* during HIV infection, although the results are somewhat conflicting. Decreased levels of reduced glutathione in HIV-infected patients have been found in plasma,[1] in lung epithelial-lining fluid,[1] in PBMC,[2] and in CD4+ and CD8+ lymphocytes.[3,4] On the other hand, in two studies, levels of reduced glutathione in PBMC from HIV-seropositive patients were not different from levels found in healthy controls.[49,50] Furthermore, in one recent study, we demonstrated that increased levels of oxidized glutathione and decreased ratio of reduced to total glutathione rather than decreased levels of reduced glutathione were the major glutathione disturbances in CD4+ lymphocytes from HIV-infected patients (Table 3.1), particularly in patients with advanced clinical and immunological disease.[5]

Table 3.1 Antioxidant Levels in CD4+ Lymphocytes, CD8+ Lymphocytes, Monocytes and Plasma

	CD4+ Lymphocytes	CD8+ Lymphocytes	Monocytes	Plasma
Total glutathione	—	—	↑	—
Oxidized glutathione	↑	—	—	↓
Reduced/total glutathione	↓	↑	—	ND
Total cysteine	ND	ND	ND	—
Reduced/total cysteine	ND	ND	ND	—
Total cysteinylglycine	ND	ND	ND	—
Reduced/total cysteinylglycine	ND	ND	ND	↑
Total homocysteine	ND	ND	ND	—
Reduced/total homocysteine	ND	ND	ND	↑
Glutamate	ND	ND	ND	—

Note: Levels as determined by the authors.[5,62,70] The CD4+ lymphocytes differ from the other cell populations by their enhanced levels of oxidized glutathione and low ratio of reduced/total glutathione. Both are parameters of increased oxidative stress. These alterations were most pronounced in the "naive" subpopulation (CD4+CD45RA+) compared to CD4+CD45RO+ memory subpopulation. In plasma, the elevated ratios of reduced/total homocysteine and cysteinylglycine should be emphasized, possibly contributing to enhanced production of ROS. (—: No significant difference compared to blood donor controls; ↑ or ↓: significant difference compared to blood donor controls; ND: not determined).

It has been claimed that depletion of reduced glutathione in HIV infection is caused by decreased availability of precursor amino acids, particularly of cysteine.[2,49,51] However, in one study, depletion of reduced glutathione in serum was accompanied by normal cysteine levels.[52] Furthermore, except for a slight decrease in oxidized cysteine, recent studies in our group did not demonstrate any abnormalities in plasma cysteine levls in HIV-infected individuals.[5] Thus, although several lines of evidence suggest that there are important glutathione abnormalities in HIV-infected individuals, several controversies exist concerning thiol status during HIV infection.

The discrepancies may at least in part be related to methodological differences. First, it is known that factors such as food intake, [53,54] age,[53,55] gender,[56] smoking,[38,57] family history of coronary heart disease,[39] and circadian fluctuations[58,59] may all influence plasma and possibly cellular levels of various thiol species. These factors have to be controlled for analysis of thiol status in HIV-infected individuals. Second, and most important, due to the high reactivity of thiols, the analysis of reduced, oxidized, and protein-bound forms of these compounds in plasma may be unreliable if proper precautions are not taken.[31,56,60] A period of 2.5 min is sufficient for oxidation of a substantial fraction of glutathione,[60] and cysteine may be oxidized even more rapidly.[61] In several studies by Dröge et al. showing decreased plasma levels of reduced cysteine during HIV infection, the time between blood collection and addition of acid to blood samples was longer than 90 min[2] and this may well lead to erroneous results. In studies from our group[5,62] plasma thiols were immediately derivatized during blood collection ensuring that essentially no oxidation could take place. Third, it has been demonstrated that protein-bound cysteine is the predominant form of this thiol in plasma with only approximately 40% being in the free form.[5,38,60,62] Thus, the results from previous studies only reporting plasma levels of free cysteine species[2,49,63,64] will not reflect the total cysteine status in plasma. Furthermore, by forming mixed disulfides with proteins,[38,56] this free cysteine fraction will further decrease *ex vivo* during storage of whole blood or plasma, both at low (4°C) and high (20°C) temperature if the thiol compounds are not derivatized during blood collection.[60,65]

With regard to measurement of intracellular levels of reduced glutathione in PBMC, a recent study[66] has demonstrated technical flaws when using flow cytometry and monochlorobimane conjugated to glutathione as done in several studies analyzing glutathione levels in lymphocytes during HIV infection.[3,4,67] It was found that a considerable fraction of the glutathione-bimane adducts is released from cells, accumulates extracellularly within minutes, and will not be measured.[66] This extracellular release of the glutathione-bimane adducts may be prevented by adding sulfosalicylic acid to the cells before derivatization with monobromobimane.[31,60] Moreover, it seems that the specificity of the flow cytometry method may be poor compared with that of the chromatographic method used in some other studies.[5,50,56,60,62]

Furthermore, it is conceivable that the degree and rate of the thiol oxidation in plasma *ex vivo* may be altered by disease activity during HIV

infection. This will further complicate the interpretation of data from studies comparing plasma cysteine levels or reduced glutathione levels in lymphocyte subsets in HIV-infected patients with levels in healthy controls.

