Glutathione Levels and Sensitivity to Apoptosis Are Regulated by Changes in Transaldolase Expression*

(Received for publication, August 1, 1996, and in revised form, September 16, 1996)

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Transaldolase (TAL) is a key enzyme of the reversible nonoxidative branch of the pentose phosphate pathway (PPP) that is responsible for the generation of NADPH to maintain glutathione at a reduced state (GSH) and, thus, to protect cellular integrity from reactive oxygen intermediates (ROIs). Formation of ROIs have been implicated in certain types of apoptotic cell death. To evaluate the role of TAL in this process, Jurkat human T cells were permanently transfected with TAL and expression vectors oriented in the sense or antisense direction. Overexpression of TAL resulted in a decrease in glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities and NADPH and GSH levels and rendered these cells highly susceptible to apoptosis induced by serum deprivation, hydrogen peroxide, nitric oxide, tumor necrosis factor-α, and anti-Fas monoclonal antibody. In addition, reduced levels of TAL resulted in increased glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities and increased GSH levels with inhibition of apoptosis in all five model systems. The effect of TAL expression on susceptibility to apoptosis through regulating the PPP and GSH production is consistent with an involvement of ROIs in each pathway tested. Production of ROIs in Fas-mediated cell death was further substantiated by measurement of intracellular ROI production with oxidation-sensitive fluorescent probes, by the protective effects of GSH precursor, N-acetyl cysteine, free radical spin traps 5,5-dimethyl-1-pyrroline-1-oxide and 3,3,5,5-tetramethyl-1-pyrroline-1-oxide, the antioxidants desferrioxamine, nordihydroguaiaretic acid, and Amytal, and by the enhancing effects of GSH depletion with buthionine sulfoximine. The results provide definitive evidence that TAL has a role in regulating the balance between the two branches of PPP and its overall output as measured by GSH production and thus influences sensitivity to cell death signals.

Apoptosis is a fundamental form of programmed cell death that is indispensable for normal development and maintenance of homeostasis within multicellular organisms (1). Defects in apoptosis may underlie the etiology of neurodegenerative diseases, cancer, autoimmune diseases, and the acquired immune deficiency syndrome (2, 3). Reactive oxygen intermediates (ROIs) have long been considered as toxic by-products of aerobic existence, but evidence is now accumulating that controlled levels of ROIs modulate cellular function and are necessary for signal-transduction pathways, including those mediating apoptosis (4–7). Many of the chemical and physical stimuli that elicit programmed cell death generate ROIs such as H₂O₂ and OH⁻ (8). Low doses of H₂O₂ induce apoptosis in a variety of cell types, thus establishing oxidative stress as a mediator of apoptosis (7). The ability of scavengers of ROIs such as N-acetylcysteine (NAC), a precursor of GSH (7, 8) and of free radical spin traps such as 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) and 3,3,5,5-tetramethyl-1-pyrroline-1-oxide (TMPO) to inhibit apoptosis, supports this hypothesis (9). bcl-2, the prototype of a novel family of proto-oncogenes that inhibit apoptosis when it is induced by many diverse stimuli, was recently demonstrated to have antioxidant behavior (5, 6). However, apoptosis and bcl-2 protection were demonstrated in very low oxygen pressure, suggesting that ROI may not be an absolute requirement for programmed cell death (10).

A normal reducing atmosphere, required for cellular integrity, is provided by reduced GSH, which protects cells from damage by ROIs (11). Synthesis of GSH from its oxidized form, GSSG, is completely dependent on NADPH produced by the pentose phosphate pathway (PPP) (11). In fact, a fundamental function of PPP is to maintain glutathione in a reduced state and thus provide protection of sulfhydryl groups and cellular integrity from emerging oxygen radicals. PPP comprises two separate branches: the oxidative and the nonoxidative. Reactions in the oxidative branch are irreversible, whereas all reactions of the nonoxidative branch are fully reversible. The two branches are functionally connected. The nonoxidative branch can convert ribose 5-phosphate into glucose 6-phosphate for the oxidative branch, and thus, indirectly, it can also contribute to generation of NADPH. The rate-limiting enzymes for the two branches are different. The oxidative phase is primarily dependent on G6PD (12). While the control of the nonoxidative branch is less well established, transaldolase (TAL) has been proposed as its rate-limiting enzyme (12, 13).

