Rapid and Specific Efflux of Reduced Glutathione during Apoptosis Induced by Anti-Fas/APO-1 Antibody*

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Although human J URKAT T lymphocytes induced to undergo apoptosis with anti-Fas/APO-1 antibody were observed to rapidly lose reduced glutathione (GSH), increased concentrations of oxidized products were not detectable. Unexpectedly, the reduced tripeptide was instead quantitatively recovered in the incubation medium of the cells. As GSH loss was blocked by bromosulfophthalein and dibromosulfophthalein, known inhibitors of hepatocyte GSH transport, a specific export rather than nonspecific leakiness through plasma membranes is proposed to be responsible. Apoptosis was delayed when GSH-diyethylesters were used to elevate intracellular GSH, although the high capacity of the activated efflux system quickly negated the benefit of this treatment. Stimulation of GSH efflux provides a novel mechanism whereby Fas/APO-1 ligation can deplete GSH. We speculate that it enhances the oxidative tonus of a responding cell without requiring an increase in the production of reactive oxygen species.

In the current paradigm for apoptotic cell death, stimulation of the proteolytic activity of a family of interleukin 1-β-converting enzyme (ICE)1-like proteases initiates several distinct events within a cell (1–3). These include chromatin fragmentation, plasma membrane blebbing, and an overall cell shrinkage, eventually culminating in either the phagocytosis or lysis of the apoptotic cell. There have also been numerous reports that apoptotic cells accumulate oxidized proteins and lipids and are presumably therefore exposed to some degree of oxidative stress (4). In addition, antioxidants have been widely reported to protect against, or delay, many different forms of apoptosis (5). However, several recent reports of apparently normal apoptosis occurring in very low oxygen environments indicate that oxidative stress independent of any increase in the production of reactive oxygen species (ROS) are unlikely to be essential mediators of this type of cell death (6–8).

Human J URKAT T lymphocytes are rapidly induced to undergo apoptosis after exposure to anti-Fas/APO-1 antibody (9–11). One of the earliest detectable events downstream of ligand-receptor binding is activation after 15 min of the ICE-like protease CPP-32/apoapain (12, 13). Plasma membrane blebbing and degradation of the cytoskeleton commence soon after, while chromatin breakdown is first detectable at 45 min with internucleosomal DNA fragments beginning to accumulate after approximately 90 min (14). Gross alterations in plasma membrane permeability develop several hours later. Although it has been reported that these changes occur with similar kinetics independently of ambient oxygen tension (6), the possibility remains that oxygen-independent modification of intracellular redox states are involved in these events. To further understand this controversial area, the glutathione metabolism of J URKAT cells exposed to anti-Fas/APO-1 antibody was investigated. Consistent with results in thymocytes (15, 16), T lymphocytes undergoing apoptosis became dramatically depleted of their reduced glutathione (GSH) coincident with the onset of chromatin fragmentation. Unexpectedly, this was caused by an accelerated efflux of GSH from the cells rather than any intracellular oxidation of the tripeptide. As GSH efflux was inhibited by bromosulfophthalein (BSP) and dibromosulfophthalein (diBSP), known inhibitors of the GSH transporter located on the canalicular membrane of hepatocytes (17), the selective opening of a GSH-specific membrane channel is proposed to be responsible. Severe depletion of GSH will lower the reducing capacity of the cell and thereby enhance oxidative stress independent of any increase in the production of ROS.

EXPERIMENTAL PROCEDURES

Cell Lines and Reagents—The human leukemic T cell line JURKAT (clone E6) was obtained from ATCC and cultured in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 100 IU penicillin/streptomycin in an atmosphere of 5% CO₂ at 37 °C. For experiments the cells were suspended at 4 × 10⁶ cells ml⁻¹. Where indicated the cells were cultured in cystine-free RPMI medium supplemented as above. Monodonal IgM anti-Fas/APO-1 antibody, Z-Val-Ala-Asp-chloromethylketone, monobromobimane, and glutathione diethylester were purchased from Medical Biological Laboratories, Enzyme System Products, Calbiochem and Bachem, respectively. The glutathione transport inhibitors BSP and phenol-3,6-dibromophenathaldehyde disulfonate = diBSP were purchased from Aldrich and Société d’Etudes et Recherches Biologiques (Paris), respectively, while a bromosulfophthalein-glutathione conjugate (BSP-GSH) was synthesized and purified according to Whelan et al. (18). The final BSP-GSH product was contaminated with less than 0.8% BSP as determined by TLC. All other chemicals were of analytical grade.