Finally, it should be underscored that the glutathione levels may differ between different cell types, and more importantly, that the response of the glutathione redox cycle to oxidative stress may be differently regulated in different cell types.[9,68] Studies from our group suggest that the regulation of the glutathione metabolism is different in monocytes and CD4+ lymphocytes[5,69] and in CD45RA+ ("naive") and CD45RO+ ("memory") CD4+ lymphocytes.[69,70] Thus, when measuring intracellular glutathione levels in PBMC as has been done in most studies on glutathione disturbances in HIV infection, the findings may merely reflect variable proportions of particular cell types in HIV-infected patients. Barditch-Crovo et al.[71] have recently reported that HIV-infected patients have increased levels of reduced glutathione in PBMC compared with controls when expressed as glutathione levels per number of cells, but decreased levels when expressed as glutathione levels per mg cell protein. We believe that this discrepancy may partly be explained by increased proportions of monocytes in PBMC from HIV-infected patients. Furthermore, our previous report of isolated CD8+ lymphocytes from HIV-infected patients showing normal glutathione redox status[5] seems partly to reflect altered distribution of naive and memory CD8+ lymphocytes in these patients. In fact, we have recently found that while there is an increase in proportion of CD45RO+CD8+ subpopulation comprising normal glutathione redox status, there is a decrease in proportion of CD45RA+CD8+ lymphocytes with raised levels of oxidized glutathione and decreased ratio of reduced to total glutathione in HIV-infected patients.[70]

Increased level of oxidized glutathione — important indicator of increased oxidative stress during HIV infection

It has been suggested that decreased levels of reduced glutathione in PBMC or lymphocyte subpopulations is an important marker of oxidative stress.[72] However, there are also reports of increased levels of this glutathione species during oxidative stress.[73-77] In fact, at least some antioxidant defenses can be induced by increased ROS generation,[78] and an increase in reduced glutathione may represent an adaptive response to oxidative stress mediated by increased activity of enzymes involved in glutathione synthesis, e.g., γ-glutamylcysteine synthetase,[74] increased uptake of precursors (e.g., cysteine), or intact glutathione.[74,77] It seems that increased levels of oxidized glutathione or decreased ratio of reduced to total glutathione are better parameters of increased oxidative stress than decreased levels of reduced glutathione.[27,73,79-82] Interestingly, it has been found that increased intracellular levels of reduced glutathione do not protect cells against oxidative damage if these cells have decreased ratio of reduced to total glutathione.[73] Thus, it appears that the capacity of the glutathione redox cycle, rather than intracellular levels

of reduced glutathione, may determine the resistance to oxidative stress, at least in some cell types.[27,73,79]

Most studies analyzing intracellular glutathione status in PBMC or lymphocyte subpopulations in HIV-infected individuals have only measured levels of reduced glutathione. However, we have recently determined the glutathione redox balance in lymphocyte subpopulations and monocytes during HIV infection by measuring intracellular levels of both reduced and total glutathione. We found a marked increase in oxidized glutathione and a considerable decrease in ratio of reduced to total glutathione as the major glutathione redox disturbances in CD4+ lymphocytes from HIV-infected individuals. In resting mammalian cells, only a small fraction of total glutathione exists in the oxidized form.[27,28] However, we found that CD4+ lymphocytes from the majority of patients with symptomatic HIV infection had a ratio of reduced to total glutathione below 0.5. Although a decrease of such magnitude has been found in some intracellular structures such as the endoplasmic reticulum,[83] this has not been reported, to our knowledge, in lymphocyte subpopulations in any human disease. Furthermore, the increase in oxidized glutathione and the decrease in ratio of reduced to total glutathione, but not the moderate decrease in levels of reduced glutathione in CD4+ lymphocytes, were significantly correlated with advanced clinical and immunological disease.[5] These changes, reflecting increased oxidized stress, may well represent important immunopathogenic factors in HIV infection. Interestingly, we have observed similar glutathione disturbances in CD4+ lymphocytes from patients with common variable immunodeficiency (CVI, a primary B-cell deficiency), and this may well be of importance for the pathogenesis of CVI.[69]

Glutathione redox disturbances in CD4+ lymphocytes during HIV infection — reflection of increased "inflammatory stress"?

Several hypotheses have been put forward to explain the disturbed glutathione metabolism during HIV infection. Lymphocytes depend on extracellular concentration of reduced cysteine for glutathione synthesis and provision of cysteine is the rate-limiting step.[31,51] It has been suggested that the decreased plasma levels of cysteine leads to disturbed glutathione homeostasis in HIV infection.[2,51] However, when appropriate methods for measuring circulating thiol levels are used, no abnormalities in plasma cysteine levels seem to be present in any group of HIV-infected patients.[5,62] As an alternative explanation for the deranged thiol status in HIV-infected patients, Dröge et al. have proposed that elevated circulating concentrations of glutamate in HIV infection may inhibit the uptake of oxidized cysteine into monocytes and macrophages which then will make less reduced cysteine available to lymphocytes for synthesis of reduced glutathione.[63,84] However, we and others[5,64] could not confirm these findings [63,84] of increased serum

levels of glutamate during HIV infection. In these studies[63,84] there are no reports of glutamine or glutamine + glutamate levels, and one cannot exclude that the demonstration of raised glutamate levels in these studies may represent *ex vivo* interconversion of glutamine to glutamate.[85]

