Glutathione deficiency and human immunodeficiency virus infection

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Introduction

Glutathione (GSH), the main intracellular defence against oxidative stress, is decreased in plasma, lung epithelial-lining fluid, and T lymphocytes in individuals infected with human immunodeficiency virus (HIV). This deficiency may potentiate HIV replication and accelerate disease progression, especially in individuals with increased concentrations of inflammatory cytokines because such cytokines stimulate HIV replication more efficiently in GSH-depleted cells. GSH deficiency also contributes to the overall depression of immune functions. Drugs that replenish GSH, such as N-acetylcysteine, counteract oxidative stress and inhibit HIV transcription and replication in models of acute and latent HIV infection. Here we review the role of GSH in HIV infection and the potential for countering GSH deficiency with drugs.

Metabolism and functions of glutathione

GSH is a cysteine-containing tripeptide (\(\text{γ-glutamyl-cysteinyl-glycine}\)) that is found in eukaryotic cells at millimolar concentrations. Its cellular functions include amino acid transport, acting as a cofactor in enzymatic reactions, and maintenance of protein sulphydryl redox status. Furthermore, GSH is a defence against electrophilic xenobiotics and intracellular oxidants (free radicals, reactive oxygen intermediates). Therefore, GSH is also a regulator of cellular redox potential. Synthesis and degradation of
GSH are part of the γ-glutamyl cycle in which GSH is synthesised by the action of two ATP-consuming enzymes: γ-glutamylcysteine synthetase and GSH synthetase (fig 1). Reduced GSH can be oxidised to GSH disulphide either non-enzymatically or through the activity of GSH peroxidase (fig 1). Under normal conditions, GSH disulphide can be reduced by GSH disulphide reductase to regenerate GSH.

Adenine concentrations of GSH are required for mixed lymphocyte reactions, T-cell proliferation, T and B cell differentiation, cytotoxic T-cell activity, and natural killer cell activity. Decreasing GSH by 10 to 40% can inhibit completely T-cell activation in vitro. Thus, an intracellular GSH deficiency in lymphocytes has profound effects on immune functions.

Decreased glutathione concentrations in HIV infection

Several groups have demonstrated depleted GSH in the tissues of HIV-infected individuals (fig 2). Eck and colleagues were the first to show that HIV-infected individuals have decreased concentrations of acid-soluble thiols (cysteine and GSH) in their plasma and in cell lysates of peripheral blood mononuclear cells (PBMCs). These changes were found in symptom-free individuals, indicating that they were not the consequence of a wasting syndrome. The same patients also had increased plasma glutamate concentrations, which inhibit the cystine uptake needed for GSH synthesis. Buhl et al. confirmed the lower plasma GSH concentrations and, in addition, showed that GSH concentrations were decreased in lung epithelial-tinining fluids of 14 symptom-free HIV-infected individuals. This finding indicates that HIV infection is accompanied by a systemic deficiency of extracellular GSH. HIV-infected children also have plasma and whole-blood GSH deficiencies.

We have examined intracellular GSH concentrations in PBMC subsets (CD4 and CD8 T cells, B cells, and monocytes) from 134 HIV-infected individuals in different stages of HIV-related disease. With a fluorescence-activated-cell-sorter-based flow cytometric method we showed that in healthy controls there are two distinct types of CD4 and CD8 T cells containing high and low intracellular concentrations of GSH. While the ratio of these two cell types varies considerably from individual to individual, the GSH concentrations of each type are highly conserved among individuals. High-GSH T cells are depleted from the CD4 and CD8 T cell subpopulations of HIV-infected individuals, even in the symptom-free stages of the disease. The selective loss of high-GSH T cells results in lower median GSH concentrations in the T cells of HIV-infected individuals. For example, in patients with symptomatic acquired immunodeficiency syndrome (AIDS), GSH concentrations in CD8 and CD4 T cells are 62 and 63%, respectively, of those found in seronegative controls. Other studies of HIV-1 seronegative individuals (eg, B cells acutely infected) do not show significant decreases in GSH concentrations. However, some changes in GSH regulation seem to occur in B cells. B-cell GSH concentrations vary considerably more among HIV-infected than among healthy individuals. Furthermore, in HIV-infected individuals, but not in uninfected controls, GSH concentrations correlate with increased expression of the pan B-cell surface antigen CD20.10

Further proof of the early depletion of GSH after HIV infection came from a study which showed that thiol depletion occurred soon after infection (within a week) in a primate model for AIDS in which macaques were infected with simian immunodeficiency virus. The same study also found alterations in serum cysteine and glutamate concentrations, similar to those found in human beings, indicating that thiol depletion may be a general consequence of simian immunodeficiency virus and HIV infection.

Glutathione concentrations and regulation of HIV transcription and replication in vitro

