

HIV-induced cysteine deficiency and T-cell dysfunction – a rationale for treatment with *N*-acetylcysteine

W. Dröge, H-P. Eck and S. Mihm

Markedly decreased plasma cystine and cysteine concentrations have been found in HIV-infected patients at all stages of the disease and in SIV-infected rhesus macaques. The elevated glutamate levels found in the same patients aggravate the cysteine deficiency by inhibiting the membrane transport activity for cystine. The intact immune system appears to require a delicate balance between pro-oxidant and antioxidant conditions, maintained by a limited and well-regulated supply of cysteine. This balance is obviously disturbed in HIV infection and may contribute to the pathogenesis of AIDS.

The importance of thiols^{1,2} and especially of glutathione (GSH, a derivative of cysteine)^{3–5} to lymphocyte function has been known for many years. Therefore, when HIV-induced cystine (the disulfide-linked cysteine dimer) and cysteine deficiencies were discovered^{6,7}, it was tempting to speculate that this deficiency may play an important role in the immunopathology of human immunodeficiency virus (HIV) infection. As T cells have an extremely weak membrane transport activity for cystine^{8–10}, it serves as a limiting factor for T-cell function. The physiological relevance of this weak cystine transport activity may be that the activation of certain T-cell functions requires reactive oxygen intermediates (ROI), notably hydrogen peroxide. These processes are antagonized by antioxidants such as cysteine and GSH (see Table 1).

The supply of cysteine to T cells

Cystine accounts for approximately 90% of the low molecular weight cysteine in the blood plasma, while reduced cysteine is present at extremely low concentrations (15 μM) in comparison with other protein-forming amino acids. Cystine is only of limited use for T cells: the membrane transport activity for cystine and glutamate is more than tenfold lower in these cells than the transport activities for cysteine, alanine or arginine^{8–10}. As a consequence, T cells have a low baseline supply of cysteine, even under healthy physiological conditions. The weak glutamate transport is not limiting for the T cells since glutamate is produced within the cell in large quantities from glutamine. However, T cells cannot produce their own cysteine^{7,11}.

The cysteine supply to T cells is regulated by at least two further mechanisms: first, cysteine transport activity (via the ASC system) is upregulated in stimulated T cells⁹; secondly, macrophages can provide T cells with elevated extracellular concentrations of cysteine⁵. Macrophages have a relatively strong membrane transport system for cystine and, regulated by stimulating agents, can release

large amounts of reduced cysteine into the extracellular space^{5,12}. This effect might be expected to favor T cells that are intimately bound to macrophages and newly engaged in specific immune responses. However, macrophages can provide T cells with cysteine and elevate their intracellular GSH level even if the two types of cells are separated by a permeable membrane³. Because of the weak transport activity for cystine the intracellular GSH concentration of T cells is strongly influenced even by relatively moderate variations of the extracellular cysteine level^{5,7,9}. The 'cysteine supply function' of the macrophages therefore appears to be part of a mechanism that enables T cells to shift from pro-oxidant to more antioxidant conditions.

ROI, cysteine and T-cell activation

The production of superoxide anion, hydrogen peroxide and other reactive oxygen intermediates (ROI) by activated macrophages and neutrophils was originally thought to serve primarily as a nonspecific first line of defense against environmental pathogens. Recent studies suggest that ROI may also play an important role in the initiation of antigen-specific immune responses: superoxide anions and hydrogen peroxide, at physiologically relevant concentrations, augment the production of interleukin 2 (IL-2) by accessory cell-depleted T-cell populations¹³. Conversely, IL-2 production and IL-2 mRNA expression were inhibited by GSH¹⁴. Cysteine and *N*-acetylcysteine (NAC) have recently been shown to inhibit the activation of nuclear factor κB (NF- κB), a transcription factor in human T cells. These thiols also inhibit the replication of HIV that is also under the control of NF- κB ^{15,18,19}. In contrast, physiological concentrations of hydrogen peroxide induce NF- κB in a human T-cell line, indicating that ROI may be among the physiological inducers of immunologically relevant NF- κB -dependent genes^{17,20}. Several well-known substances which activate NF- κB include IL-1, tumor necrosis factor α (TNF- α), and the tumor promoter