Thus, it appears that increased levels of oxidized glutathione and decreased ratio of reduced to total glutathione are the major intracellular glutathione redox disturbances in CD4+ lymphocytes from HIV-infected individuals. Among the CD4+ lymphocytes, the most pronounced glutathione abnormalities were found in the "naive" (CD45RA+) subpopulation.[70]

Several factors may influence the intracellular levels of oxidized glutathione during oxidative stress, including increased generation, the activities in the glutathione reductase and glutathione transhydrogenase (e.g., thioredoxin), and the ability to export oxidized glutathione from cells.[27,28,83]

Recent studies have focused on the cooperation between the glutathione and the thioredoxin system.[86,87] Indeed, thioredoxin appears to be of importance for maintenance of glutathione in a reduced state and vice versa.[28,88] Furthermore, it seems that some of the effects seen after increasing the intracellular ratio of reduced to total glutathione may at least in part be mediated by raised thioredoxin levels (e.g., inhibition of apoptosis).[86,89] Masutani et al. found that thioredoxin high-producer cells were selectively lost in lymph nodes from AIDS patients.[90] In a recent study, elevated plasma thioredoxin levels were found in HIV-infected patients, especially in those with advanced disease.[91] Thus, the combined redox disturbances of the glutathione and the thioredoxin system seen in HIV-infected patients may represent a vicious circle contributing to the markedly disturbed intracellular redox balance.

Oxidative stress may lead to increased formation of oxidized glutathione, and recent studies have suggested that enhanced ROS generation in lymphocytes may be an important immunopathogenic factor in HIV infection as indicated in Figure 3.4.[92] Furthermore, we have recently demonstrated a significant positive correlation between serum level of TNFα and levels of oxidized glutathione in CD4+ lymphocytes from HIV-infected patients.[5,70] This may suggest that increased inflammatory stress, which in turn may result in increased ROS generation,[93] is responsible for disturbed glutathione redox cycle in CD4+ lymphocytes during HIV infection. Indeed, TNFα stimulation both *in vitro* and *in vivo* has been shown to increase the level of oxidized glutathione.[94,95]

The activity of the glutathione reductase system is of particular importance in the defense against oxidative stress by regeneration of reduced from oxidized glutathione.[27] The markedly decreased ratio of reduced to total glutathione in CD4+ lymphocytes from HIV-infected individuals may indicate marked impairment of this enzyme system during HIV infection. The glutathione reductase system is dependent on NADPH, which is resupplied by a reduction in NADP+ via the pentose-phosphate pathway.[28] Interestingly,

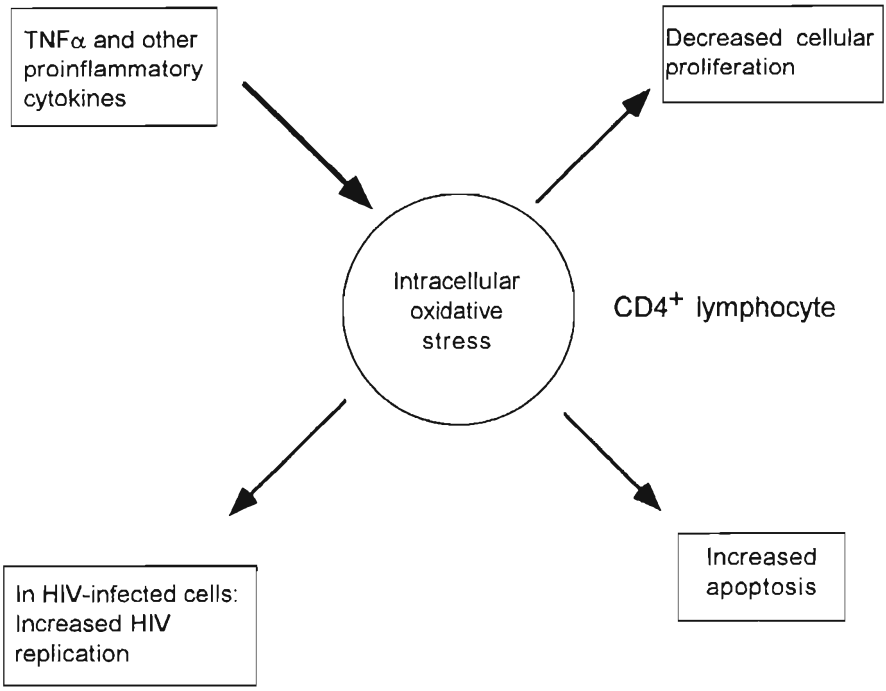

Figure 3.4 Hypothetical scheme regarding the causes and effects of intracellular oxidative stress in CD4+ lymphocytes from HIV patients. Chronic "inflammatory stress" due to overproduction of TNFα and other proinflammatory cytokines causes intracellular oxidative stress. This leads to impaired lymphocyte proliferation and possibly increased apoptosis. In HIV-infected cells, oxidative stress can also result in increased HIV replication due to activation of the transcription factor NF-κB.

low level of NADPH compromising the activity of glutathione reductase has been demonstrated in AIDS-related Kaposi's sarcoma cells.[96]

Last, but not least, TNFα and other pro-inflammatory cytokines, which enhance ROS production, may also inhibit this reductase system by depleting intracellular NADPH levels.[93] Thus, we believe that increased "pro-inflammatory stress", and in particular increased TNFα activity, is of major importance for the glutathione redox disturbances during HIV infection.

