Biomedical Reviews 2: 9-13 (1993) Published by the Bulgarian - American Center, Varna, Bulgaria

GLUTATHIONE DEFICIENCY IN HIV INFECTION

W Droge, H-P Eck, S Roth, and S Mihm
Division of Immunochemistiy, Deutsches Krebsforschungszentrum, Heidelberg, Germany

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 SIV_{mac}2$]- 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 NFkB 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-INFECT-ED 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 piM. 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 LYMPHO- CYTE FUNCTIONS

• The importance of thiols (9JO) 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.l. 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. . . .

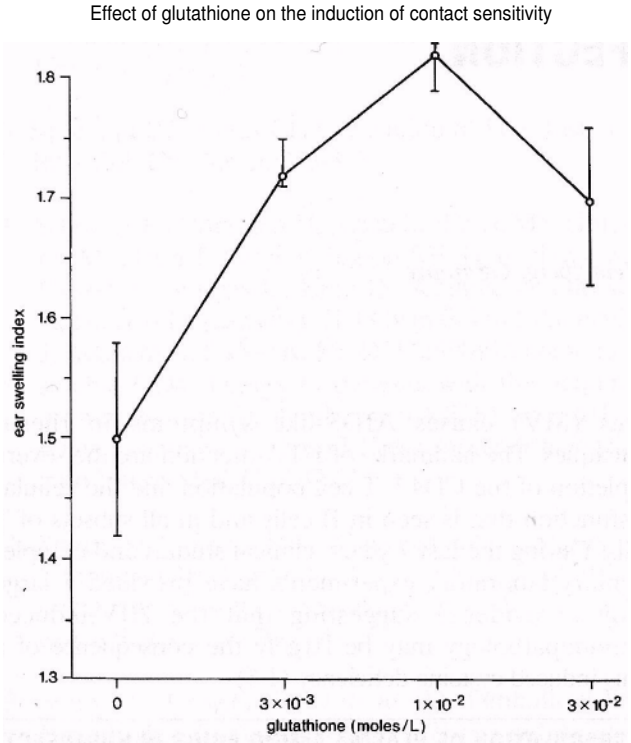

Figure 1. Effect of glutathione on the induction of contact sensitivity.
Two-month-old male C3Hmice were immunized by skin painting with 6x10 \d 5% TNCB on the four footpads and two abdominal sites (day 0). The mice received also ip. injections of 1 ml BSS containing the indicated concentrations (GSH) on day 0 and on day 2. Five days after immunization, the mice were painted on both sides of the left ear with 2x10 \jl3% TNCB in acetone, and the are swelling index was determined 1 day later.

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

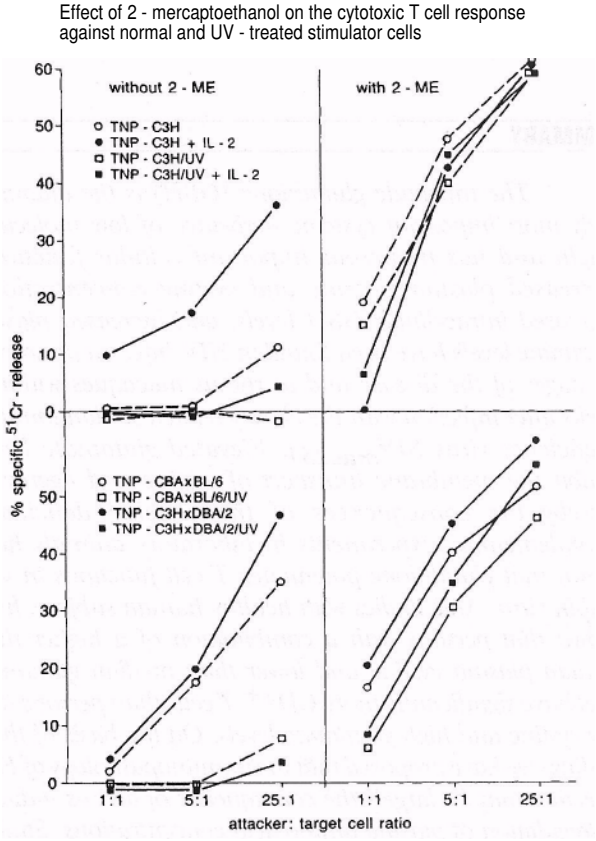

Figure 2. Effect of 2-mercaptoethanol on the cytotoxic T cell response against normal and UV-treated stimulator cells.

