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Review

Intracellular Glutathione in Cellular Immunology

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ABSTRACT

Glutathione is the most abundant intracellular tripeptide thiol and acts as a reducing agent and an antioxidant. Glutathione may regulate many enzymatic and pharmacological activities by changing the intracellular redox condition. Recent studies have accumulated evidence for important roles of glutathione in full competence of lymphocytes for such as activation, proliferation and induction of cytotoxicity. A decrease in intracellular glutathione may cause malfunction of lymphocytes, immunosuppression and apoptosis. A variety of immunological functions are determined on the basis of the balance between synthesis and consumption of intracellular glutathione.

Key words: Glutathione, T cell, Signal transdnction, Apoptosis

INTRODUCTION

Glutathione (GSH), γ-glutamyl-cysteinyl glycine, is composed from three amino acids, glutamate, cysteine and glycine. Among the amino acids, cysteine is the most important in terms of the synthesis and function of tripeptides. Intracytoplasmic GSH functions as a scavenger of free radicals, a reducing agent, an antioxidant and transport of amino acids by using the sulfhydryl residue of cysteine. By donating the proton, GSH is able to regulate intracytoplasmic reducing and oxidative conditions (redox). Recent studies demonstrate that many physiological functions of lymphocytes are also subject to the redox condition. Intracellular superoxide dismutase (SOD), thioredoxin/ATL-derived factor (ADF) and the other catalytic enzymes may retain similar functions as GSH in the regulation of the redox condition(1). This review is focused on the significance of intracellular glutathione in various immunological functions including activation, signal transduction, proliferation, differentiation and cell death (apoptosis).