Increased TNFα activity and disturbed glutathione redox status — a vicious cycle operating in HIV infection

Several lines of evidence suggest that the correlation between increased TNFα activation and intracellular glutathione redox disturbances in CD4+ lymphocytes from HIV-infected patients[5] may reflect important immunopathogenic mechanisms in HIV infection. TNFα stimulation may decrease levels of reduced glutathione by causing enhanced ROS production which

in turn leads to consumption of this glutathione species.[94,97,98] TNFα stimulation both *in vivo* and *in vitro* has also been demonstrated to increase oxidized glutathione levels and to impair the activity of the glutathione reductase system.[93-95,99] Moreover, it seems that antioxidants such as *N*-acetylcysteine (NAC) and glutathione impair TNFα production from PBMC.[100,101] Furthermore, glutathione redox disturbances as found in CD4+ lymphocytes from HIV-infected individuals may possibly increase the inflammatory cellular response to TNFα stimulation,[102] and proper glutathione redox status is of major importance in protecting against the toxic effects of TNFα.[6,93]

Thus, at normal glutathione redox status, proliferation and antigenic stimulation will be favored ("antigenic mode"), whereas at lower ratio of reduced to total glutathione, inflammatory-type responses are more likely to occur ("inflammatory mode").[102] Finally, increased TNFα activation may in turn increase the sensitivity of cells to ROS exposure.[94] Thus, TNFα activation with enhanced ROS generation and disturbed glutathione homeostasis may represent a vicious cycle leading to increased levels of oxidative stress with important clinical, immunological, and virological consequences in HIV infection (Figure 3.4).

Immunological consequences of glutathione redox disturbances in HIV infection

Disturbed intracellular glutathione metabolism in lymphocytes may result in immunological dysfunction either as a direct effect of decreased level of reduced glutathione,[103,104] as a direct effect of increased levels of oxidized glutathione,[105,106] or indirectly through intracellular redox disturbances and oxidative stress.[83,107] Furthermore, as enhanced ROS production may induce glutathione redox disturbances, it is difficult to separate the effects of increased ROS generation from disturbed glutathione homeostasis. Thus, while depletion of intracellular reduced glutathione with buthionine sulfoximine (a glutathione synthesis inhibitor) may inhibit lymphocyte proliferation,[103,104] it fails to induce T cell apoptosis.[86] However, ROS generation may lead to glutathione redox disturbances *and* apoptosis in these cells.[86]

Several immunological functions related to HIV infection are dependent on adequate intracellular glutathione redox balance, e.g., lymphocyte activation by mitogens, natural killer cell activitation, and T-cell-mediated cytotoxicity.[103,108,109] Furthermore, a shift from Th1 (e.g., IL-2) to a Th2 (e.g., IL-4 and IL-10) cytokine profile has been suggested to be of importance in the immunopathogenesis of HIV infection.[110,111] Interestingly, recent studies have found that thiol supplementation or reducing, in contrast to oxidizing conditions, may suppress Th2 cytokine production.[112,113]

Decreased T cell proliferation or anergy upon antigen stimulation is an important immunological feature of HIV infection.[114] This hypoproliferation may be manifest even long before any decline in the absolute numbers of

CD4+ lymphocytes is observed.[115,116] Disturbed intracellular glutathione homeostasis may be of importance for this defect in T cell proliferation (Figure 3.4), and this will be discussed in somewhat more detail.

Disturbed intracellular redox status may alter functions of several enzymes and cofactors, particularly those with free SH groups in important positions,[28,88] and may also influence other intracellular redox systems.[20,88] Furthermore, optimal concentrations of thiol species are required for rapid and complete refolding of many proteins.[83,107] Thus, important cellular functions may indeed be affected by intracellular glutathione redox disturbances. In fact, a recent study has demonstrated that a decrease in intracellular levels of reduced glutathione by as little as 10 to 30% almost completely abrogated the intracellular calcium flux and the proliferative response when T cells were stimulated through the T cell receptor(TCR)/CD3 complex.[102] This may seem in some contrast with the observed increased or maintained tyrosine phosphorylation after TCR/CD3 stimulation in glutathione depleted cells.[117,118] However, examination of the complex signal transduction pathway triggered by anti-CD3/TCR stimulation indicates that increases in tyrosine phosphorylation of inhibitory sites of $p59^{fyn}$ and $p56^{lck}$, regulated by CD45 tyrosine phosphatase in concert with $p50^{csk}$, will suppress the activities of these src kinases and result in decreased phosphorylation of other proteins (e.g., phospholipase C-γ).[119,120] Interestingly, it has been suggested that increased levels of oxidized glutathione may inhibit tyrosine phosphatases and thereby inhibit dephosphorylation of inhibitory sites of src kinases.[106] In fact, decreased CD45 tyrosine phosphatase activity has been found in CD4+ lymphocytes from HIV-infected patients, and this impaired phosphatase activity was partly restored by antioxidant supplementation.[121] Thus, it seems that glutathione redox disturbances with a decreased ratio of reduced to total glutathione may markedly impair stimulation through the TCR/CD3 complex.