Measurement of Apoptosis—Plasma membrane integrity was monitored either by release of cytosolic lactate dehydrogenase as monitored with the Live-Dead cytotoxicity kit supplied by Molecular Probes. The ability of cells to reduce an exogenous substrate was assayed by adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 1 mg ml⁻¹) into their incubation medium. Formazan production was measured 40–60 min later in a spectrophotometer (592 nm) after first

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1 The abbreviations used are: ICE, interleukin 1-β-converting enzyme; ROS, reactive oxygen species; BSP, bromosulfophthalein; diBSP, dibromosulfophthalein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HPLC, high performance liquid chromatography; dansyl, 5-dimethylaminonaphthalene-1-sulfonfonyl; DTT, dithiobisretol.

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lysing the cells in 0.5% SDS, 40 mM HCl in 90% isopropanol. High molecular weight chromatin fragments were detected by field inversion gel electrophoresis (19). DNA extracted from approximately 0.2 × 10^6 cells was electrophoresed using the conditions described in Nobel et al. (20). DNA migration on the gels was calibrated with Pulse-marker™ molecular weight standards (Sigma). Intercellular DNA fragmentation was quantitated by the diphenylamine assay (21) and visualized using the agarose gel electrophoresis protocol described by Wyllie et al. (22).

Glutathione and Protein Thiol Determination—Intracellular GSH was assayed using monobromobimane as described by Cotgreave and Moldéus (23). In short, 4 × 10^6 cells were collected and washed in 1 ml of isotonic buffer (24 mM NaHCO_3, 110 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM CaCl_2, 10 mM glucose, 1 mM MgSO_4, pH 7.2) and centrifuged for 6 min at 700 × g. The supernatant was discarded and the cell pellet resuspended in 100 µl of buffer. An equal volume of 8 mM monobromobimane in 50 mM N-ethylmorpholine, pH 8.0, was added and the sample allowed to stand 3 min in the dark. Protein was then precipitated by adding 10 µl of 100% trichloroacetic acid. After centrifugation at 10,000 × g the low molecular weight thiols in the supernatant were analyzed by HPLC. In several experiments protein thiols in the pellet were also quantitated relative to a BSA standard (23). For simultaneous determination of reduced and oxidized intracellular glutathione, 2.5 × 10^6 cells were washed twice and precipitated for 2 h in 350 µl of 5% perchloric acid, 0.2 N boric acid. Protein was removed by centrifugation, and primary amines in the supernatant derivatized with dansyl chloride after first alkylating free thiols with iodoacetic acid. The dansylated derivatives of GSH and GSSG were then separated by HPLC essentially as described by Fariss and Reed (24) for the corresponding diethylphosphat adducts.

To measure GSH in the culture medium, 6 × 10^6 cells (150 µl total volume) were centrifuged and two 60-µl aliquots of the supernatant collected. One was derivatized immediately with 8 mM monobromobimane, whereas the other was first reduced with 5 mM DTT before derivatization with 30 mM monobromobimane (23). In order to facilitate detection of reduced thiols some experiments were also performed in a cysteine-free RPMI medium.

RESULTS

The intracellular GSH content of human JURKAT T lymphocytes was observed to fall after exposure to anti-Fas/APO-1 antibody (Fig. 1A). This GSH loss began after a lag period of approximately 30 min and was almost complete by 3 h. In five independent experiments the half-life of intracellular glutathione, 2.5 × 10^6 cells were washed twice and precipitated for 2 h in 350 µl of 5% perchloric acid, 0.2 N boric acid. Protein was removed by centrifugation, and primary amines in the supernatant derivatized with dansyl chloride after first alkylating free thiols with iodoacetic acid. The dansylated derivatives of GSH and GSSG were then separated by HPLC essentially as described by Fariss and Reed (24) for the corresponding diethylphosphat adducts.

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The experiment was carried out to measure the average ratio in untreated cells during the test period. By HPLC, data points show mean ± S.D. from triplicate samples. Inset, the average ratio of GSH:GSSG is shown for Fas/APO-1-stimulated cells at each time point. The average ratio in untreated cells during the experiment was 27 ± 6 (mean ± S.D.; n = 7).

