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N-Acetylcysteine: Potential for AIDS Therapy

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Abstract

The observations that people infected with HIV suffer not only from an inflammatory stress but also from depleted glutathione levels have led to a general hypothesis that these two are causally related, and that treatment of AIDS should include thiol-replenishment therapy. In particular, inflammatory stimulations are dependent on intracellular thiol levels, as they are potentiated at low glutathione levels (oxidative stress) and inhibited at high glutathione levels. Inflammatory stress may itself lead to decreased levels of glutathione. HIV has taken advantage of inflammatory signals to regulate its own replication; thus, the HIV infection is exacerbated by low levels of glutathione. We have shown that N-acetylcysteine can inhibit inflammatory stimulations, including that of HIV replication. Since N-acetylcysteine can replenish depleted glutathione levels in vivo, we suggest that it be used as an adjunct in the treatment of AIDS.

Key Words
HIV
Inflammatory stress
Oxidative stress
Reactive oxygen intermediates
Glutathione
NF-κB
AIDS treatment
Signal transduction

Introduction

The etiological cause of AIDS is the lentivirus termed human immunodeficiency virus (HIV). However, many of the pathological aspects of the disease are not directly due to infection by the retrovirus; rather, they are secondary effects due to the host response to the infection. We have made the argument that one of the more important aspects of this disease is the chronic inflammatory and oxidative stresses that accompany the infection [1]. These stresses not only result in detrimental effects on the general health of the infected individual, but eventually contribute to the loss of CD4 (helper) T cells, increased opportunistic infections, immunodeficiency in general, wasting disease and death.

Eck et al. [2] first demonstrated the possible connection between oxidative stress and AIDS: that HIV-infected invididuals have decreased levels of thiols in their blood. Glutathione is the major intracellular antioxidant, and is the main defense against oxidative stresses such as those presented by the production of reactive oxygen intermediates.

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Buhl et al. [3] confirmed these results, further demonstrating that the lung epithelial lining fluid from HIV-infected individuals had severely depleted glutathione levels.

Fauci and colleagues [4] have suggested that the inflammatory cytokines, such as tumor necrosis factor-alpha (TNF), play a major role in the progession of AIDS. Such cytokines can directly stimulate viral replication [5]. Our research, confirmed recently by several groups, demonstrates that the stimulation of HIV replication brought about by inflammatory cytokines can be abrogated by N-acetylcysteine (NAC) [6–10]. NAC is an antioxidant which replenishes glutathione; thus, we suggested that it functions by relieving the oxidative stress induced by TNF. These data led us to suggest that NAC, a safe, commonly used drug, be used as an adjunct in the therapy of AIDS.

Inflammatory Stress Accompanies AIDS

Sera from individuals infected with HIV often show elevated levels of cytokines, especially those involved in inflammatory responses. Specifically, elevated levels of TNF [11–16], IL-1α [16], IL-1β [17], IL-2 [16], IL-6 [17, 18], inteferon-α [15, 19], and inteferon-γ [20] have been shown to occur with AIDS. The level of neopterin, a molecule produced by macrophages principally in response to interferon-γ, correlates not only with the progression of disease, but is also a strong predictor for subsequent progression to AIDS [21–25].

Functional studies on lymphocytes from infected individuals also suggest that AIDS is characterized by an inflammatory stress. Polyclonal B cell activation was noted as early as 1983 [26]. Several observations confirm that B cells in HIV-infected individuals are activated, including hypergammaglobulinemia, elevated expression of B cell activation markers (and CD20), increased frequency of B lymphomas, and elevated levels of plasma IL-6 [18, 26–31].

In contrast to the B cells, T cells and monocytes are less responsive if not anergic in HIV-infected individuals. Both in vivo and in vitro functions are defective in comparison to uninfected controls [32]. This deficiency is probably brought about by excessive (or chronic) stimulation with cytokines [33–35]