IL-2 production is of major importance for an adequate lymphocyte proliferative response, and decreased intracellular levels of reduced glutathione seem to impair IL-2 production in lymphocytes,[122,123] although the results are somewhat conflicting.[124,125] In fact, it has been suggested that H_2O_2 may stimulate IL-2 production through activation of the transcriptional factor NF-κB in T cell lines.[126] These discrepancies may have several explanations. First, activation of NF-κB through phosphorylation of the inhibitor I-κB, which thereby dissociates from the NK-κB complex, seems to be dependent on increased tyrosine phosphorylation.[127] Although glutathione redox disturbances may enhance phosphorylation of I-κB after some stimuli (e.g., TNFα), this phosphorylation may be impaired after antigenic stimuli.[117,128] Second, although physiological levels of H_2O_2 may activate NF-κB and induce IL-2 production and proliferation in lymphocytes, unphysiologically high ROS production and increased levels of oxidized glutathione may impair these functions partly by inhibiting DNA binding of NF-κB and NF-AT, another transcriptional factor of importance for IL-2 production.[106,129] Third, it seems that the stimulating effect of H_2O_2 on NF-κB is restricted to

some T cell lines.[130] Fourth, HIV-infected patients are characterized by persistently enhanced oxidative stress *in vivo*.[5,7,131] Although short time (hours) exposure to physiological concentrations of ROS may enhance IL-2 production *in vitro*, chronic exposure to increased oxidative stress seems to impair IL-2 production.[129,132]

It appears that enhanced ROS production and glutathione redox disturbances as found in HIV-infected patients will markedly impair rather than enhance IL-2 production in lymphocytes and will markedly suppress lymphocyte proliferation upon antigen stimulation. Indeed, we have recently demonstrated a significant correlation between intracellular redox disturbances in CD4+ lymphocytes and both impaired lymphocyte proliferation and decreased IL-2 production in HIV-infected patients.[5] Furthermore, recent studies of Cayota et al. have demonstrated that restoration of glutathione redox imbalance by anti-oxidant supplementation was able to revert the impaired proliferative activity of CD4+ lymphocyte from HIV-infected patients on CD3 stimulation.[67]

Glutathione redox disturbances in CD4+ lymphocytes during HIV infection — possible role in apoptosis

Apoptosis has been suggested to play an important immunopathogenic role in HIV infection, both in the depletion of CD4+ and CD8+ lymphocytes in advanced disease.[133-138] Importantly, a recent study demonstrated a significant correlation between disease progression and apoptosis of CD4+ and CD8+ lymphocytes in HIV-infected individuals.[139]

Several lines of evidence suggest that the markedly disturbed intracellular glutathione homeostasis, in particular when combined with increased TNFα activity, may be of major importance for this inappropriate induction of apoptosis (Figure 3.4). TNFα may induce apoptosis in both neoplastic and nonneoplastic cell types.[93,140,141] It seems that the factors determining whether cells will undergo apoptosis after TNFα stimulation at least partly depend on the extent of ROS formation as well as the cell's redox buffering capacity, of which glutathione redox status is of major importance.[93,142] Furthermore, recent studies have suggested that reduction in mitochondrial potential associated with increased ROS production is an early irreversible step of apoptosis in lymphocyte *in vivo*, further supporting a role for redox status in regulation of apoptosis.[82,143] Interestingly, such mitochondrial dysfunction has recently been demonstrated in circulating T lymphocytes from HIV-infected individuals.[92] In addition, independent of TNFα, it seems that intracellular redox disturbances mediated by increased levels of oxidized glutathione may activate redox sensitive transcriptional factors leading to induction of apoptosis.[140] Moreover, the bcl-2 proto-oncogen is rather unique among cellular genes in its ability to block apoptotic death in multiple contexts which include TNF and possibly also fas-ligand mediated apoptosis.[144,145] Hockenbery et al.[146] have suggested that bcl-2 protects against apoptosis by regulating an

antioxidant pathway at the sites of ROS production. Interestingly, they also found that overexpression of glutathione peroxidase and supplementation with NAC, but not overexpression of manganese superoxide dismutase, could prevent apoptosis in a similar fashion as bcl-2.[146] Of importance, *N*-acetylcysteine has also been found to inhibit apoptosis in HIV-infected cells.[147]

The importance of ROS generation in the induction of apoptosis and the role of bcl-2 as an "antioxidant" have recently been questioned. Two laboratories have shown that bcl-2 protects against apoptosis in near-anaerobic conditions.[148,149] Nevertheless, although ROS generation may not represent a final common pathway in induction of apoptosis, it may be of great importance for induction of apoptosis through certain stimuli (e.g., TNF).[93,140,141] Notably, glutathione may be involved in regulation of apoptosis in some other ways than operating as an antioxidant. Glutathione redox status may modify the sulphydryl groups of several proteins including enzymes and thereby alter their functions. The DNA-binding capacity of certain transcriptional factors [e.g., Fos-Jun (AP-1)], with possible function in the induction of apoptosis, may also be influenced by glutathione redox status.