Table 1. Some relevant ROI and cysteine derivatives

Type	Structure
Superoxide anion and hydrogen peroxide augment the expression of the IL-2 (Ref. 13) and hemeoxygenase gene ²³ and the activity of NF- κ B ¹⁷	O_2^- H_2O_2
Glutathione (GSH) Important intracellular thiol and radical scavenger. GSH peroxidase removes hydrogen peroxide	$ \begin{array}{c} \text{CO-NH-CH}_2\text{-COOH} \\ \\ \text{HS-CH}_2\text{-CH} \\ \qquad \qquad \qquad \\ \text{NH-CO-CH}_2\text{-CH}_2\text{-CH} \\ \qquad \qquad \qquad \qquad \\ \qquad \qquad \qquad \qquad \text{COOH} \end{array} $
Cysteine. Limiting precursor for GSH biosynthesis	$ \begin{array}{c} \text{COOH} \\ \\ \text{HS-CH}_2\text{-CH} \\ \\ \text{NH}_2 \end{array} $
N-acetylcysteine. Cysteine derivative with well-documented pharmacokinetics and safety ⁴⁷⁻⁴⁹	$ \begin{array}{c} \text{COOH} \\ \\ \text{HS-CH}_2\text{-CH} \\ \\ \text{NH-CO-CH}_3 \end{array} $

tetradecanoyl-phorbol acetate (TPA), also induce ROI production (reviewed in Ref. 20).

NF- κ B is involved in the inducible transcription of several immunologically important genes, including the ones encoding the IL-2 receptor α -chain and TNF- α , the major histocompatibility complex (MHC) genes, and the *c-fos* gene (reviewed in Ref. 21). Paradoxically, the DNA-binding activity of NF- κ B from nuclear extracts is augmented by thiols and inhibited by oxidation or methylation of cysteine residues²². This discrepancy is presently not understood and deserves further investigation.

T cells are not the only cells that respond to hydrogen peroxide and neither is NF- κ B the only transcription factor responsive to ROI. Hydrogen peroxide or depletion of GSH induces expression of heme oxygenase mRNA in various cell types²³, although there is no κ B-like sequence in the promoter region of the corresponding gene.

Low cystine transport activity brings with it certain hazards: it raises the probability of oxidative damage and promotes cysteine availability to a rate-limiting factor for certain T-cell functions, such as IL-2-dependent proliferation, the development of large CD8⁺ T-cell blasts, and cytotoxic T lymphocyte (CTL) activity. These functions require high intracellular GSH levels and are strongly inhibited by buthionine sulfoximine, a specific inhibitor of the GSH biosynthesis^{5,9,24}. The induction of IL-2 production, in contrast, is not inhibited even by severe depletion of intracellular GSH (Ref. 25).

Taken together, these studies indicate that certain T-cell functions are favored by ROI and inhibited by cysteine or cysteine derivatives, while others are favored by high extracellular cysteine and intracellular GSH concentrations.

The pro-oxidant-antioxidant balance in HIV-infected individuals

HIV-infected patients, at all stages of disease, have markedly elevated plasma concentrations of glutamate, markedly decreased plasma cystine and cysteine concentrations^{6,7}, and decreased intracellular GSH (Refs 7,26,27). Some HIV-infected individuals have less than one fifth of the normal levels of cysteine and/or five times the normal level of plasma glutamate. It is possible that this cysteine deficiency is responsible for the progressive destruction of the immune system in these patients. In rhesus macaques, cysteine levels begin to decrease and glutamate levels increase within one week of infection with simian immunodeficiency virus (SIV_{mac251})²⁸. The mechanism of the simultaneous dysregulation of plasma glutamate and cystine/cysteine levels is not known.

