GLUTATHIONE DEFICIENCY IN HIV INFECTION

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SUMMARY

- The tripeptide glutathione (GSH) is the quantitatively most important cysteine derivative of low molecular weight and has numerous important cellular functions. Decreased plasma cysteine and cystine concentrations, decreased intracellular GSH levels, and increased plasma glutamate levels have been found in HIV-infected persons at all stages of the disease and in rhesus macaques within 2 weeks after infection with the closely related simian immunodeficiency virus SIVmac251. Elevated glutamate levels inhibit the membrane transport of cystine and aggravate thereby the consequences of the cysteine deficiency. Complementary experiments in laboratory animals have shown that glutathione potentiates T cell functions in vivo and in vitro. And studies with healthy human subjects have shown that persons with a combination of a higher than median plasma cystine and lower than median glutamate level have significantly more CD4+ T cells than persons with low cystine and high glutamate levels. On the basis of these findings we have proposed that the immunopathology of HIV infection may be largely the consequence of a virus-induced dysregulation of plasma amino acid concentrations. Studies on the mechanistic details revealed that the cysteine and intracellular glutathione deficiency may have several immunologically relevant consequences that affect the antigen presenting cells as well as the responding T lymphocytes. The redox regulation of the transcription factor NFκB accounts at least for some of the consequences.

INTRODUCTION

- The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) which belongs to the family of non-transforming retroviruses. A closely related simian immunodeficiency virus (SIV) causes AIDS-like symptoms in rhesus macaques. The hallmarks of HIV infection are the severe depletion of the CD4+ T cell population and the cellular dysfunction that is seen in B cells and in all subsets of T cells. During the last 7 years, clinical studies and complementary laboratory experiments have provided a large body of evidence suggesting that the HIV-induced immunopathology may be largely the consequence of a virus-induced cysteine deficiency (1-5).

DYSREGULATION OF PLASMA AMINO ACIDS IN HIV-INFECTED PERSONS

- The plasma thiol levels represent mainly cysteine and - to a minor extent - glutathione. Blood plasma of healthy human subjects contains about 15 pM thiol with a range of about 10-20 pM. HIV-infected persons were found to have, on the average, thiol levels of less than 10 MM and in some cases less than 3 yM. This decrease of plasma cysteine and glutathione levels was associated with a significant decrease of intracellular glutathione both in the peripheral mononuclear cells and the monocyte population (2). HIV-infected persons have also, on the average, markedly elevated plasma glutamate levels (1-3). Some of the patients were found to have more than 5-fold the normal level. Glutamate levels are important in this context because extracellular glutamate inhibits competitively the membrane transport of cystine (6-8). Cell culture experiments have shown that a 5-fold increase of extracellular glutamate concentrations causes a substantial decrease of intracellular cysteine and glutathione levels.

THE EFFECT OF CYSTEINE AND GLUTATHIONE ON LYMPHOCYTE FUNCTIONS

- The importance of thiols (9J0) and especially of...
glutathione (11-13) for lymphocyte functions has been known for many years. The effect of glutathione in vivo on the induction of contact sensitivity in C3H mice is illustrated in Fig.1. A similar immunopotentiating effect has been observed in mice with respect to the activation of cytotoxic T cells (12). Complementary studies in vitro in lymphocyte cultures with approximately physiological amino acid concentrations in graded concentrations of cysteine have shown that several lymphocyte functions increase strongly with the extracellular supply of cysteine (2, 7, 13). Cysteine starvation causes under these conditions a marked depletion of intracellular glutathione levels and a strong decrease of DNA synthesis in T cell clones and in mitogenically stimulated ex vivo derived lymphocytes, indicating that cysteine is indeed limiting for the magnitude of the proliferative T cell response. The decrease of the cysteine concentration in the plasma of HIV-infected persons is therefore expected to affect amongst others the IL-2 dependent proliferation of the T cells.