Recently, Herzenberg et al. suggested that Fas antigen stimulation is of major importance for the increased apoptosis of T lymphocytes in HIV-infected patients.[150] Fas- and TNF-induced apoptosis seem to operate at least partly through different intracellular pathways,[141] and the role of glutathione in protection against fas-mediated apoptosis has been questioned.[151] However, in a recent study Chiba et al. found that fas-mediated apoptosis in human T cells, but not perforin-mediated apoptosis, is impaired by increasing intracellular level of reduced glutathione in these cells.[152] Kohno et al. have recently shown that fas-mediated apoptosis is related to GSH in adult T cell leukemia cells,[142] while van den Dobbelsteen et al. found that fas-induced apoptosis in a T cell line was associated with loss of GSH due to enhanced export out of the cells.[153] Further studies are needed to clarify the role of intracellular redox status in fas-mediated apoptosis.

While HIV itself or interaction of CD4+ lymphocytes with gp120 may represent the "priming signal" for subsequent induction of apoptosis,[136] indirect mechanisms, which include activation of the TNF system and intracellular glutathione redox disturbances, appear also to be important in mediating increased apoptosis of T lymphocytes during HIV infection.[154]

Intracellular redox disturbances in combination with persistent immune activation: important role in enhancing HIV replication

Persistent immune activation and, in particular, TNFα activation together with increased oxidative stress seem to be major characteristics of HIV-infected patients. Recent studies indicate that HIV infection, even in the

asymptomatic phase, is characterized by an active process in which cells are being infected and dying at a high rate and in large numbers, suggesting that AIDS is primarily a consequence of continuous, high-level replication of HIV.[155-157] However, this view does not exclude an important role for sustained immune activation and intracellular redox disturbances in the immunopathogenesis of HIV infection. There is a growing body of evidence indicating that an activated immune system is needed to maintain a high level of virus replication.[133,158] Both TNFα and ROS are potent stimulators of HIV replication through activation of NF-κB which is essential for the expression of genes controlled by the LTR of HIV.[97,126,159] Interestingly, it has been suggested that increased oxidized glutathione or decreased reduced to total glutathione ratio may directly enhance HIV replication through this mechanism.[160,161]

Furthermore, NAC, glutathione, and glutathione-esters have been demonstrated to inhibit HIV replication *in vitro*, both in acutely and chronically infected cell lines and in lymphocytes and monocyte/macrophages from HIV-seronegative donors.[97,162-168] However, there are to our knowledge no studies demonstrating inhibitory effect of glutathione supplementation on HIV replication in cells from HIV-infected patients, either *in vivo* or *in vitro*. It seems that the antiviral effect of glutathione is mediated by inhibition of NFκB activation.[97,162,166] However, Bergami et al.[163] have reported that cystamine, which may enhance intracellular levels of reduced glutathione,[169] inhibits HIV replication in human lymphocytes and macrophages by interfering with the orderly assembly of HIV virions. Furthermore, oltipraz (4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione), which also may increase intracellular levels of reduced glutathione has been found to be a potent inhibitor of HIV reverse transcriptase.[170,171] By whatever mechanisms, restoring of glutathione redox status in HIV-infected patients may not only have immunomodulating effects such as inhibition of apoptosis and restoring of impaired IL-2 production and lymphocyte proliferation, but may also possibly have direct anti-HIV effects.

Homocysteine in HIV infection

In a recent study, we found that all but one of 21 patients with HIV infection had plasma levels of reduced homocysteine above the range of the control group.[62] However, no difference in the plasma concentration of total homocysteine was found when patients and controls were compared. As a result, the reduced-total homocysteine ratio was considerably increased in the HIV-infected patients. Our data on plasma levels of total homocysteine in HIV patients are in accordance with a previous study showing normal serum levels of total homocysteine in 20 AIDS patients.[52] Elevated levels of reduced homocysteine in plasma have been found in various other conditions such as homocystinuria,[172] cobalamin deficiency,[173] and after administration of homocysteine[174] or methionine.[175] In all these conditions, the elevation

of reduced homocysteine has been associated with increased concentrations of total homocysteine in plasma. To our knowledge, the combination of elevated levels of reduced homocysteine and normal total homocysteine concentrations has not previously been observed in any clinical condition.

Depending on parameters such as pH, concentration, and the presence of metal ions, reduced homocysteine may function either as an antioxidant or as a pro-oxidant.[37,38] In patients with elevated plasma concentration of reduced homocysteine and early-onset arteriosclerosis, reduced homocysteine has been suggested as a possible thrombogenic agent due to its pro-oxidant effect.[38]