Spleen cells (2x10 [7]) from 2-month-old male C3H mice were incubated with 5x106 irradiated (1500 rad) and trinitrophenylated C3H spleen cells with or without interleukin-2 (1,3 U/ml) and with or without 3x10' M 2-mercaptoethanol (2-ME) in RPMI1640 medium plus 10% fetal calf serum. Some of the cultures were stimulated with trinitrophenylated (CBA x BL/6) Fl cells, and some of the cultures were stimulated with trinitrophenylated cells which had been treated with UV-light for 15 min prior to the culture.*

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 respons 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.

CYSTEINE AS A REGULATORY MEDIATOR IN T CELL-MEDIAT- ED IMMUNE RESPONSES

• Macrophages are the antigen presenting cells *par excellence.* They process and present antigenic fragments in the context of their major histocompatibility antigens to the specific antigen receptors of the responding T limphocytes. In the process of antigenic stimulation, the T cell receptor binds to the antigen on the macrophage surface and causes thereby an intimate contact between the plasma membranes of the macrophage and the responding lymphocyte. Macrophages take this opportunity to deliver several regulatory mediators to the lymphocyte including the hormone-like cytokines interleukin-1 and TNF . In addition, we have provided evidence that cysteine plays a role similar to that of the cytokines, since it is also produced by the macrophage at a variable and regulated rate and modulates the function of the responding T cell. This metabolic cooperation is based on several key facts. Firstly, most of the cysteine is present in the blood plasma mainly in its oxidized form cystine. The cystine concentration in the plasma is equivalent to about 150 pM cysteine. T-lymphocytes, however, have only a very weak membrane transport activity for this relatively large amino acid (6,7) and can hardly take advantage of this relatively abundant extracellular source of cysteine. Lymphocytes have a relatively strong membrane transport activity for the smaller amino acid cysteine and other small neutral amino acids. However, the plasma concentration of cysteine is only about 15 ijM, which is one of the lowest concentrations among all protein forming amino acids. Macrophages, in contrast to lymphocytes, have a rather strong membrane transport activity for cystine (6,7) and take up more cystine than they need for their own metabolism. The excess is reduced intracellularly and released into the extracellular space. This enables the macrophage to provide adjacent lymphocytes with considerably higher extracellular concentrations of cysteine and to increase thereby their intracellular glutathione levels (13). Elevated extracellular concentrations of glutamate, as they are found in the plasma of HIV-infected persons, inhibit competitively the uptake of cystine and the release of cysteine into the extracellular space (13).

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 interleuken-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 NFicB 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~$^{m}i^{cr\circ}$gl°bulin and c-fos (19). The overexpression of TNFa, interleukin-2 receptor oc-chain cleavage products and J^-microglobulin that 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 NFicB. As expected from the effect of cysteine on NFicB activity, we found that cysteine and N-acetyl-cysteine inhibit the replication of HIV in a dose-dependent fashion (26).

CONCLUSIONS AMD 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 limphocyte 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 NFicB. The overexpression of TNFoc 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.

ACKNOWLEDGEMENTS

• The assistance of Mrs. I. Fryson in the preparation of this manuscript is gratefully acknowledged.

REFEREMCES

1. Droge W, Eck H-P, Naeher H, Pekar U, Daniel V (1988) Abnormal amino acid concentrations in the blood of patients with acquired immunodeficiency syndrome (AIDS) may contribute to the immunological defect. Biol Chem Hoppe-Seyler 369:143-148

2. Eck H-P, Gmiinder H, Hartmann M, Petzoldt D, Daniel V, Droge W (1989) Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol Chem Hoppe-Seyler 370:101-108

3. Eck H-P, Mertens T, Rosokat H, Fatkenheuer G, Pohl C, Schrappe M, Daniel V, Naher H, Petzoldt D,Drings P, Droge W (1992) $T4^+$ cell numbers are correlated with plasma glutamate and cystine levels: association of hyperglutamataemia with immunodeficiency in diseases with different aetiolgies. Int Immunol 4:7-13

4. Eck H-P, Stahl-Hennig C, Hunsmann G, Droge W (1991) Metabolic disorder as early consequence of simian immunodeficiency virus infection in rhesus macaques. Lancet 338:346-347

5. Droge W, Eck H-P, Mihm S (1992) HIV-induced cysteine deficiency and T-cell dysfunction - a ratinale for treatment with N-acetyl-cysteine. Immunol Today 13:211-214

6. Watanabe H and Bannai S (1987) Induction of cystine transport acitivity in mouse peritonealmacrophages. J Exp Med 165:628-640.

