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Dysregulation of plasma amino acid levels in HIV-infection and cancer and its relevance for the immune system

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Summary. T cells have a weak membrane transport actitivity for cyst*i*ne but strong transport activity for cyst*ei*ne. Even moderate variations of the cysteine concentration affect T cell functions in spite of the high concentration of cystine in cultures with physiological amino acid concentrations. The IL-2 dependent DNA synthesis and the activation of cytotoxic T cells are positively regulated by cysteine, while the activity of the transcription factor NFκB and the production of IL-2 are stimulated by active oxygen species and inhibited by cysteine or GSH. Macrophages, in contrast to T cells, take up more cystine than they need and release the excess after intracellular reduction as cysteine into the extracellular space. This "cyst*ei*ne pumping activity" of macrophages raises intracellular GSH levels and DNA synthesis of T cells in the vicinity. The difference between the cystine transport activities of T cells and macrophages, therefore, enables T cells to switch between prooxidant and antioxidant states. The "cysteine pump" favors selectively the antigen-specific T cells that are about to be stimulated by antigen-presenting macrophages. The capacity of macrophages to take up cystine and to release cysteine is inhibited, however, by elevated extracellular glutamate concentrations. Elevated plasma glutamate levels have been found in several pathological conditions including cancer and HIV-infection. In HIV-infected patients, the hyperglutamataemia is aggravated by hypocystinaemia and hypocysteinaemia. Our studies, therefore, suggest that the cysteine supply is impaired in several pathological conditions with immunodeficiencies including AIDS. N-acetyl-cysteine (NAC) is a safe and well established drug that may be considered for the treatment of patients with HIV-infection.

Keywords: Amino acids – HIV-induced cysteine deficiency – HIV-induced glutathione deficiency – Cysteine, role in AIDS – Glutamate, role in cancer and AIDS – N-Acetyl-cysteine as a treatment of HIV-infection

Introduction

HIV-infected patients at all stages of the disease were found to have, on the average, markedly elevated plasma glutamate and decreased plasma cystine and cysteine concentrations (Dröge et al., 1988b; Eck et al., 1989b) and decreased intracellular glutathione (GSH) levels (Eck et al., 1989b). Decreased GSH levels have been found subsequently also by other laboratories (Buhl et al., 1989; Roederer et al., 1991). Elevated glutamate levels are expected to aggravate the cysteine deficiency in HIV-infected persons, since glutamate inhibits competitively the membrane transport of cystine (Watanabe and Bannai, 1987; Bannai, 1986; Makowske and Christensen, 1982; Takada and Bannai, 1984; Hishinuma et al., 1986). Even a moderate increase of extracellular glutamate levels was found to cause a substantial decrease of intracellular cysteine levels (Gmünder et al., 1991b; Eck and Dröge, 1989a) and to inhibit lymphocyte functions (Dröge et al., 1988b; Dröge et al., 1988a) in cultures with otherwise approximately physiological amino acid concentrations.

The conclusion that the dysregulation of plasma amino acid levels is indeed the consequence of retroviral infection was supported also by the observation that plasma glutamate levels increase and cysteine levels start to decrease within 1 week after inoculation of SIV_{mac} into rhesus macaques (Eck et al., 1991). In this review we describe the available evidence showing that cysteine has both positive and negative regulatory effects on the immune system and that macrophages play a key role in regulating the supply of cysteine to the responding T cells.

The limiting baseline supply of cysteine in T cells

The weak membrane transport activity for cystine in T cells is the key element of a mechanism that ensures a limited baseline supply of cysteine to T cells and that allows T cells to switch between prooxidant states and antioxidant states. The major cysteine derivative in the blood plasma is the disulfide cystine (120–160 μM 1/2 cystine), while the concentration of reduced cysteine (10–20 μM) is extremely low in comparison with other amino acids. Studies with a large series of human and murine T cell clones and T cell tumors and ex vivo derived lymphocyte preparations showed, however, that T cells and T cell tumors have generally a strong transport activity for cysteine and only a weak transport activity for the amino acids cystine and glutamate (Gmünder et al., 1991a) which share the same transport system (Watanabe and Bannai 1987; Bannai, 1986; Makowske and Christensen, 1982; Takada and Bannai, 1984; Hishinuma et al., 1986). These observations confirmed and extended earlier studies with unfractionated murine spleen cell populations (Ishii et al., 1987).