![Figure 2](image2.png) **Fig. 2.** GSH loss induced by anti-Fas/APO-1 antibody is not associated with an accumulation of oxidized GSSG. Cells (5 × 10^6 cells) were left untreated (open symbol) or exposed to 250 ng/ml anti-Fas/APO-1 antibody (closed symbol). At various times after treatment cells were collected by centrifugation, and GSH (●) and GSSG (△) derivatized with dansyl chloride prior to separation and detection by HPLC. Data points show mean ± S.D. from triplicate samples. Inset, the average ratio of GSH:GSSG is shown for Fas/APO-1-stimulated cells at each time point. The average ratio in untreated cells during the experiment was 27 ± 6 (mean ± S.D.; n = 7).

Experiments indicate that the decrease of GSH in JURKAT cells after treatment with anti-Fas/APO-1 antibody is too rapid to be accounted for by an inhibition of precursor uptake or biosynthesis. It is more likely that its rate of excretion is increased in response to stimulation of the Fas/APO-1 cell surface receptor.

Two approaches were adopted to investigate the possibility that the tripeptide was being extruded from cells. First, cells were depleted of intracellular GSH by incubation in a cystine-free medium (GSH content falls to approximately 40% of control levels after 6 h; see Fig. 3) and then allowed to resynthesize the tripeptide by adding 200 μM [35S]cystine. GSH levels recovered (and actually slightly overshot) control levels within 3 h (data not shown). Anti-Fas/APO-1 antibody was then added to the cells and radioactivity in the incubation medium monitored. Extracellular 35S increased markedly 40–70 min after adding the antibody, suggesting that labeled cysteinyl groups were being expelled from the cells (Fig. 4A). In a more direct experiment, the incubation medium of cells was collected at various times after adding anti-Fas/APO-1 antibody and then reduced with DTT and derivatized with monobromobimane prior to analysis by HPLC. (NB, Due to a rapid thiol-disulfide exchange with cystine, GSH has a half-life of less than 3 min in standard RPMI medium. Reduction with DTT is therefore required before GSH can be detected as a monobromobimane conjugate.) Glutathione was quantitatively recovered in the incubation medium coincident with its disappearance from the antibody-treated cells (Fig. 4C). We conclude that JURKAT cells exposed to anti-Fas/APO-1 antibody expel a large fraction of their intracellular GSH during the first hour of their treatment. This phenomenon occurs at about the same time as chromatin begins to be cleaved but several hours before any gross increase in plasma membrane leakiness.

Although the experiments described above identify an anti-Fas/APO-1 antibody-induced accumulation of intact glutathione outside cells, they do not allow identification of the redox form (GSH or GSSG) actually transported. To investigate this issue cells were incubated in cystine-free medium to prevent thiol-disulfide exchange between GSH and cystine. A small depletion of intracellular GSH, therefore, directly results from cystine withdrawal, as can clearly be seen at the later time points in the untreated cells (Fig. 5A). Consistent with previous studies in other lymphoid cells (31), untreated JURKAT T lymphocytes were observed to only very slowly release GSH into the culture medium (Fig. 5A). GSH release from anti-Fas/APO-1 antibody-treated cells remained at background levels for the first 30 min, but then dramatically increased coincident with its loss from the intracellular compartment (Fig. 5B). Almost all of the GSH lost from the cells was quantitatively recovered in the medium. In addition, small amounts of the GSH breakdown products cysteinyl-glycine and cysteine were also detected (not shown). During the initial phase of glutathione release, there was no difference in the efficiency of trapping GSH as its monobromobimane conjugate in the presence or absence of DTT, indicating that it was present in its reduced form at this time. As GSSG added to culture medium is not spontaneously reduced (data not shown), these results can only be explained by glutathione being exported from the Fas/APO-1-stimulated cells as an intact reduced tripeptide. At later time points some recovery of oxidized thiol was also recorded (illustrated by the difference in amount of GSH measured before and after DTT reduction; Fig. 5B). This is likely to be secondary to a slow oxidation of extracellular GSH in the cystine-free medium.