In addition, there is evidence that the consequences of the cysteine deficiency for a given T cell in the course of an immune response may depend on the nature of the stimulator cell. This has been illustrated amongst others in experiments with 2-mercaptoethanol (2-ME). This thiol compound is being added routinely to lymphocyte cultures by many immunologists, and Bannai and colleagues have...
shown that it forms in cystine-containing culture medium a mixed cysteine/2-ME disulfide which is transported into lymphocytes much more effectively than the cysteine disulfide cystine (14). The experiment in Fig.2 illustrates that spleen cells from C3H mice generate a profound cytotoxic T cell activity against trinitrophenylated syngeneic target cells if incubated with trinitrophenylated C3H spleen cells plus interleukin-2 (upper left panel), or with trinitrophenylated semi-allogeneic C3H x DBA/2 stimulator cells in culture medium without 2-ME. When the stimulator cells are treated with UV-light, however, the responses in cultures without 2-ME is completely suppressed. In 2-ME-containing cultures all types of stimulator cells produce high and virtually indistinguishable cytotoxic T cell responses, indicating that the cysteine dependency is not only determined by the responder cell per se but also by the nature of the antigen presenting stimulator cell (Fig.2). Since macrophages have a rather strong membrane transport activity for cystine and release reduced cysteine into the extracellular space, there is clearly the possibility that this cysteine delivering function may be compromised by UV-light (13,15). These findings may be relevant with respect to the exposure of HIV-infected patients to solar UV-irradiation.

**THE REQUIREMENT OF PROOXIDANT STATES DURING THE ACTIVATION OF T LYMPHOCYTE**

- The most important physiological implication of the metabolic cooperation between macrophages and lymphocytes may be that cysteine is delivered preferentially to those T cells that are about to be recruited into a specific immune response. This mechanism discriminates against bystander lymphocytes of unrelated specificities. Moreover, there are certain aspects of the immune response that appear to require prooxidant conditions. The induction of interleukin-2 production in T cells, for example, was found to be strongly augmented by hydrogen peroxide (16). And Baeuerle and colleagues have show that the activation of the nuclear transcription factor NF-κB is facilitated by reactive oxygen intermediates (ROI) (17,18). This transcription factor is involved in the inducible transcription of several immunologically relevant genes including the genes for the interleukin-2 receptor a-chain, tumor necrosis factor a (TNFa), B2-microglobulin and c-fos (19). The overexpression of TNFa, interleukin-2 receptor oc-chain cleavage products and J-chain microglobulin has been observed in HIV infected persons (20-25) thus may also be a consequence of the virus-induced cysteine deficiency. Last not least, the HIV-proviral DNA is also known to contain two binding sites for the transcription factor NFκB. As expected from the effect of cysteine on NFκB activity, we found that cysteine and N-acetyl-cysteine inhibit the replication of HIV in a dose-dependent fashion (26).

**CONCLUSIONS AND CLINICAL IMPLICATIONS**

- Since certain aspects of T cell mediated immune responses require prooxidant conditions while others require antioxidant conditions and relatively high cysteine and glutathione levels, we hypothesized (i) that the func-
tion of the immune response may require a delicate balance between prooxidant conditions, and (ii) that the metabolic cooperation between macrophages and lymphocytes may allow the lymphocyte to switch from prooxidant to antioxidant conditions in the course of the immune response. This delicate balance between prooxidant and antioxidant conditions is apparently disturbed in HIV-infected persons by the HIV-induced cysteine deficiency and increased plasma glutamate levels. The cysteine deficiency and the decrease of intracellular glutathione levels are expected to affect primarily the IL-2-dependent proliferation and may contribute to the cellular dysfunction that is observed already in the very early stages of HIV infection. In addition, the cysteine deficiency is also expected to cause an overactivation of functions that are favored by prooxidant states and may be responsible for an abnormal expression of certain cytokine genes that are under the control of NF-kB. The overexpression of TNFα is widely believed to contribute to the progressive loss of muscle mass (cachexia) in these patients.

In view of the important role of cysteine in the immune system and in view of the various consequences of a cysteine deficiency for lymphocyte functions, we have proposed to consider cysteine or cysteine derivatives such as N-acetyl-cysteine (NAC) for the treatment of HIV-infected persons. The first anecdotal observations by us and by others revealed that patients with manifest AIDS may improve substantially on NAC therapy but cannot be cured. Apparently, there are already too many irreversible metabolic deficiencies and immunological abnormalities in the late stages of the disease that cannot be corrected by this treatment. It is possible, however, that treatment of HIV-infected persons in the early stages of the disease with a cysteine derivative may prevent the devastating effects of HIV infection and the progression to full blown AIDS. About 90% of HIV-infected individuals are in the pre-AIDS stages. And N-acetyl-cysteine is a safe and inexpensive drug with well documented pharmacology and pharmacokinetics.

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