TAL catalyzes the transfer of a 3-carbon fragment, corresponding to dihydroxyacetone, to D-glyceraldehyde 3-phosphate, D-erythrose 4-phosphate, and a variety of other acceptor aldehydes, including nonphosphorylated trioses and tetroses. Enzymatic activity of TAL is regulated in a tissue-specific (13, 14) and developmentally specific manner (15). In the brain, TAL is expressed selectively in oligodendrocytes at high levels.

The abbreviations used are: ROIs, reactive oxygen intermediates; TAL, transaldolase; TK, transketolase; G6PD, glucose 6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; GSH, reduced glutathione; PPP, pentose phosphate pathway; TNF, tumor necrosis factor; NO, nitric oxide; NAC, N-acetylcysteine; BSO, buthionine sulfoximine; DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; TMPO, 3,3,5,5-tetramethyl-1-pyrroline-1-oxide; DCFH-DA, 5,6-carboxy-2′,7′-dichlorofluorescein-diacetate; DHR, dihydrorhodamine 123.

* This work was supported in part by National Institutes of Health Grant RO1 DK 49221, National Multiple Sclerosis Society Grant RG 2466A1/3, the Arthritis Foundation, and the Central New York Community Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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TAL Activities—The reverse reaction catalyzed by TAL was tested in the presence of 3.2 mM D-fructose-6-phosphate, 0.2 mM TAL-H cDNA and dephosphatase, 0.1 mM NADH, and 10 μg of α-glycerophosphate dehydrogenase/triosephosphate isomerase at a 1:6 ratio in 40 mM triethanolamine, pH 7.6, 5 mM EDTA at room temperature by continuous absorbance reading at 340 nm for 8 min (18). The forward reaction catalyzed by TAL was measured at room temperature in the presence of 50 mM triethanolamine, pH 7.4, 5 mM MgCl₂, 5 mM ribose-5-phosphate, 0.9 mM xylulose-5-phosphate, 0.5 mM NADPH, 0.2 mM thiamine pyrophosphate, 0.2 units/ml transketolase, 0.4 units/ml phosphoglucone isomerase, and 0.3 units/ml G6PD, following a 10-min lag period, by continuous absorbance reading at 340 nm for 8 min (26). The enzyme assays were conducted in the activity range of 0.001—0.01 units/ml. Unless indicated otherwise, TAL activity refers to enzyme activity measurements conducted in the reverse reaction.

Transketolase (TK), G6PD, and 6PGD Activities—TK activity was measured in 50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 0.06 mM thiamine pyrophosphate, 0.1 mM NADH, 10 μg of α-glycerophosphate dehydrogenase/triosephosphate isomerase at a 1:6 ratio, 5 mM ribose 5-phosphate, and 1.25 mM xylulose 5-phosphate (13). G6PD was measured in the presence of 120 mM Tris, pH 8.4, 10 mM MgCl₂, 2 mM glucose 6-phosphate, 0.9 mM NADP, and 0.1 units/ml G6PD (27). 6PGD activity was determined in 120 mM Tris, pH 7.7, 10 mM MgCl₂, 0.9 mM NADP, and 2 mM 6-phosphogluconate (27).

Glutathione, NADPH, and NADH Levels—Total glutathione content was determined by the enzymatic recycling procedure essentially as described by Tietze (28). 10⁶ cells were resuspended in 100 μl of 4.5% 5-sulfosalicylic acid. The acid-precipitated protein was pelleted by centrifugation at 4 °C for 10 min at 2000 × g. The total protein content of each sample was determined using the Lowry assay (29). GSH content of the aliquot assayed was determined in comparison to reference curves generated with known amounts of GSH. For NADPH and NADH assays, 5 × 10⁵ cells were washed in phosphate-buffered saline and resuspended in 125 μl of H₂O₂, and pyridine nucleotides were extracted by adding 62.5 μl of freshly prepared 1 M KOH in ethanol as earlier described (30).