Inflammatory Stress Leads to Oxidative Stress

Stimulation with inflammatory cytokines such as TNF leads to the intracellular production of reactive oxygen intermediates in a wide variety of cell types [36]. The production of these intermediates in response to stimulation occurs not only in granulocytes, whose activity relies on this production, but also in other cell types such as B and T cells [37–40]. It is now becoming clear that the production of reactive oxygen intermediates in these latter cell types is not a 'side effect', but may rather represent an important aspect of the signal transduction pathways for inflammatory cytokines [41]. The main defense against the toxicity of reactive oxygen intermediates in the cell is glutathione. Glutathione, a cysteine-containing tripeptide, is present in 1–10 mmol/l concentrations in the cytoplasm, and thereby represents the major redox buffering moiety. Through enzymatically-catalyzed reactions, it reduces reactive species to nontoxic species through its conversion to the disulfide GSSG. GSSG is subsequently exported from the cell (or alternatively reduced back to glutathione by glutathione reductase) [42]. Thus, the redox status within the cell is dependent on the (competing) levels of glutathione and oxidative species.

Increasing Glutathione Levels Inhibit Toxic Inflammatory Responses

Several studies have shown that glutathione levels (and the production of reactive oxygen intermediates) are critically involved in TNF-induced cytotoxicity. While supplementation of glutathione levels with NAC protects cells against TNF cytotoxicity [43], depletion with buthionine sulfoximine potentiates cytotoxicity [44]. This is true not only for cell lines, but in vivo: rats given lethal doses of TNF are rescued by coadministration of NAC [45], indicating that the oxidative stress induced by TNF can directly lead to death.

We suggested that the production of reactive oxygen intermediates may be a prerequisite for the signal transduction of TNF. Using a cultured cell line to study the stimulation by TNF, we demonstrated that addition of NAC inhibited the TNF-stimulated expression of gene products responsive to this cytokine (e.g. HIV core protein p24) [6]. This inhibition was evident at the mRNA level [7], suggesting that the block was in the signal transduction pathway leading to increased transcription. This hypothesis was confirmed by the observation that NAC inhibits the activation of NF-κB [8, 10, 41], the enhancer-binding protein responsible for increasing transcription of TNF-responsive genes. These results suggested the role of a redox-sensitive step in the signal transduction pathway for inflammatory cytokines.

More recently, Baeuerle's group made the crucial connection between the production of reactive oxygen intermediates and the activation of NF-κB. They demonstrated that hydrogen peroxide (which is one of the reactive species normally produced in cells) could directly and by itself activate NF-κB [41]. They also showed that exogenous addition of NAC inhibited the activation of NF-κB by a wide variety of agents. On the basis of these results, they hypothesized that reactive oxygen intermediates are second messengers for the signal transduction of inflammatory signals.

HIV has taken advantage of signalling pathways by inflammatory cytokines to regulate its own transcription. Indeed, the HIV long terminal repeat, which contains the transcription-enhancing elements, has two binding sites for NF-κB [46]. Activation and binding of NF-κB is necessary and sufficient to mediate the stimulation of viral transcription as induced by TNF, IL-1, and phorbol esters [5, 46–49]. This mode of regulation introduces a positive feedback loop during the progression to AIDS: HIV infection (in an unknown fashion) produces an inflammatory stress, including elevated levels of inflammatory cytokines; this stress, among other effects, can stimulate viral replication.

We have shown that inhibiting the TNF-induced NF-κB activation with NAC leads to inhibition of stimulated viral transcription and replication. This inhibition was evident both in several acute and chronic infection models. For example, peripheral blood mononuclear cells (PBMC) from uninfected individuals were infected with cultured HIV, in the presence or absence of NAC. Even submillimolar concentrations of NAC could completely inhibit the stimulated viral replication induced by TNF [6]. For models of chronic infection, we used the cell lines developed by Fauci's group, ACH-2 (T cells) and U1 (promonocytes) [4, 47]. These lines produce minimal virus until stimulated by a variety of agents including TNF and phorbol 12-myristate-13-acetate diester (PMA) [50]. We showed that NAC inhibits the TNF- and PMA-stimulated evolution of virus from these lines [7]. Poli et al. [51] further demonstrated that NAC also inhibited the stimulation by IL-6 and GM-CSF. These results suggest that NAC may be effective in maintaining viral latency by inhibiting the stimulated expression of the integrated virion.