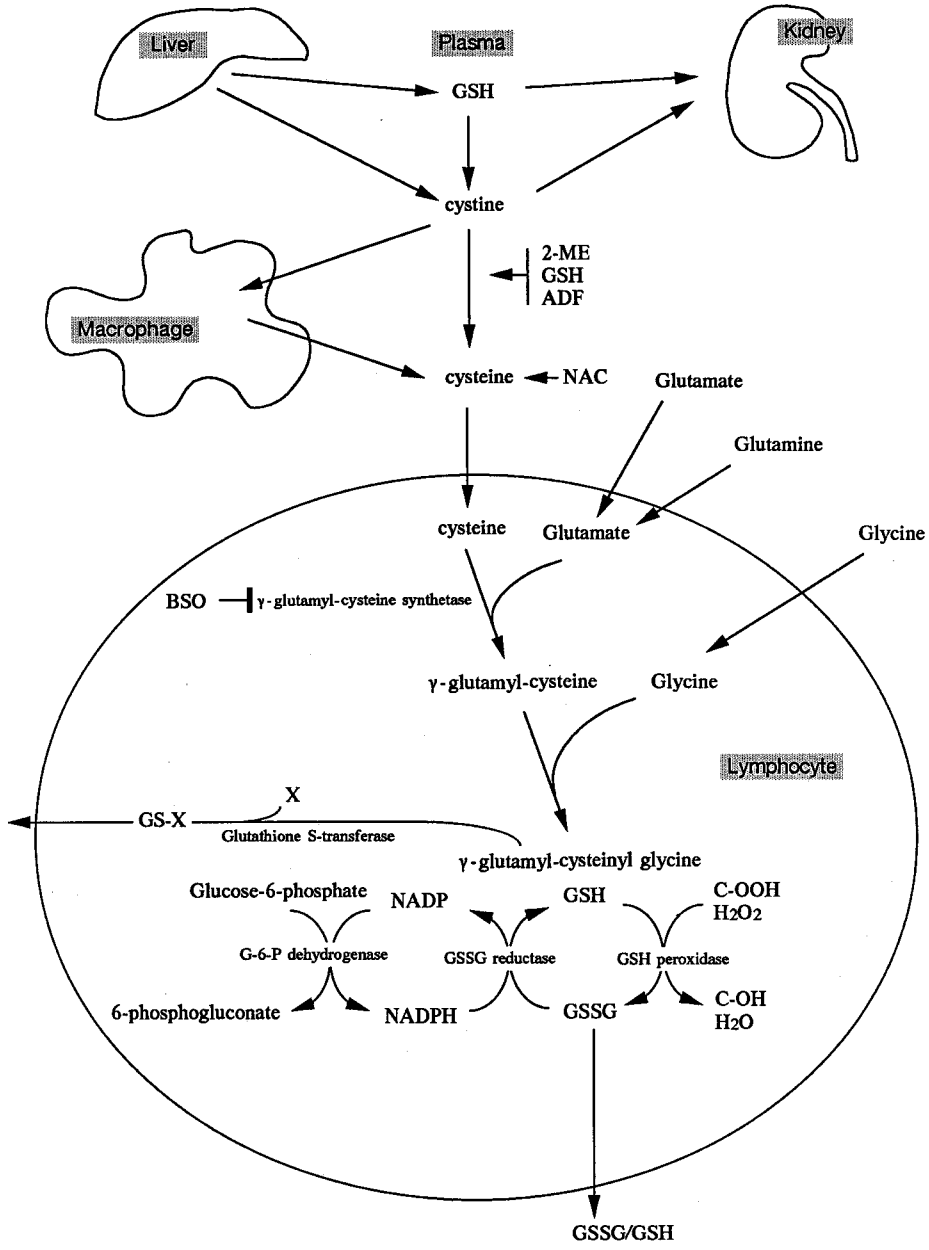

Fig. 1 The regulation of intracytoplasmic glutathione in lymphocytes. See text for detail.

GLUTATHIONE METABOLISM IN LYMPHOCYTES.

In vivo, most of the plasma GSH is provided by the liver(2, 3). Lymphocytes lack the ability to synthesize cysteine and little GSH can be taken up directly by the cells because of weak membrane transport activity for cystine (disulfide-bonded form of cysteine) and GSH(4). Accordingly, lymphocytes need to take up extracellular cysteine to produce intracellular GSH (Fig. 1) and are dependent on the enzymatic synthesis of GSH to increase intracytoplasmic GSH.

γ-glutamyl-cysteine synthetase is the limiting enzyme in various steps of the GSH synthesis and appropriate supply of cysteine is necessary as a substrate for the enzyme. Cystine is mostly reduced to cysteine by the reducing conditions in cytoplasm, while in contrast, the serum cystine concentration is much higher than that of cysteine. Thus, the presence of reducing agents in the extracellular environment is important for the efficient transport of cysteine into the lymphocytes (3).

Elucidation in GSH synthesis has been eased as it became possible both in vivo and in vitro to manipulate the GSH content in cells. Intracellular GSH can be decreased by blocking γ-glutamyl-cysteine synthetase with buthionine sulfoximine (BSO) or by decreasing the supply of cysteine(3). There are several methods to increase intracellular GSH: the reducing agents N-acetyl cysteine (NAC), GSH, and 2-mercaptoethanol are able to increase intracellular GSH synthesis by reducing extracellular cystine into cysteine. L-2-oxothiazolidine-4-carboxylate (OTC) also delivers cysteine into cells. Some of these methods may be used for experimental and therapeutic purposes for changing the systemic or intracellular GSH(3).

The intracellular ratio between the reduced (GSH) and oxidized (GSSG) forms of glutathione is subject to the activity of various enzymes, such as glutathione peroxidase and glutathione reductase. Under normal conditions, glutathione reductase is active and most of the intracellular glutathione is the reduced form (GSH). However, accumulation of oxidants in the cytoplasm may cause unbalanced production of GSSG, which can be transferred to outside the cells, resulting in a drop in intracellular GSH(3). Glutathione S-transferase can conjugate GSH with various drugs and oxidative substrate and the GSH-conjugated substrates can be transferred out of cells as well. It has been shown that the activated macrophages were able to excrete cysteine and increase intracytoplasmic GSH in lymphocytes near by(5). Thus, macrophages not only present antigens and produce lymphokines, they are also local feeders of cysteine to lymphocytes.

GLUTATHIONE IN LYMPHOCYTE ACTIVATION.

Recent studies have demonstrated that appropriate amount of intracellular GSH is essential in full activation in T-lymphocytes(6—11). T cells activated by mitogen stimulation such as anti-CD3 antibodies, phytohemagglutinin (PHA) and pokeweed mitogen (PWM), caused blastic transfomation. When T cells were incubated with BSO before activation with mitogens, T cells could not induce full activation to proliferate, this was true both for T cells with artificially reduced GSH by BSO and for T cells sorted on the basis of GSH content(7, 8). T cells with low GSH levels were partially activated to increase in nuclear size and were able to secrete IL-2 and express IL-2 receptor, suggesting that a shift from G0 to G1 phase was not affected by BSO(7, 9, 10, 12).

Similar results were obtained by culturing the lymphocytes in L-cystine- and GSH-free media, though the expression of transferrin receptor under GSH-depleted condition was varied among reports(12, 13). Those studies consistently demonstrated that the GSH-depleted lymphocytes failed to synthesize new DNA and RNA resulting in late G1 arrest. G1 arrested T cells did not recover after the addition of exogenous IL-2; thus G1 arrest may not attribute to the failure of production of lymphokines(7, 9, 11). However, the removal of BSO from the tissue culture reversed the inhibitory effects on DNA and GSH synthesis. The GSH levels of normal lymphocytes may be decreased less than 5% of the control values by BSO treatment for 24 hours and the lymphocytes may restore most of the GSH level by removing BSO within 24 hours. That explains why G1 arrest is specifically opposed by low levels of GSH and is rapidly reversed by restoration of GSH(7, 9, 11). The studies have demonstrated that GSH is not required for the G1 activation of lymphocytes but is necessary for transition from G1 to S phase.