Inflammatory cytokines (interleukins 1 and 6, and tumour necrosis factor alpha) and phorbol esters can stimulate HIV production in vitro. N-acetylcysteine, which represses and enhances intracellular GSH, inhibits cytokine-stimulated HIV replication in acutely infected Molt4 T cells, chronically infected cells, and in normal PBMCs infected in vitro. GSH and GSH esters also block cytokine-stimulated HIV transcription.13 The mechanism for thiol regulation of HIV activation can largely be explained by the influence of thiols on the nuclear factor k B (NF-κB) transcription factor. NF-κB is a nuclear factor that binds to the enhancer of the X light chain and greatly increases HIV transcription and replication. Stimulation of NF-κB with tumour necrosis factor alpha with or without phorbol myristate acetate, which can generate intracellular oxidants, is effectively inhibited by N-acetylcysteine. Moreover, oxidants (eg, H2O2) activate NF-κB and HIV transcription directly and N-acetylcysteine blocks this activation. By contrast, dexamethasone pre-treatment oxidises intracellular GSH and facilitates cytokine-stimulated HIV-directed transcription. Thus, transcription of the NF-κB (and HIV) activation signal is more likely to be inhibited when thiol concentrations are low and is less likely or inhibited when thiols are maintained at high concentrations. Thus, redox mechanisms regulate HIV activation. We do not know if N-acetylcysteine acts to replete GSH concentrations or to scavenge oxidants directly. Under certain conditions, N-acetylcysteine is a direct scavenger of reactive oxygen intermediates (ROIs). When de-novo GSH synthesis is inhibited by buthionine sulphoxime (an irreversible inhibitor of
gamma-glutamylcysteine synthetase), and GSH concentrations are lowered. N-acetylcysteine still inhibits HIV transcription and replication (P. Staal, M. Roederer, unpublished). The degree of inhibition by N-acetylcysteine is essentially the same without buthionine sulfoximine. These results accord with those of Milun et al., who have suggested that cysteine itself may have a direct regulatory role.

Chronic oxidative stress and decreased glutathione concentrations during HIV infection

HIV replication can be expected to proceed more rapidly in immunosuppressed individuals who have infections that induce inflammatory responses. Indeed, HIV-infected individuals often have increased serum and plasma concentrations of inflammatory cytokines (which stimulate NF-kB and thereby HIV activation).16 These cytokines stimulate the production of ROIs in certain cell types.17 The principal defence against oxidative stress within cells is GSH, which scavenges ROIs and is consumed by this process, resulting, potentially, in lower intracellular GSH concentrations. Therefore, inflammatory responses in HIV-infected individuals can cause thiol loss so that NF-kB activation and HIV transcription are favoured. Whatever the exact mechanism of the decrease in GSH concentrations, it is likely that GSH deficiency contributes to the pathogenesis of AIDS. Exactly how HIV causes immunodeficiency is unclear and several hypotheses, including autoimmune changes18 and the presence of an HIV-encoded superantigen,19 have been advanced. However, in view of the requirement for well-regulated GSH concentrations for immune function, GSH deficiency has to be included among the factors leading to the symptoms of HIV infection and immune-cell depletion. Lowered GSH concentrations are probably not restricted to HIV infection, but may occur whenever concentrations of inflammatory cytokines are increased (eg, in acute respiratory distress syndrome,20 cerebral malaria,21 and multiple sclerosis22).

Therapeutic strategies

Restoration of GSH concentrations will decrease ROIs, inhibit HIV stimulation by inflammatory cytokines, and (partly) block viral production. Replenishment may also restore some immune functions of T lymphocytes and alleviate the cachexia that is often seen in the late stages of AIDS. Many drugs with antioxidant properties (not only GSH-replenishing drugs) may be suitable. Pentoxifylline inhibits HIV production in vitro and in vivo, but has unwanted side-effects.23 Pentoxifylline, an effective inhibitor of tumour necrosis factor alpha, decreases HIV replication and has been proposed for treatment of AIDS.23 Vitamin C can also inhibit HIV replication in vitro in synergy with N-acetylcysteine but not with GSH.22 Whatever the merits of the drugs listed above, GSH prodrugs are most likely to be effective at alleviating GSH deficiency. We and others have proposed using N-acetylcysteine as a therapeutic agent in AIDS.24-26 N-acetylcysteine has antiretroviral effects in vitro, low toxicity in vivo, a long history of use in patients, can be given orally in a palatable form, and is inexpensive. The pharmacokinetics of this drug are well-established26 and high doses have been used without side-effects.25,26
antidote for paracetamol (acetaminophen) overdose,28 for example. Several clinical trials to establish the efficacy of N-acetylcysteine in the treatment of acetaminophen-induced hepatic necrosis are under way. Other GSH produgs include L-2-oxothiazolidine-4-carboxylic (OTC), GSH esters, and GSH itself. OTC is a cysteine prodrug requiring intracellular conversion by N-acetyltransferase. Although the pharmacokinetics of this drug are known,29 no in-vitro studies of its effects on HIV replication have been reported. Buhl et al.29 have proposed the use of acetylated GSH esters to correct GSH deficiency in the lung epithelial-lining fluid of AIDS patients. Halliwell and Cross29 have suggested that antioxidant therapy with an N-acetylcysteine and vitamin C is merited given the low toxicities of these drugs.

GSH replacement therapy, especially with N-acetylcysteine, is attractive because it inhibits expression of integrated HIV and thus offers a novel route for interfering with progression of HIV infection. Castle and colleagues19 have suggested that GSH or GSH produgs are reverse-transcriptase inhibitors and do not inhibit HIV production once it is integrated into the host genome. Because of its mode of action, GSH replacement therapy may be effective in extending latency and in delaying the development of opportunistic infections. A combination of N-acetylcysteine (or other GSH produgs) with reverse-transcriptase inhibitors and other antiviral drugs (e.g., pentamidine for Pneumocystis carini pneumonia) may prove to be a useful treatment for HIV infection.

We thank "Project Inform" for support and Digital Equipment Corp. for a gift of equipment. M. R. is a Leukemia Society of America Special Fellow. P. T. S. is supported by Demarest of Genetics funds. M. T. A. by a University-wide AIDS Research Program fellowship (PWS70194) and L. A. H. was supported in part by National Institute of Health grant CA21709.

REFERENCES


Smith CV, Hansen IN, Hansen IC, Shaw EE, Glutathione concentrations in plasma and blood are markedly decreased in HIV-infected children. Sixth International Conference on AIDS; June 1989; San Francisco, Ill.