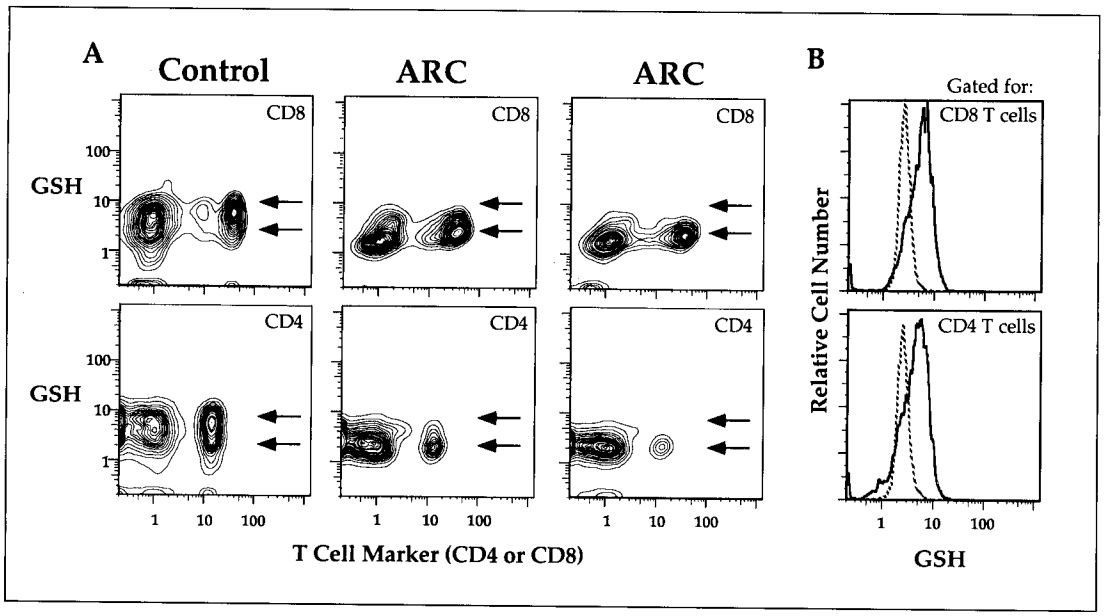

Fig. 1. FACS analysis of intracellular glutathione in $CD4^+$ and $CD8^+$ T cells. **A** Dual parameter plot of monochlorobimane (MCB) fluorescence (measuring glutathione) on the ordinate versus CD4 or CD8 fluorescence on the abscissa. Left panel: HIV-healthy control; middle and right panels: HIV^+ individuals classified as ARC. Note that the scale is logarithmic. Arrows indicate the approximate positions of the low- and high-glutathione T cells (as found in the normal controls). The high-glutathione containing T cell classes are virtually missing in these individuals. While a significant loss of CD4 T cells was observed in these HIV^+ individuals, there was no decline in the number of CD8 T cells; however, the high-glutathione T cells are missing in both subsets (cell counts: CD4: 806, 304, and 53/μl, respectively; CD8: 524, 974, and 393/μl). **B** Histograms of the glutathione in the CD8 (top) or CD4 (bottom) cells from the HIV^- control (solid line) and the second HIV^+ individual (hatched line). Note that the low end of the distributions are at the same level of glutathione for both individuals, indicating that the low-glutathione T cells have not decreased in intracellular glutathione content; rather, the representation of cells has shifted from predominantly high-glutathione to exclusively low-glutathione. Reproduced from Staal et al. [54] by permission; see this reference for experimental methodology.

HIV-Infected Individuals Have Depleted Glutathione Levels

The decreased glutathione levels shown by Eck et al. [2] and Buhl et al. [3] were measured in serum samples or bulk lysates of cells. We adapted a fluorescence-activated cell sorter (FACS)-based assay [52] to measure intracellular glutathione on a cell-by-cell basis in PBMC, in order to study the depletion in greater depth. We demonstrated a stark contrast in the intracellular glutathione levels in PBMC from uninfected healthy individuals, and those infected with HIV. In uninfected individuals, glutathione levels are highly conserved in different PBMC subsets [53]. However, in HIV-infected individuals, there is a much broader range of glutathione levels [54], indicating a systemic dysregulation of intracellular glutathione levels. Furthermore, as figure 1 shows, HIV-infected individuals display a specific loss of a class of T cells with