Our findings of elevated concentration of reduced homocysteine may be relevant for current theories implicating oxidative stress in the immunopathogenesis of HIV infection.[5,7,8,72] Elevated levels of reduced homocysteine might contribute to the production of ROS as the sulfhydryl group of homocysteine is believed to act catalytically with cupric or ferric ions to generate hydrogen peroxide and various homocysteinyl radicals.[35,36,61] In one of these studies, homocysteine plus an increasing concentration of copper led to hydrogen peroxide production in a dose-dependent manner.[36] Although data on serum copper levels in HIV patients are discrepant,[176,177] a longitudinal study has shown that patients progressing to AIDS had significantly higher serum copper levels compared to the nonprogressors.[176] In the presence of metal ions, e.g., copper or iron ions, hydrogen peroxide can react to form the highly reactive hydroxyl radical.[61] Recent studies suggest that it is the hydroxyl radical that is responsible for the NF-κB activation causing the stimulation of HIV replication in HIV-infected cells.[178] Thus, one may speculate that elevated circulating levels of reduced homocysteine in HIV patients is one of several contributing factors to an enhanced production of ROS, which in turn could lead to stimulation of HIV replication through NF-κB activation. Alternative to the hypothesis suggested above, elevated levels of reduced homocysteine might be a consequence of other redox disturbances in patients with HIV infection. Also, it is uncertain whether the measured reduced homocysteine in plasma from these patients is trapped in a form reacting with the derivatizing agent or exists as authentic homocysteine in the circulation.

No significant differences in reduced homocysteine levels were noted when asymptomatic and symptomatic HIV patients were compared, and we did not find any relationship between reduced homocysteine levels and other markers of immunodeficiency.[62]

Cobalamin or folate deficiency leads to hyperhomocysteinemia.[39,173] Most patients in our study had cobalamin levels in the lower normal range. While only two of the patients had cobalamin levels in serum below normal, their plasma concentration of total homocysteine was slightly elevated. Our findings of subnormal cobalamin levels in some HIV-infected patients are in accordance with recent studies.[177,179,180] However, the total homocysteine concentrations in the patient and control groups were similar.

Also, the HIV-infected patients in our study had reduced methionine concentration in plasma. This is in accordance with previous reports.[2,64] As methionine is an essential amino acid, malabsorption may lead to methionine deficiency. However, none of the patients in this study had clinical signs or symptoms suggesting malabsorption.

A significant but modest decrease in the total cysteinylglycine plasma concentration was found in the HIV patients compared to controls. As cysteinylglycine is a degradation product of glutathione,[27] the decreased concentration of cysteinylglycine might reflect low glutathione turnover. In fact, plasma glutathione tended to be lower in this group of HIV patients compared to controls.[5] The concentration of reduced cysteinylglycine tended to be slightly elevated in the patient group resulting in an increased reduced-total ratio. As with homocysteine, elevated levels of reduced cysteinylglycine might contribute to ROS generation due to the presence of a free sulfhydryl group.[61]

Correction of thiol redox status — therapeutic approaches in HIV-infected patients

The cornerstone in treatment of HIV-infected patients is antiviral agents such as nucleoside analogs and protease inhibitors.[181-183] However, there is a growing body of evidence suggesting that immunomodulating agents may be of importance in combination with antiviral agents in the treatment of these patients.[114,184] One immunomodulatory strategy is treatment that restores the dysregulated thiol redox balance in patients with HIV infection. In particular, modulation of the intracellular glutathione redox status in CD4+ lymphocytes is promising.

Cysteine prodrugs

N-acetyl-L-cysteine (NAC) is a direct ROS scavenger and is metabolized *in vivo* to cysteine that restores intracellular GSH levels.[185,186] Presently, NAC is used as an antidote for paracetamol (acetaminophen) overdose[187] and in the treatment of various respiratory diseases.[188,189] It can be given orally and is well tolerated.[190] NAC effectively inhibits TNFα-induced activation of the HIV long terminal repeat, leading to reduced HIV replication in infected cells.[162,166] This effect of NAC is due to a specific blocking of the induction of NF-κB.[97,191] Also, NAC has been shown to enhance antibody-dependent cellular cytotoxicity of cells from patients with HIV infection.[192] In a recent *in vitro* study, the impaired proliferative capacity of CD4+ lymphocytes from HIV-infected patients was restored by NAC.[67] In cultured T cells from HIV-seronegative donors, NAC enhanced both the proliferative capacity and IL-2 production.[122] Furthermore, NAC has been shown to inhibit apoptosis of T lymphocytes and monocytoid cells[193,194] and also in HIV-infected monocytoid

cells.[147,195] However, this anti-apoptotic effect of NAC may not be mediated by increase in intracellular GSH.[196]

Only a few trials with NAC have been performed in patients with HIV infection. de Quay et al. treated 9 HIV-infected patients with a single oral dose of NAC and found a transient and moderate increase in GSH in mononuclear cells in some patients.[49] However, the glutathione redox balance in CD4+ lymphocytes was not assessed in this study. In a study over 14 weeks, Walker et al. treated HIV patients with 4 different dosage levels of NAC and did not observe serious toxicities.[138] Finally, Witschi et al. treated 6 AIDS patients with oral NAC for 2 weeks and found a significant increase of plasma cysteine, while the GSH level in mononuclear cells and plasma was unaltered[197]

There may be several reasons for the lack of GSH response to NAC treatment. First, the bioavailability of NAC is low by oral treatment.[190] Second, the control point in glutathione synthesis, γ-glutamylcysteine synthetase, is subject to feedback inhibition by GSH, and this may inhibit the effect of supplementation with NAC or other cysteine prodrugs.[27] Third, it has been suggested that the glutathione imbalance in HIV-infected patients is due to inhibition of systemic synthesis of GSH.[181,198] If so, it is not likely that GSH precursors such as NAC would lead to a marked increase in GSH concentration.