RESULTS

Regulation of the PPP and GSH Levels by TAL—To assess whether TAL expression may influence biochemical processes regulating apoptosis, cell lines producing increased and suppressed levels of TAL were generated. Jurkat human T cells were stably transfected with eukaryotic expression vectors containing full-length transaldolase cDNA in the sense (pL26-3) or antisense (pL18-3) orientation. Western blot analysis revealed...
Using actin as a baseline, TAL expression was increased by several cell lines carrying pL26-3 and pL18-3 that had either untransfected Jurkat cells (control). Sense (L26-3/4) and antisense (L18-3/1) orientations of TAL expression vectors oriented in the NADH levels were unchanged in these cell lines. L26-3/4 and L26-3/2D1 cells, NADH levels were decreased in L26-3/4 and L26-3/2D1 cells in comparison to control Jurkat cells. GSH levels were depleted in L26-3/4 and L26-3/2D1 cells. Since these cell lines, TK activity remained unchanged in L18-3/1 cells, whereas TAL activity was increased in L18-3/1 cells and L18-3/1D9 cells, whereas TAL activity was increased in L26-3/4 and L26-3/2D1 cells. Suppression of TAL was associated with a decrease of TK activity in L18-3/1 cells, while TK activity in the reverse reaction was augmented by 210% (3.1-fold) in L26-3/4 cells and by 24.9% (1.25-fold) in L26-3/2D1 cells. TAL activity in the reverse reaction was increased by 127% (2.3-fold) in L26-3/4 cells and by 24.9% (1.25-fold) in L26-3/2D1 cells.

| Cell      | TAL-FOR (pmol/μg protein) | TAL-REV (pmol/μg protein) | Δ (FOR-REV) | TK (pmol/μg protein) | G6PD (mU/μg protein) | 6PGD (mU/μg protein) | NADH (pmol/μg protein) | NADPH (pmol/μg protein) | GSH (μmol/g protein) | Viability |
|-----------|--------------------------|---------------------------|-------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|----------------------|-----------|
| Jurkat    | 23.0 ± 1.3               | 20.1 ± 0.9                | 2.9 ± 0.9   | 9.0 ± 0.6            | 42.6 ± 7.1           | 44.3 ± 2.9           | 0.36 ± 0.03           | 0.36 ± 0.06           | 58.6 ± 3.6           | 60.8 ± 3.6  |
| L18–3/1   | 17.7 ± 1.2***            | 14.4 ± 1.1***             | 3.3 ± 1.3   | 4.9 ± 0.3***         | 58.6 ± 3.6***        | 66.8 ± 2.9***        | 0.33 ± 0.02           | 0.99 ± 0.2            | 149.5 ± 22**        | 95.5 ± 3.4** |
| L18–3/1D9 | 13.5 ± 0.9***            | 9.6 ± 2.4***              | 3.9 ± 1.3   | 9.7 ± 0.3            | 48.6 ± 3.8*          | 58.0 ± 3.1*          | 0.28 ± 0.04           | 0.95 ± 0.2            | 123.2 ± 31**        | 82.0 ± 9.8** |
Sensitivity to Apoptotic Signals Is Influenced by Levels of TAL Expression—Stably transfected cell lines with increased and depressed TAL activities have been maintained in culture for over 2 years showing viability similar to that of control Jurkat cells (>99%). However, levels of TAL expression had a dramatic influence on susceptibility to apoptotic cell death induced by H₂O₂, NO, TNF-α, anti-Fas monoclonal antibody, or withdrawal of fetal calf serum from the culture medium (Fig. 2). Apoptosis was monitored by DNA ladder formation (Fig. 3) and flow cytometry as shown for Fas simulation (Fig. 4). Cell death was particularly accelerated in TAL-overproducing cells in comparison to control Jurkat cells. Suppressed TAL expression inhibited cell death produced by all stimuli tested. Cell survival inversely correlated with TAL expression levels (Table I).