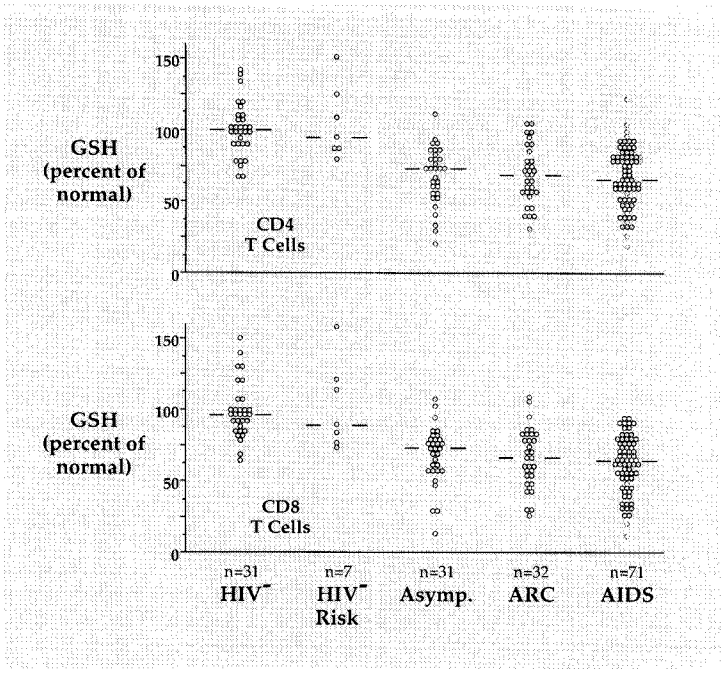

Fig. 2. Intracellular glutathione levels in $CD4^+$ and $CD8^+$ T cells are lower in HIV^+ than in HIV^- individuals. Intracellular glutathione is determined as the median of MCB fluorescence, with the average median of normal, healthy subjects (random control) normalized to 1.0 for each T cell subset. Each individual is indicated as a small circle; the median glutathione level for each category is shown with a bar. Reproduced from Staal et al. [54] by permission.

high glutathione (of both CD4 and CD8 lineages) [53, 54]. While uninfected individuals always have these high-glutathione T cells, they are virtually absent in HIV-infected individuals, even at the asymptomatic stage. This loss leads to the observed average decline in intracellular glutathione levels in T cells in infected individuals (fig. 2).

There are several possible explanations for the loss of the high-glutathione T cells: (i) these cells are more sensitive than the low-glutathione T cells to some factor induced by HIV, and are selectively killed; (ii) these cells are removed from the periphery in response to the HIV infection; (iii) the high-glutathione T cells are converted into low-glutathione T cells through loss or oxidation of glutathione; (iv) the generation of new high-glutathione T cells is abrogated, such that only low-glutathione T cells are produced. Any explanation must account for the observations that high-glutathione cells of both the CD4 and CD8 lineages are lost, and that the fraction of high-glutathione CD4 T cells correlates extremely well with the fraction of high-glutathione CD8 T cells, irrespective of the HIV-infected status of the individual.

We feel that it is most likely that the loss of the high-glutathione T cells is directly related to the inflammatory and thus oxidative stress present in these individuals. Chronic stimulation and production of reactive oxygen intermediates in a variety of cell types could lead to systemic glutathione depletion; it may be that the high-glutathione T cells are most sensitive to this process. It is also possible that the dysregulation of glutathione levels leads (at least in part) to the anergy [32, 55] of T cells and monocytes from infected individuals. In view of the sensitivity of T cell function and proliferation on appropriate intracellular glutathione levels [56–58], we expect that the

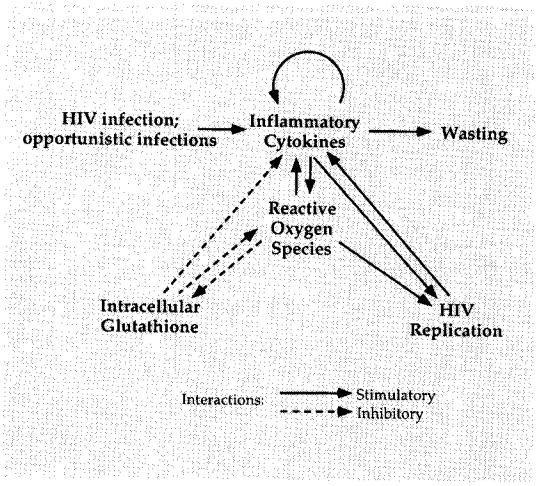

Fig. 3. Dynamics of inflammatory cytokines, reactive oxygen intermediates, and glutathione during the progression of the HIV infection. Positive interactions (stimulations) and negative interactions (inhibitions) result in positive feedback loops between inflammatory cytokines (such as TNF), the production of reactive oxygen intermediates, decreases in intracellular glutathione, and increases in HIV replication. Opportunistic infections can pump this cycle through stimulation of the immune system; overproduction of TNF (and possible other cytokines) may lead to progressive wasting and eventual death. Reprinted from Roederer et al. [1] by permission.