To investigate whether GSH efflux was activated by the same proteolytic cascade believed to initiate apoptotic chromatin fragmentation, JURKAT cells were preincubated with Z-Val-Ala-Asp-chloromethylketone, an irreversible inhibitor of ICE family proteases (32). Consistent with reports by Enari et al. (10) and Los et al. (33), inhibiting the activity of this protease family was found to prevent both the fall in MTT reduction and the increase in DNA fragmentation that occurs after cells are exposed to anti-Fas/APO-1 antibody (Table I). Irrespective of the presence of the antibody, intracellular GSH levels were also fully maintained when Z-Val-Ala-Asp-chloromethylketone was included in the preincubation medium (Table I). This indicates that the rapid efflux of GSH observed after stimulation of the Fas/APO-1 receptor is a distinct cytoplasmic event directly or indirectly activated by a member of the ICE family of cysteine proteases.

Several GSH-specific transporters located in both plasma and mitochondrial membranes of cells have been described...
Rat hepatocytes are the best characterized cell system with respect to this type of transporter. The sinusoidal membrane of rat hepatocytes contains a GSH transporter distinguished by its sensitivity to L-methionine, cystathionine, and the BSP-GSH conjugate (34, 37, 38), while their canalicular plasma membranes contain both high affinity/low capacity

| Cells                  | MTT reduction | DNA fragmentation | GSH     |
|------------------------|---------------|-------------------|---------|
| Untreated              | % of control  | % of total        | mmol·mg⁻¹·protein⁻¹ |
| Untreated + Z-Val-Ala-Asp-cmk | 102 ± 4       | 7 ± 1              | 30.0 ± 0.7 |
| Anti-Fas/APO-1 antibody | 24 ± 1        | 87 ± 2             | 1.6 ± 0.1 |
| Anti-Fas/APO-1 antibody + Z-Val-Ala-Asp-cmk | 99 ± 5       | 7 ± 1              | 26.9 ± 0.6 |

previously (34–37). Rat hepatocytes are the best characterized cell system with respect to this type of transporter. The sinusoidal membrane of rat hepatocytes contains a GSH transporter distinguished by its sensitivity to L-methionine, cystathionine, and the BSP-GSH conjugate (34, 37, 38), while their canalicular plasma membranes contain both high affinity/low capacity
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and low affinity/high capacity GSH transporters that are inhibited by BSP and diBSP (17, 39). When BSP was applied to untreated JURKAT cells, intracellular GSH was slowly depleted (Fig. 6A), presumably due to the previously reported glutathione S-transferase catalyzed conjugation of BSP and GSH (40). We therefore concentrated on studying the effects of BSP on anti-Fas/APO-1 antibody-induced GSH efflux during short incubations (30–60 min). Under these conditions, BSP was observed to give a concentration-dependent inhibition of GSH efflux from cells exposed to the antibody, with an EC_{50} of approximately 100 μM (Fig. 6A). This closely resembles the concentration at which BSP inhibits the hepatocyte canalicular membrane GSH transporter (17). Compared with BSP, the analog diBSP is a relatively poor substrate for glutathione S-transferases, and therefore leaves the intracellular GSH levels of control cells unaffected for a longer time (Fig. 6B). 2 mM diBSP completely inhibited the anti-Fas/APO-1-induced decrease in intracellular GSH for up to 2 h (Fig. 6B), although in longer incubations it again slowly depleted GSH from otherwise untreated cells (data not shown). These experiments suggest that a GSH transporter with inhibitor sensitivity resembling the hepatocyte canalicular GSH transporter is activated in JURKAT cells undergoing apoptosis in response to stimulation of Fas/APO-1.

To further identify the characteristics of this inducible GSH transporter, the effects of other known inhibitors of various GSH transporters were studied. Inhibitors of the liver sinusoidal membrane GSH transporter, such as L-methionine and cystathionine (34, 37, 38), failed to inhibit the Fas/APO-1-induced loss of intracellular GSH (Table II). In contrast, another inhibitor of the sinusoidal membrane GSH transporter, the BSP-GSH conjugate, did partially inhibit GSH efflux (Table II). However, as Ballatori and Dutczak (17) have shown that this agent also has some inhibitory effects on GSH transport in canalicular liver membrane vesicles, the absolute specificity of this reagent is unclear. It is also possible that GSH transport in JURKAT T cells has some difference in its profile of inhibitor sensitivity compared with the better characterized rat hepatocyte model. Neither GSSG nor any of the six S-alkylglutathione conjugates tested were able to significantly inhibit GSH loss in response to antibody treatment (Table III). These negative results exclude both the high affinity/low capacity canalicular GSH transporter and the multidrug resistance-associated protein from being involved (17, 41, 42). As the low affinity/high capacity canalicular-like GSH-transporter (in contrast to the transporter on sinusoidal membranes) is also present in numerous other cell types (35, 43), we propose that it is a plausible candidate for the membrane channel controlling GSH efflux during Fas/APO-1-stimulated apoptosis.