Fas-mediated Apoptosis Is Associated with Production of ROI—The effect of TAL expression on susceptibility to apoptosis through regulating the PPP and GSH production suggested the involvement of ROIs in each pathway tested. While the production of ROIs has been associated with apoptosis induced by H₂O₂, NO, TNF, and serum deprivation, involvement of ROIs in Fas-dependent signaling has been controversial. To assess changes in intracellular ROIs we used oxidation-sensitive fluorescent probes DCFH-DA and DHR (32, 33). DCFH-DA is nonfluorescent, readily accumulates within cells, and following deacetylation to DCFH is oxidized to the fluorescent compound dichlorofluorescein. Similarly, DHR is nonfluorescent, uncharged, and readily taken up by cells, whereas R123, the product of DHR oxidation, is fluorescent, positively charged, and trapped within cells. We evaluated the rates of increase in

Fig. 2. Rate of apoptotic cell death induced by serum withdrawal, 100 μM H₂O₂, 5 mM sodium nitroprusside, 20 ng/ml TNF-α, and 50 ng/ml anti-Fas monoclonal antibody in L26-3/4, L18-3/1, and control Jurkat cells. Data represent the mean ± S.E. of four independent experiments.
fluorescence of cells treated with 100 mM H₂O₂ and 50 ng/ml anti-Fas monoclonal antibody. As shown in Fig. 5A, relative to H₂O₂ a smaller but consistent increase in ROI was detected in Fas-stimulated cells as compared with untreated cells. In agreement with earlier data (33), DHR was a significantly more sensitive detector of increases in ROI levels than DCFH.

Production of ROI correlated with the rate of cell death (p < 0.01; Fig. 5B).

**Effect of Antioxidants on TAL and G6PD Activities, GSH Levels, and Fas-induced Cell Death**—The involvement of ROI in Fas-dependent signaling was suggested by our observation of (i) an increased sensitivity to Fas-induced death of cells with increased TAL expression and decreased GSH content and (ii) the production of ROI during Fas-mediated apoptosis. We
therefore examined whether changes in GSH levels can influence Fas-induced programmed cell death. Under the conditions utilized, none of the agents had a significant effect on the binding of anti-Fas antibody to its receptor based on flow cytometry (data not shown). Intracellular GSH levels were raised by as much as 2-fold using NAC, a precursor of glutathione (34), or suppressed to less than 15% of baseline by BSO, an inhibitor of γ-glutamyl-cysteine synthetase (34) (Fig. 6A). Neither NAC nor BSO influenced TAL activity. In contrast, G6PD activity was increased by both NAC (p < 0.01) and BSO (p < 0.05) after 24 h incubation. In accordance with earlier observations (35), cell viability was not affected up to 4 days in culture by NAC (up to 3 mM), BSO (up to 1 mM), or any of the other antioxidants tested (data not shown). However, Fas-mediated cell death was markedly influenced by NAC and BSO (Fig. 6B). Prior to stimulation with anti-Fas antibody, Jurkat cells were pretreated with NAC or BSO for 24 h. 1 mM BSO substantially accelerated apoptosis, while 3 mM NAC inhibited Fas-mediated cell death (p < 0.01; Fig. 6B). The antioxidants amytal, desferrioxamine, and nordihydroguaiaretic acid also inhibited Fas-induced apoptosis (Fig. 6B). DMPO and TMPO are nitrones that react with ROI to form more stable nitroxide radical products (36) and have protected thymocytes against apoptosis (9). While DMPO or TMPO had no significant effect on GSH levels (not shown), they also inhibited Fas-induced apoptosis (p < 0.01; Fig. 6B).

**DISCUSSION**

The PPP fulfills two essential functions consisting of the formation of pentose phosphates for synthesis of nucleotides, RNA, and DNA and the generation of NADPH for biosynthetic reactions and to maintain glutathione at a reduced state, thus protecting sulphydryl groups and cellular integrity from oxygen radicals. A number of different approaches have been applied to delineate the mechanism by which PPP is controlled: 1) identification of the rate-limiting enzymes, 2) comparison of both the mass action ratios with the equilibrium constant and steady-state concentrations with the actual flux of intermediates for each enzyme, and 3) identification of enzymes under hormonal or environmental control (12). Finding a unifying approach has been complicated by the fact that PPP is comprised of two separate branches, oxidative and nonoxidative. Reactions in the oxidative branch are irreversible, whereas all reactions of the nonoxidative phase are fully reversible. The rate-limiting enzymes for the two branches are different. The oxidative branch is primarily dependent on G6PD (12). The
control of the nonoxidative branch, between transaldolase and transketolase, is less well established based on enzyme kinetic studies. Tissue-specific variations in enzymatic activities suggested that TAL may be a rate-limiting enzyme of the nonoxidative branch of the PPP (13, 14). This study provides evidence that TAL may have a pivotal role in regulating the entire pathway. Overexpression of TAL in Jurkat human T cells resulted in down-regulation of G6PD and 6PGD activities and a decrease of NADPH and GSH levels. NADH levels were also reduced in TAL-overproducing cells, which was consistent with the tendency to maintain NADPH at the expense of NADH by transhydrogenases (37). Alternatively, decreased TAL expression led to up-regulation of G6PD and 6PGD activities and increased GSH levels.