GSH IS NECESSARY IN CYTOTOXIC T CELL FUNCTION.

As mentioned above, GSH-depleted lymphocytes do not proliferate in response to mitogens and when the intracellular GSH levels are restored, the lymphocytes regain the ability to respond to IL-2. Thus intracellular GSH is necessary for lymphocytes to proliferate.

Recent studies have demonstrated that GSH is vital to certain functions of T cells as well as their proliferation. In the human system, GSH was required to induce LAK activity and NK cells(14). In addition, the activity of granzyme A, an enzyme necessary for cytolysis, was markedly reduced under GSH-depleted condition. In an experimental model, the cytotoxicity of LAK cells was markedly reduced in the presence of BSO and was enhanced by NAC(15). In vivo

experiments in mice administered with N-acetyl cysteine to increase systemic GSH showed a significant decrease in tumor progression; a small number of mice had complete tumor regression(15). This was most likely due to the selective enhancement of the cytotoxic function of T cells by the increase of GSH. The data demonstrate the importance of GSH in the induction of cytotoxic function of lymphocytes.

GSH AFFECTS INTRACELLULAR SIGNAL IN LYMPHOCYTES.

It has been shown that intracytoplasmic GSH may regulate the activity of various enzymes involved in signals from surface receptors (Fig. 2). As stated above, GSH is necessary for T cells to induce full activation to start proliferation by various mitogenic stimulation. Stimulation of T cell receptor/CD3(TcR/CD3) complex has been shown to induce various intracytoplasmic changes including tyrosine phosphorylation and calcium flux leading to activation of various kinase cascades, such as protein kinase C. In GSH-depleted T cells by BSO, there were no apparent changes in tyrosine phosphorylation by crosslinking of TcR/CD3; however, subsequent calcium flux was markedly reduced(16). In contrast, tyrosine phosphorylation mediated by the stimulation of tumor necrosis fac-

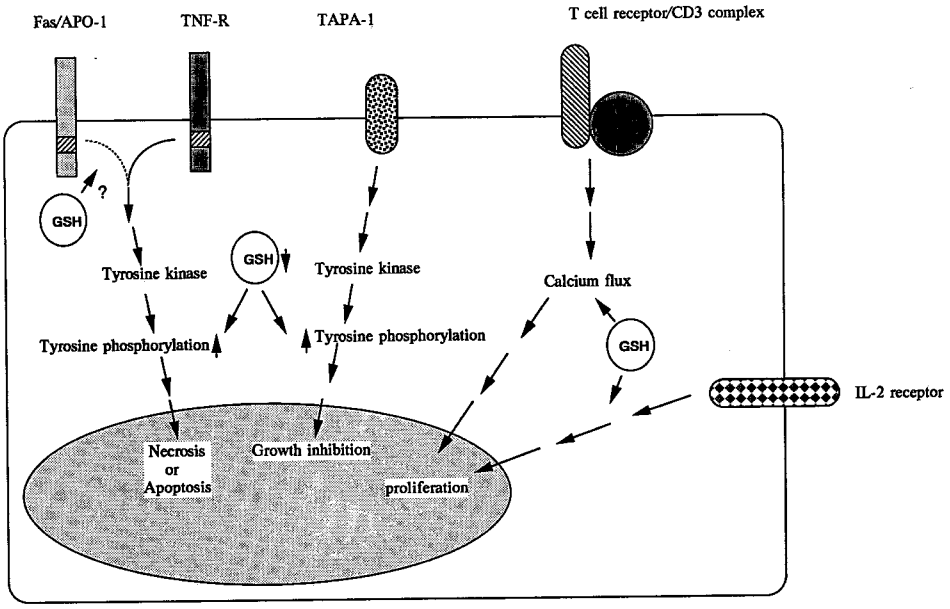

Fig. 2 The biological effects of intracytoplasmic glutathione on various signals. See text for detail.

tor receptor (TNFR) was markedly enhanced in the GSH-depleted T cells. These results indicate that certain types of tyrosine phosphorylation may be selectively regulated by the redox condition in the T cells. There was a report showing that the calcium flux of T cells by mitogens, such as anti-CD3 antibodies and PHA, was unaffected in a thiol-depleted medium(13). The discrepant results regarding to the calcium flux might be attributable to the effects of BSO. Though the results about the effects of GSH-depletion on the signal transductions are presently inconclusive, most results suggested that the depletion of intracellular GSH may contribute to the incompetence of immunological function and enhancement of inflammatory reaction.