Evidence that the cysteine supply is indeed a *limiting* factor that determines the magnitude of T cell *functions* is based mainly on laboratory experiments in vitro. It has been known for almost two decades that both T cell and B cell responses can be strongly augmented in vitro by high concentrations of cystine, cysteine or other sulfhydryl compounds such as 2-mercaptoethanol (2-ME). However, culture systems with approximately physiological amino acid concentrations have been studied only recently (Eck et al., 1989b; Gmünder et al., 1990a).

These studies revealed i) that T cell clones, T cell tumors and ex vivo derived T cells and cytotoxic T lymphocytes are influenced even by relatively small variations of the extracellular cysteine concentration in the physiological range and ii) that T cells usually fail to respond to comparable variations of the extracellular cystine concentration (Eck et al., 1989b).

Variations of the intracellular concentration of the cysteine derivative glutathione (GSH) affect some but not all T cell functions and T cell subsets

The biochemical and functional parameters of T cells that are most strongly affected by cysteine starvation are the intracellular GSH level and the rate of DNA synthesis (Eck et al., 1989b; Gmünder et al., 1990a; Ishii et al., 1987). It was, therefore, of interest to determine the importance of the intracellular GSH level for different T cell functions and T cell subsets. A detailed analysis showed that treatment of ex vivo derived T cell preparations and T cell clones with buthionine sulfoximine, a specific inhibitor of GSH biosynthesis, inhibits the activation of cytotoxic T lymphocytes and the IL-2 dependent DNA synthesis of ex vivo derived T cells and most (but not all!) T cell clones (Gmünder et al., 1990a; Gmünder et al., 1990b). Buthionine sulfoximine has practically no influence on the BLT-esterase activity and perforin activity which is associated with cytotoxic T cell activity (Gmünder et al., 1990a). In contrast to the cysteine and GSH dependent function of DNA synthesis and cytotoxic T cell activation, IL-2 production is not inhibited even by severe depletion of intracellular GSH (Gmünder et al., 1990b). Moreover, it was found that $CD8^+$ T cells are markedly more sensitive against intracellular GSH depletion than $CD4^+$ T cells (Gmünder and Dröge, 1991a). These results suggest that the decrease of intracellular GSH levels in HIV-infected patients may contribute to the cellular dysfunction but not to the selective loss of $T4^+$ cells in these patients.

The "cysteine pumping activity" as a novel immunoregulatory function of macrophages

Macrophages are important stimulator and accessory cells in T cell-mediated immune responses and come into intimate contact with these cells in the course of antigen presentation and stimulation. In contrast to lymphocytes, macrophages have a rather strong transport activity for cystine (Watanabe and Bannai, 1987), and they take up more cystine than they need for their own metabolism (Eck and Dröge, 1989a). The excess of cystine is reduced intracellularly and released in the form of reduced cysteine at a variable and regulated rate (Gmünder et al., 1990a; Eck and Dröge, 1989a). Stimulation of macrophages with bacterial lipopolysaccharide (LPS) or TNFα strongly augments the amount of cysteine released (Gmünder et al., 1990a). Antigen-specific T cells which are about to be stimulated by antigen presenting macrophages are thus provided with an additional supply of cysteine. This "cysteine pumping activity" is even demonstrable in double chamber experiments when macrophages are separated from lymphocytes by a porous membrane (Gmünder et al., 1990a). In this experimental system macrophages have been shown to increase the intracellular

GSH level and the rate of DNA synthesis of activated T cells. However, the uptake of cystine by macrophages and their capacity to release cysteine into the extracellular space is markedly inhibited even by moderately elevated extracellular glutamate concentrations (Eck and Dröge 1989a; Gmünder et al., 1991b). This effect is associated with a substantial decrease of intracellular cyst(e)ine levels within the macrophages (Eck and Dröge, 1989a). In view of the increased mean plasma glutamate concentrations in HIV-infected persons and cancer patients, this effect is believed to be clinically important.

The importance of a prooxidant state in the course of *T* cell activation and the characterization of disadvantageous effects of cysteine and cysteine derivatives

The limited baseline supply of cysteine for T cells and the "cysteine pumping activity" of the macrophages serve the purpose that cysteine is delivered preferentially to antigen specific T cells that bind to antigen presenting macrophages in the course of an antigen specific immune response. Importantly, however, this mechanism also enables T cells to acquire a prooxidant state at certain times during T cell activation and to switch between prooxidant and antioxidant states.