Another cysteine prodrug, *L-2-oxothiazolidine-4-carboxylic acid* (OTC; also called Procysteine) has been shown to increase GSH levels in lymphocytes when given to healthy volunteers.[199] OTC enters the cells independently of the cystine transport pathway and is converted to cysteine by oxoprolinase inside the cell.[188] While NAC has the dual ability to replenish GSH and scavenge oxidants, OTC is a strict glutathione precursor. When NAC and OTC were compared with respect to inhibition of cytokine-induced HIV replication in various cell lines, it was observed that NAC was far more effective than OTC.[167] Also, NAC fully replenished intracellular GSH, while OTC did not.[167] In a phase I/II trial of intravenous OTC for 6 weeks in 24 asymptomatic HIV-infected patients, no change in CD4+ lymphocyte count or HIV viral load was observed, although in the subgroup of patients receiving the highest OTC dose, whole blood glutathione was significantly higher at the end of the study compared to baseline levels.[200]

Another thiol compound, *cystamine*, has been shown to inhibit HIV replication both in lymphocytes and macrophages *in vitro*.[163,201] Cystamine can increase GSH.[169] In addition, cystamine inhibits HIV replication by interference with two steps of the viral life cycle, namely inhibition of proviral DNA formation and assembly of HIV virions, causing the production of defective viral particles.[163] Interestingly, cystamine also inhibited lipopolysaccharide (LPS)-induced TNFα production in macrophages.[201] However, cystamine has not been used in humans while the structurally related form, cysteamine, has a low toxicity in man.[202] In a recent study, cysteamine was found to be a potent inhibitor of HIV replication *in vitro* at

similar concentrations to those obtained by oral administration for the treatment of cystinosis, an inherited disorder.[203]

Treatment with glutathione

In one study, intravenous infusion of GSH was given to eight HIV-infected patients, the plasma level of both cysteine and GSH increased, but the intracellular GSH concentration in *mononuclear cells* did not increase.[198] The authors suggest that GSH remained low due to a decreased systemic synthesis of GSH.[198] However, as we have underscored above, glutathione levels may differ between different cell types, and measurements of intracellular glutathione in *mononuclear cells* may thus merely reflect increased or decreased proportions of particular cell types.

Modulation of glutathione peroxidase

The activity of the selenium-dependent glutathione peroxidases is important for the intracellular protection against oxidative damage. Patients with HIV infection have an impaired selenium status,[204-206] and this may well affect the glutathione peroxidase activity. In fact, selenium supplementation has been shown to increase glutathione peroxidase activity in HIV-infected T lymphocytes.[207] Thus, selenium supplementation in patients with HIV infection may be of importance and may also have a synergistic effect in combination with GSH replenishment, e.g., with NAC.

Modulation of glutathione reductase

The elevated intracellular level of oxidized glutathione in CD4+ lymphocytes from HIV-infected patients may well be due to impaired activity of glutathione reductase. FAD is synthesized from riboflavin and is required as cofactor for glutathione reductase. Thus, one might speculate that reduced uptake of riboflavin due to malabsorption could lead to reduced activity of glutathione reductase. However, no data are available from HIV patients regarding this subject.

Also, glutathione reductase is dependent on NADPH, and it is of importance that pro-inflammatory cytokines such as TNFα deplete intracellular NADPH levels[93] and thus may inhibit the reductase. The effects of TNFα are further potentiated by the HIV regulatory protein tat.[208] Furthermore, tat caused a decrease in the intracellular glutathione level in different T cell lines.[208]

Thus, anti-TNFα treatment may well enhance the activity of glutathione reductase in HIV-infected patients. Several clinical trials with thalidomide[209,210] or pentoxifylline[211-213] have been performed, but glutathione status has not been evaluated in these trials. It is thus important that the intracellular glutathione redox balance is evaluated in future trials where anti-TNFα treatment is given.

Other antioxidants with effects on the glutathione redox balance

In vitro studies with alpha-lipoic acid and vitamin E derivatives have shown that they inhibit TNFα induced NF-κB activation in T cell lines, and it has been suggested that these compounds may be possible candidates for clinical trials in HIV-infected patients.[214,215]

Ascorbic acid inhibits HIV replication *in vitro*.[164,216] Furthermore, ascorbic acid has a synergistic effect in combination with NAC with regard to reduction of HIV replication.[164] The antiviral effect of ascorbic acid seems not to be mediated by suppression of NF-κB activation.[217]

Based on these data and the fact that ascorbic acid has a low toxicity,[218] it would be interesting to evaluate ascorbic acid in combination with thiols such as NAC in clinical trials of HIV-infected patients. In a current study in our department, HIV-patients receive a combination of NAC and ascorbic acid while clinical, immunological, and virological parameters are evaluated.

Conclusion

Two lines of evidence suggest that patients with HIV infection may benefit from antioxidant treatment: First, the impaired glutathione status in their CD4+ lymphocytes, and second, *in vitro* data indicating that oxidative stress leads to impairment of important lymphocyte functions and causes increased HIV replication in infected cells. Whether treatment with antioxidants such as *N*-acetylcysteine will prove to be useful in the treatment of patients with HIV infection awaits the results of future clinical trials.

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