The protective effect of BSP and diBSP against GSH efflux were lost after longer incubation (>2 h), while both compounds also slowly depleted intracellular GSH by direct conjugation. We were therefore unable to determine whether they improve the long term viability of cells exposed to anti-Fas/APO-1 antibody (JURKATS retain their membrane integrity for at least 4 h after the addition of antibody; see Fig. 1A). Furthermore, BSP and diBSP directly interfere with the MTT assay such that it was not possible to use this method to search for any protective effects of inhibiting GSH transport on cellular reducing capacity. However, diBSP could be used to investigate the...
Inhibition of GSH efflux has no effect on Fas/APO-1-induced chromatin fragmentation. Jurkat cells were incubated in the absence (lanes 1–3) or presence (lanes 4–6) of 250 ng/ml anti-Fas/APO-1 antibody. After 25 min control medium (lanes 1 and 4), 2 mM diBSP (lanes 2 and 5) or 1 mM BSP-GSH conjugate (lanes 3 and 6) were added and the incubation continued for 95 min. 1 x 10^6 cells were harvested for agarose gel electrophoresis, and 3 x 10^6 cells were harvested for quantitation of DNA fragmentation using diphenylamine (n = 4, S.D. values ranging between 0.6 and 5.2 are not shown).

Table III. Effect of various glutathione S-conjugates and GSSG on intracellular GSH levels following exposure to anti-Fas/APO-1 antibody. JURKAT cells (4 x 10^6/ml) were incubated with 1 mM of the glutathione S-conjugate or GSSG 60 min before the addition of 250 ng/ml anti-Fas/APO-1 antibody at 37 °C. Intracellular GSH levels were determined 60 min later by the monobromobimane method. Results are expressed as mean ± S.D. (n = 4), and the percent GSH content of anti-Fas/APO-1-treated cells relative to their control is written in parentheses.

| Intracellular GSH level | Untreated | Anti-Fas/APO-1 antibody (%) | n mol·mg^(-1)·protein |
|-------------------------|-----------|----------------------------|----------------------|
| Control                 | 27.6 ± 0.4| 15.7 ± 0.3 (57)            |
| S-Methylglutathione     | 27.1 ± 1.2| 14.3 ± 1.5 (53)            |
| S-Propylglutathione     | 26.7 ± 1.3| 15.6 ± 1.5 (58)            |
| S-Butylglutathione      | 26.3 ± 0.9| 13.6 ± 0.2 (52)            |
| S-Pentylglutathione     | 24.7 ± 0.8| 16.3 ± 0.2 (66)            |
| S-Hexylglutathione      | 25.7 ± 0.6| 13.5 ± 0.4 (53)            |
| S-Nonylglutathione      | 23.2 ± 1.7| 7.5 ± 0.7* (33)            |
| GSSG                    | 26.4 ± 0.8| 12.6 ± 1.0 (48)            |

* p < 0.05, significantly different from control cells as determined by analysis of variance.

Effect of maintaining normal GSH levels on DNA degradation induced by ligation of Fas/APO-1. In two experiments, although GSH depletion in anti-Fas/APO-1 antibody-treated cells was almost completely prevented in the presence of 2 mM diBSP (Fig. 6B), apoptotic degradation of nuclear DNA in the same cells was indistinguishable in the presence or absence of the inhibitor (Fig. 7). The BSP-GSH conjugate also failed to inhibit chromatin fragmentation. Thus, inhibition of GSH efflux has no apparent effect on the kinetics of DNA degradation in these cells.