The increased plasma glutamate levels are relevant because glutamate competitively inhibits the membrane transport of cystine^{8,9,12,29}. Experiments *in vitro* using physiological amino acid concentrations and graded concentrations of extracellular glutamate have shown that even a moderate increase in extracellular glutamate levels, analogous to the high plasma levels in HIV infection, causes a substantial decrease of intracellular cyst(e)ine and GSH concentrations^{5,12}. Thus, the elevated plasma glutamate levels in HIV-infected people probably aggravate the consequences of the decreased cystine and cysteine levels and contribute to the decrease of intracellular GSH levels in these patients. Elevated extracellular glutamate levels also inhibit 'cysteine supply function' of the macrophages, inhibiting their uptake of cystine and decreasing their intracellular cysteine/cystine level¹².

The effects of cysteine on the NF- κ B activity¹⁵ suggested that a cysteine deficiency may result in over-expression of genes with κ B-like sequences in the promoter region. This is indeed the case. Abnormally high and variable concentrations of TNF- α ^{30,31}, IL-2 receptor α chain cleavage products^{32,33} and β_2 -microglobulin^{34,35} are found in HIV-infected persons, even in early stages of the disease. The elevated TNF- α levels are generally believed to contribute to the weight loss (cachexia) of these patients. Furthermore, the genome of HIV itself is under the control of the transcription factor NF- κ B^{36,37} and will also benefit from the cysteine deficiency in these patients; cysteine and NAC were found to inhibit HIV replication *in vitro*^{15,18,19}. Detailed analysis of the role of cysteine in the regulation of gene expression is the subject of ongoing research.

Another consequence of the cysteine deficiency is the decrease in intracellular GSH levels⁷. GSH levels in normal lymphocytes are bimodal. CD4⁺ and CD8⁺ T cells with high GSH levels are selectively lost as HIV infection progresses²⁷. In view of the dependency of IL-2-induced proliferation and CTL activation on GSH, it is possible that the decrease in intracellular GSH contributes to the cellular dysfunction characteristic of HIV infection^{38,39}. Like the amino acid dysregulation, this cellular dysfunction is observed in the early, asymptomatic stage of the disease³⁹. The decreased intracellular GSH levels and the resulting decreased radical-scavenging activity can explain the increased production of ROI by granulocytes during early stages of HIV infection⁴⁰. Cysteine deficiency also explains the increased production of

malondialdehyde in HIV-infected patients, a strong indicator of oxidative damage⁴¹. Since the lymphoid cells are generally endowed with a low membrane transport activity for cystine, they may be particularly vulnerable to oxidative damage.

Cysteine supply and CD4⁺ T-cell numbers

CD4⁺ T cells, *in vitro*, are markedly less sensitive to intracellular GSH depletion than CD8⁺ T cells, at least in mice²⁴. These short-term experiments reveal no connection between selective depletion of CD4⁺ T cells and decreased intracellular GSH levels. CD4⁺ T-cell depletion in HIV-infected patients is very slow, perhaps too slow to be analysed in short-term experiments *in vitro*.

Is there a connection between cysteine supply and CD4⁺ T-cell numbers *in vivo*? When groups of healthy human individuals were divided into four subgroups according to the median glutamate and median cystine levels, the subgroup with the low glutamate and high cystine levels had a significantly higher CD4⁺ T-cell count than the other three subgroups, while the mean numbers of CD8⁺ T cells were not different among the four subgroups⁴². Another study with a different cohort has shown that in the subgroup with low glutamate and high cystine levels there was a significantly higher response to a T-cell mitogen than in the other three subgroups⁴². These studies indicate that the transport of cystine into T cells does play a limiting role even in the immune system of healthy human individuals. In view of the influence of the extracellular glutamate level on the intracellular cysteine level^{9,12} and of the simultaneous correlation of CD4⁺ cell counts with glutamate and cystine levels, these findings are consistent with the idea that glutamate and cystine levels have a direct influence on immune responsiveness in healthy human individuals.