7. Gmiinder H, Eck H-P, Droge W (1991) Low membrane transport activity for cystine in resting andmitogenically stimulated human lymphocyte preparations and human T cell clones. Eur J Biochem 201:113 117

8. Eck H-P and Droge W (1989) Influence of the extrcellular glutamate concentration on the intracellular cysteine concentration in macrophages and on the capacity to release cysteine. Biol Chem Hoppe-Seyler 370:109-113

9. Fanger MW, Hart DA, Wells JV, Nisonoff A (1970) Enhacement by reducing agents of the transformation of human and rabbit peripheral lymphocytes. J Immunol 105:1043-1045

10. Cerottini J-C, Engers HD, MacDonald HR, Brunner KT (1974) Generation of cytotoxic T limphcytes *in vitro.* I. Response of normal and immune mouse spleen cells in mixed leucocyte cultures. J Exp Med 140:703-717

11. Hamilos DL, and Wedner HJ (1985) The role of glu-

tathione in lymphocyte activation. I. Comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1-one by nuclear size transformation. J Immunol 135:2740-2747

12. Droge W, Pottmeyer-Gerber C, Schmidt H, Nick S (1986) Glutathione augments the activation of cyto toxic T lymphocytes *in vivo.* Immunobiol 172: 151-156

13. Grminder H, Eck H-P, Benninghoff B, Roth S, Droge W (1990) Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. Cell Immunol 129:32-46

14. IshiiT, Bannai S, Sugita Y (1981) Mechanism of growth stimulation of L1210 cells by 2-mercaptoethanol *in vitro.* Role of the mixed disulfide of 2-mercaptoethanol and cysteine. J Biol Chem 256: 12387-12392

15. Droge W, Eck H-P, Gmiinder H, Mihm S (1991) Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivtives. Am J Med 91 (suppl 3C):140S-144S

16. Roth S and Droge W (1987) Regulation of T-cell growth factor (TCGF) production by hydrogen peroxide. Cell Immunol 108:417-424

17. Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NFKB transcription factor and HIV-1. EMBO J 10:2247-2258

18. Schreck R, Meier B, Mannel DN, Droge W, Baeuerle PA (1992) Dithiocarbamates as potent inhibitors of NFkB activation in intact cells. J Exp Med 175:1181-1194

19. Ullmann KS, Northrop JP, Verweij CL, Grabtree GR (1990) Transmission of signals from the T lymphocyte antigen receptor to the genes 18 responsiblefor cell proliferation and immune function: the missing link. Annu Rev Immunol 8:421-452

20. Lahdevirta J, Maury CPJ, Teppo A-M, Repo H (1988) Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodefciency syndrome. Am J Med 85:289-291

21. Scott-Algara D, Vuillier F, Marasescu M, de Saint Martin J, Dighiero G (1991) Serum levels of IL- 2, IL-la, TNFa and soluble receptor of IL-2 in HIV-1-infected patients. AIDS Res Hum Retrovir 7:381-386

22. Kloster BE, John PA, Miller LE, Rubin LA, Nelson DL, Blair DC, Tomar RH (1987) Soluble interleukin 2 receptors are elevated in patients with AIDS or at risk of developing AIDS. Clin Immunol Immunopathol 45:440-446

23. Prince HE, Kleinman S, Williams AE (1988) Soluble IL-2 receptor levels in serum from blood donors seropositive for HIV. J Immunol 140:1139-1141

24. Fahey JL, Taylor JMG, Detels R, Hofmann B, Melmed R, Nishanian P, Giorgi JV (1990) The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type I. New EnglJMed322:166-172

25. Osmond DH, Shiboski S, Bacchetti P, Winger EE, Moss AR (1991) Immune activation markers and AIDS prognosis. AIDS 5:505-511

26. Mihm S, Ennen J, Pessara U, Kurth R, Droge W (1991) Inhibition of HIV-1 replication and NFkB activity by cysteine and cysteine derivatives. AIDS 5:497-503

Received for publication 30 March 1993

Address for correspondence:
Prof. Dr W. Droge
Division of Immunochemistry,
Deutsches Krebsforschungszentrum
im Neuenheimer Feld 280
D-6900 Heidelberg J, GERMANY