CD81, also known as TAPA-1, is associated with Leu-13 and forms a multimolecular complex which is important in signal transduction of CD19/CD21 in B cells(17, 18, 19). Tyrosine phosphorylation in GSH-depleted lymphocytes induced by anti-TAPA-1 antibodies was enhanced and the antiproliferative effect was increased (Fig. 2). On the other hand, GSH-increased lymphocytes showed reduced tyrosine phosphorylation and resistance to the antiproliferative effect of the antibodies(20). Although the antiproliferative effects of CD81 were reversible and were not likely to be apoptosis, both CD81 and TNFR share common biological responses, tyrosine phosphorylation and antiproliferative effects, modulated by intracellular GSH. Though further biochemical analysis is necessary to elucidate the role of intracellular GSH on the signaling, most studies consistently suggest that the decreased intracellular GSH modulates the signals toward the immunosuppressive conditions.

GSH WORKS AS AN ANTI-APOPTOSIS REAGENT.

Apoptosis is a morphologically and etiologically distinct form of cell death from necrosis. Apoptosis can be induced by many physiological and pharmacological stimulations and requires active metabolic process to finally exert the nuclear degradation; thus it is also referred to as programmed cell death. Many oxidants may induce apoptosis in lymphocytes; in other words, many antioxidants may inhibit apoptosis(2, 3). Stimulation of TcR/CD3 by cross linking with antibodies or by specific peptide antigens may cause apoptosis in thymocytes, leukemia T cells and T cell hybridomas(22, 23). Although it is not well known whether the stimulation of TcR/CD3 complex can actually induce oxidants, it has been reported that the preincubation of T cell hybridomas with NAC abrogate the apoptosis of myelin basic protein specific T cell hybridomas (22). NAC also blocked the cell death of the T cell hybridoma induced by mitogens, PHA and PWM, thus NAC provided common protection against activation-induced apoptosis. The results imply that production of oxidants might

be involved in the activation induced apoptosis. TNF has been shown to induce both apoptotic and necrotic cell death, although it is not well known by what mechanism the form of cell death may exert. TNF has been demonstrated to produce oxidants in sensitive cells(21, 24). Fas/Apo-1 molecule is a member of the TNF receptor family and cross-linking of Fas molecules by antibodies induces apoptosis in the Fas-sensitive cells. Intracelluar domains of Fas/Apo-1 and 55kDa TNF-receptor are homologous and required to induce cytotoxicity, suggesting that both receptors may induce apoptosis by a similar mechanism such as the production of oxidants. Indeed, ADF/thioredoxin reduced the cytotoxicity of both TNF and anti-Fas in a human histiocytic lymphoma cell line, U937(25). However, recent work has suggested that Fas/Apo-1 and 55kDa TNF receptor mediates cytotoxicity through defferent pathways(26, 27). We have previously established a monoclonal antibody, 2D1, which reacts with Fas molecules and Fas -resistant variants from human leukemia T cell lines(28). All of the Fas-resistant variant cells demonstrated higher concentrations of intracellular GSH than in the original cells, suggesting a protective role of GSH in Fas-mediated apoptosis (data not shown). The discrepant results among reports might be due to the diversity of the cell types. In any case, the biological significance of GSH and redox condition with respect to Fas- mediated apoptosis is still controversial.

GSH AND VIRUS INFECTION.

In the pathogenesis of AIDS, many studies suggest that apoptosis is responsible for the depletion of CD4+ T cells(29). In HIV-infected T cells, GSH is depleted as well as the other antioxidants including superoxide dismutase (SOD) and thioredoxin(30, 31, 32). In addition, HIV-infected T cells frequently express Fas/APO-1 molecules which may mediate apoptosis. All of these factors may contribute to susceptibility to apoptosis. Indeed, by mitogenic stimulation, such as PHA and PWM, normal peripheral lymphocyte may start to proliferate and HIV-infected T cells undergo apoptosis by the same mitogen stimulation, most likely due to the strengthened oxidative stress under low levels of intracellular GSH. Thus, the strategy of increasing the intracellular GSH in the HIV-infected patients has been seriously considered(33).

CONCLUSION

Glutathione is the most abundant intracellular tripeptide thiol and acts as a reducing agent and an antioxidant. Glutathione is not only a substrate for enzymes, such as glutathione S-transferase and glutathione peroxidase, but also regulates many enzymatic and pharmacological activities by changing the intracellular redox condition. Recent studies have accumulated evidence for

important roles of glutathione in the field of cellular immunology including activation, proliferation and induction of many immunological functions. Accordingly, a decrease in intracellular glutathione may cause malfunction of lymphocytes, immunosuppression and apoptosis. A variety of immunological functions are determined on the basis of the balance between synthesis and depletion of intracellular glutathione.

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