Sensitivity to apoptosis was effectively controlled by regulating the activity of TAL, a pivotal enzyme of the PPP. Overexpression of TAL increased sensitivity, while suppression of TAL decreased sensitivity to five different apoptotic signals indicating that TAL expression levels can profoundly influence susceptibility to programmed cell death. The mechanism of this regulatory function may be explained by a considerable difference in forward TAL catalytic activity that favors production of glucose 6-phosphate, compared with reverse TAL activity that depletes glucose 6-phosphate (11). Maximal velocity of TAL in the forward direction was only about one-third of that of the reverse direction in the yeast (38). Reversibility of the TAL reaction has been proposed as a possible control mechanism for the entire pathway in the yeast (26). In L26-3/4 and L26/2D1 cells overexpressing TAL, the nonoxidative branch was pushed in the reverse direction, depleting glucose 6-phosphate (not shown). This effect may be directly responsible for diminished G6PD activities and GSH levels and increased sensitivity to apoptotic signals.

The involvement of ROIs in each of the apoptosis signaling pathways examined here was suggested by the finding that TAL expression, via regulation of the PPP and of GSH production, modulated susceptibility to apoptosis. Apoptosis triggered by serum withdrawal (39) and NO has been associated with the tendency to maintain NADPH at the expense of NADH by transhydrogenases (37). Alternatively, decreased TAL expression led to up-regulation of G6PD and 6PGD activities and increased GSH levels.

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27. Rudack, D., Chisholm, E. M., and Holten, D. (1971) J. Biol. Chem. 246, 1249–1254
28. Tietze, F. (1969) Anal. Biochem. 27, 502–522
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
30. Zerez, C. R., Lee, S. J., and Tanaka, K. R. (1987) Anal. Biochem. 164, 367–373
31. Burrow, S., and Valet, G. (1987) Eur. J. Cell Biol. 43, 128–133
32. Royall, J. A., and Ischiropoulos, H. (1993) Arch. Biochem. Biophys. 302, 348–355
33. Packham, G., Ashmun, R. A., and Cleveland, J. L. (1996) J. Immunol. 156, 2792–2806
34. Meister, A., and Anderson, M. E. (1983) Annu. Rev. Biochem. 52, 711–760
35. Hamilos, D. L., and Wedner, H. J. (1985) J. Immunol. 135, 2740–2747
36. Finkelstein, E., Rosen, G. M., and Rauckman, E. J. (1980) Arch. Biochem. Biophys. 200, 1–16
37. Rydstrom, J. (1977) Biochim. Biophys. Acta 463, 155–184
38. Wood, T. (1972) FEBS Lett. 25, 153–155
39. Sandstrom, P. A., and Butke, T. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4708–4712
40. Meier, B., Radeke, H. H., Selle, S., Younes, M., Sies, H., Resch, K., and Habermehl, G. G. (1989) Biochem. J. 263, 539–545
41. Hennet, T., Richter, C., and Peterhans, E. (1993) Biochem. J. 299, 587–592
42. Schulze-Osthoff, K., Krammer, P. H., and Droge, W. (1994) EMBO J. 13, 4587–4596
43. Hug, H., Enari, M., and Nagata, S. (1994) FEBS Lett. 351, 311–313
44. Chinnaiyan, A. M., Tepper, C. G., Seldin, M. F., O'Rourke, K., Kischkel, F. C., Heibardt, S., Krammer, P. H., Peter, M. E., and Dixit, V. M. (1996) J. Biol. Chem. 271, 4961–4965