That certain aspects of T cell mediated immune responses require indeed a prooxidant state and are inhibited by antioxidant substances is suggested by several lines of evidence. Roth and Dröge reported that the production of interleukin-2 is augmented by superoxide anion or hydrogen peroxide at the physiologically relevant concentration of 10 μM (Roth and Dröge, 1987). In line with these findings, IL-2 production and IL-2 mRNA expression were found to be inhibited by the administration of GSH (Roth and Dröge, 1991). Dröge and colleagues have also shown that cysteine and cysteine derivatives inhibit the induction of the transcription factor NFκB (Mihm and Dröge, 1990; Mihm et al., 1991), and similar effects have been reported for N-acetyl-cysteine by different laboratories (Mihm et al., 1991; Staal et al, 1990; Schreck et al., 1991). More recent studies have shown that physiological concentrations of hydrogen peroxide (10–50 μM) induce NFκB, indicating that active oxygen derivatives may be the physiological inducers of immunologically important NFκB-dependent genes (Schreck et al., 1991). There is complementary evidence that tumor necrosis factor α (TNFα) and the tumor promoter tetradecanoylphorbolacetate (TPA) induce the endogenous production of active oxygen derivatives (Rosen and Freeman, 1984; Matsubara and Ziff, 1986; Klebanoff et al., 1986; Meier et al., 1989; Meier et al., 1990). It is, therefore, reasonable to assume that certain aspects of the T cell-mediated immune response require a prooxdidant state and are inhibited by antioxidant substances such as cysteine and cysteine derivatives.

The transcription factor NFκB is involved in the inducible transcription of immunologically important genes including the gene for the interleukin-2 receptor α-chain, TNFα and last not least the gene of the human immunodeficiency virus HIV-1 (Cross et al., 1989; Collart et al., 1990; Osborn et al., 1989; Rosenberg and Fauci, 1989). The cysteine deficiency in HIV-infected patients (Dröge et al., 1988b; Eck et al., 1989) provides therefore an explanation for the relatively high

systemic concentrations of interleukin-2 receptors and TNFα in these patients (Reddy and Grieco, 1988; Lahdevirta et al., 1988). It was also shown that the replication of HIV-1 is inhibited in latently infected cells (Mihm et al., 1991) and acutely infected cells (Roederer et al., 1990) by cysteine or N-acetyl-cysteine. Other putative NFκB binding sites have been found to be associated with the genes for interferon-β, interferon-γ, IL-2, IL-3, IL-4, IL-6, GM-CSF, genes of the major histocompatibility complex, and c-fos, some of which are induced during T cell activation (reviewed in Ullmann et al., 1990).

The weak membrane transport activity of T cells for cystine and the corresponding low baseline supply of cysteine may thus be essential to ensure this prooxidant state. And the "cysteine pumping function" of the macrophages may be important to terminate the prooxidant state and to prevent oxidative damage in these cells. The "cysteine pumping function" optimizes in particular the cysteine and GSH dependent functions including the IL-2 dependent DNA synthesis. In view of these requirements for prooxidant and antioxidant states for different aspects of the T cell mediated immune response, we propose that the strength of the immunological reactivity may depend decisively on the strength of the prooxidant state, which is particularly well expressed in the inflammatory environment, and by the capacity of the macrophages to shift the T cells from the prooxidant state back to the antioxidant state which saves them from oxidative damage and supports the IL-2 dependent DNA synthesis.

Clinical implications

Elevated plasma glutamate levels as they are found in HIV-infected persons and patients with advanced malignancies compromize this cysteine pumping function of the macrophages and may, therefore, play a key role in the immunopathology of HIV-infection and malignancies. Studies with cancer patients revealed indeed a highly significant inverse correlation between individual glutamate levels and lymphocyte reactivity (Dröge et al., 1988a). Preliminary evidence suggests that the immunological damage that is caused by the dysregulation of plasma amino acid levels may be long-lasting (Eck et al., 1990). We therefore propose that the progressive deterioration of the T cell system in HIV-infected patients during the rather long and variable latency period is caused by a series of consecutive episodes with extremely high glutamate and/or low cystine and cysteine levels, the consequences of which may accumulate over time.

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