Studies with HIV-infected patients confirmed that a combination of high plasma cystine and low plasma glutamate levels yields the highest CD4⁺ T-cell count⁴²; again, CD8⁺ T-cell counts were unaffected. These findings are consistent with the hypothesis that the elevated mean glutamate levels and decreased cystine and cysteine levels in HIV-infected persons are responsible for the selective decrease of CD4⁺ T cells. However, CD4⁺ T-cell counts decline slowly and progressively with the stages of the disease, whereas glutamate, cystine and cysteine levels show strong inter-individual and intra-individual variations at all stages of the disease⁴². We propose, therefore, that the damaging immunological consequences of the occasional episodes with extremely high glutamate and/or low cystine and cysteine levels on the CD4⁺ T-cell population are essentially irreversible and accumulate over many years. There is presently no evidence that the depletion of CD4⁺ T cells may be reversible under any condition.

Elevated glutamate levels and decreased immunological reactivity in cancer patients

Patients with certain advanced malignancies have markedly elevated plasma glutamate levels and a strongly decreased lymphocyte reactivity (for a review see Ref. 43). A study of 134 persons with different types of cancers revealed a highly significant inverse correlation between individual glutamate levels and lymphocyte re-

activity⁴³. The plasma glutamate level of patients with colorectal carcinoma returns to normal levels within one week of a potentially curative surgical operation, whereas lymphocyte reactivity remains low for several months, indicating again that the metabolically induced damage of the immune system may be largely irreversible⁴⁴.

A potentially curative surgical operation is usually not possible for patients with lung cancer. In these cases it was found that mortality was significantly higher for patients with high glutamate levels than for patients with low glutamate levels⁴⁵. This correlation was particularly striking for patients with non-small cell carcinoma of the lung⁴². When the patients were divided into four groups on the basis of their median glutamate and median cystine levels, it was found that all patients who survived for more than eight months belonged to the group with high cystine and low glutamate levels, that is, to the group with a high cellular cysteine supply. All patients of the other groups died in less than nine months⁴².

In view of the causative effects of the extracellular glutamate concentrations on the intracellular cysteine level and lymphocyte functions, these findings suggest that the cellular cysteine supply may also play a causative role in the disease progression of patients with non-small cell carcinoma. This does not necessarily mean that the immune system is protective against cancer. It is quite conceivable that the decreased cysteine supply may cause an overexpression of certain cytokine genes which in turn may either stimulate the growth of lung cancer cells or (as in the case of TNF- α) contribute to the development of cachexia. Individual glutamate and cystine concentrations of lung cancer patients may prove to be a useful diagnostic tool with a high prognostic value.

Treatment of HIV-infected patients with NAC

In view of the importance of thiols^{1,2} and particularly GSH³⁻⁵ for lymphocyte function, we have suggested replenishment with cysteine or cysteine derivatives for the treatment of HIV-infected patients^{15,46}. This may be extended to certain types of cancer. NAC is favored by us: it is an established drug with well-documented pharmacokinetics and safety⁴⁷⁻⁴⁹.

Samples of NAC were supplied by us, in 1988, to colleagues in countries where oral formulations of NAC were not available. The resulting anecdotal observations by us and by others revealed that patients with manifest AIDS may improve substantially on NAC therapy but cannot be cured. The numerous additional metabolic deficiencies and immunological abnormalities in the late stages of disease may not be correctable by a single substance. However, it is possible that treatment of HIV-infected patients in the early stages of the disease with NAC may help to prevent the progression to AIDS. About 90% of the HIV-infected individuals are still in the pre-AIDS stages, and NAC is a relatively safe and inexpensive drug.

W. Dröge, H-P. Eck and S. Mihm are at the Division of Immunochimistry, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG.

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