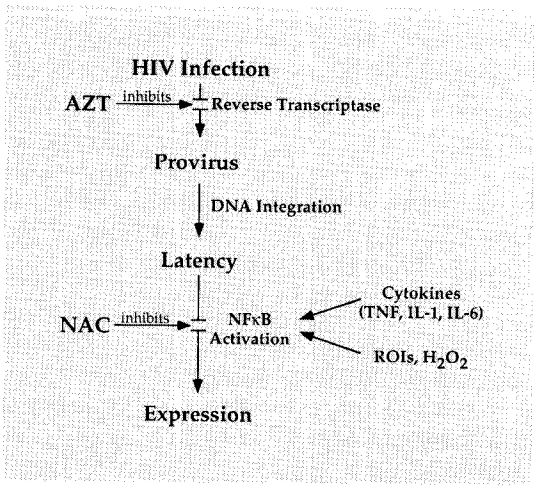

Fig 4. NAC is a novel approach to anti-HIV therapy. NAC inhibits the host-mediated stimulation of viral transcription and production. Current therapies (AZT, ddI, ddC) are directed at inhibition of the reverse transcriptase and are ineffective in the inhibition of viral production after latency has been established. Reprinted from Roederer et al. [1] by permission.

loss of high-glutathione T cells would have a profound effect on the functionality of the T cell compartment.

Whether or not decreased glutathione levels are due to inflammatory stimulations manifest at the level of the T cells remains to be determined. Certainly, there are systemic abnormalities that may lead to the deficiency at the cellular level. For instance, Eck et al. [2] also demonstrated that plasma cysteine levels were depressed. Since cysteine can be the limiting precursor for glutathione synthesis, low cysteine levels can lead directly to low glutathione levels. (Alternatively, oxidative stresses could deplete both glutathione and cysteine). Eck et al. also found increased levels of plasma glutamate, which may exacerbate intracellular cysteine/glutathione deficiencies by virtue of its inhibition of cysteine uptake by cells.

Treatment of HIV Infection Should Include Glutathione-Replacement Therapy

It is on the basis of these results that we suggest that glutathione-replacement therapy be used as an adjunct in the treatment of HIV infection. Such therapy may have beneficial

effects at several sites (fig. 3): (1) Regulation of viral stimulation by inflammatory cytokines: Optimal stimulation of HIV occurs at decreased glutathione levels; exogenous thiol sources can effectively block the stimulation of viral replication. (2) Maintenance of latency: NAC effectively blocks the cytokine-induced production of virus from latently infected cells, and may thus extend latency in minimally viremic individuals. (3) Restoration of glutathione levels in T cells: Preliminary results demonstrate that glutathione levels are restored within one week of taking oral NAC. Replenishment of glutathione may be critical for restoration of leukocyte function, by alleviating anergy. (4) Reduction of TNF levels and activity: Besides inhibiting the stimulation of HIV, thiol replacement therapy may alleviate the distressing wasting (cachexia) that often accompanies late-stage AIDS.

While many antioxidants may function in these regards, we feel that the most effective will be glutathione-replenishing pro-drugs, e.g. NAC, glutathione itself, and glutathione ester. This is because these compounds not only inhibit stimulation of HIV replication in vitro, but can also restore depleted glutathione levels in vivo. NAC has a long history of use in humans, and its safety and pharmacokinetics are well established [59–62]. The treatment of HIV infection with NAC represents an attempt to control the host-mediated stimulation of viral replication, rather than specifically aimed at HIV itself (fig. 4). This type of therapy may have many positive effects on the symptomatology of the HIV infection, e.g. reducing leukocyte anergy, immunosuppression, development of opportunistic infections, and wasting.

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