Although glutathione itself is relatively poor at permeating into normal lymphoid cells, intracellular levels can be elevated by incubation with diethylersters of the tripeptide (44). This method provided a less toxic alternative than BSP/diBSP to investigate whether manipulation of intracellular glutathione concentration would have any impact on anti-Fas/APO-1 antibody-induced apoptosis. Preincubating JURKAT cells for 1 h with 20 mM glutathione diethylester was found to consistently elevate intracellular GSH about 4-fold (Fig. 8A; using the HPLC conditions of Levy et al. (44), both diethyl- and particularly monoethyl-GSH can also be detected inside the cells). After addition of anti-Fas/APO-1 antibody, a significant delay was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis. For example, their ability to reduce MTT was observed in the rate at which the GSH-loaded cells underwent apoptosis.
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DISCUSSION

A depletion of intracellular GSH has been reported to occur in several different apoptotic systems (15, 16, 46). For example, thymocytes undergoing apoptosis after exposure to glucocorticoid hormone or DNA damaging agents lose intracellular GSH with similar kinetics to internucleosomal chromatin fragmentation (15). It is usually assumed that GSH depletion reflects an intracellular oxidation, although direct evidence for an increased production of ROS or other radicals in cells undergoing apoptosis has been difficult to obtain. The results presented here suggest a different interpretation: GSH is directly exported from a cell undergoing apoptosis via activation of a transmembrane channel rather than depleted by oxidation.

Consistent with our findings, Ghibelli et al. (46) have recently reported glutathione extrusion from U937 cells triggered to undergo apoptosis by puromycin or etoposide, although interpretation of their data is complicated by the large amounts of cell lysis also occurring. In Fas/APO-1-stimulated JURKAT cells activation of GSH efflux is apparently under the control (direct or indirect) of cytoplasmic proteases related to ICE. If GSH efflux and a resulting shift in intracellular GSH:GSSG redox state can be shown to occur in other apoptotic systems, it may represent a common mechanism by which activation of ICE-like proteases induces oxidative stress in the absence of an increased production of ROS.

The physiological benefit of rapid GSH export during Fas/APO-1-stimulated apoptosis is unknown, although several possibilities can be imagined. First, it may be necessary for a responding cell to lose reducing capacity, and that the least damaging way of achieving this is to export the most important cytotoxic antioxidant rather than to generate oxidants with their attendant nonspecific reactivities. As external antioxidants would be expected to lessen the impact of GSH depletion, this could also explain why they are sometimes observed to delay cell death in this system (47). However, it must be stressed that supplementing intracellular GSH does not protect against apoptosis induced by anti-Fas/APO-1 antibody (Ref. 45; see results presented here). At least in JURKAT cells this can be explained by their high capacity for GSH efflux rapidly removing any benefit that might be gained by having an elevated intracellular GSH content (Fig. 8B). We rather propose that GSH efflux is one of several cytoplasmic events activated during Fas/APO-1-stimulated cell death and that it contributes to optimal functioning of the overall process. Second, GSH export may be of nutritional significance for normal cells surrounding an apoptotic partner (the anti-FAS/APO-1 antibody-induced apoptosis studied in this paper is nonphysiological in the sense that almost all of the cells die in synchrony). GSH released into plasma would be a substrate for lymphocyte plasma membrane γ-glutamyl transpeptidase and thereby rapidly become available for re-uptake into other cells. Reducing agents (particularly 2-mercaptoethanol) are widely known to enhance the function of lymphoid cells in vitro (48). It is, therefore, possible that GSH efflux is valuable in locally stimulating the activity of phagocytic cells in vivo. Finally, as a minimum concentration of intracellular GSH is required for mitochondrial function (29), GSH depletion will eventually limit the ability of apoptotic cells to maintain ATP levels. As continued ATP production is required to maintain cell membrane integrity, GSH depletion may, therefore, contribute to an eventual lysis of apoptotic cells (this is often observed in vitro when phagocytes are absent).

GSH efflux after ligation of Fas/APO-1 on the surface of T lymphocytes may also be of physiological importance during viral infection. There is now convincing evidence that T lymphocytes infected with the human immunodeficiency virus exhibit an enhanced susceptibility to undergo apoptosis (49, 50). GSH levels in circulating T lymphocytes of patients infected with this virus are also reported to be significantly lower than normal (51, 52). This is usually attributed to an increase in the amount of oxidative stress experienced by these cells. For example, the Tat protein, known to be secreted from human immunodeficiency virus-infected cells, can permeate into uninfected lymphocytes and reduce both expression and activity of the mitochondrial enzyme manganese superoxide dismutase (53, 54). In JURKAT T lymphocytes this has been shown to correlate with decreased levels of intracellular GSH (54). However, in view of the recent finding that the Fas/APO-1 system may be activated during human immunodeficiency virus infection (55), it is also possible that an associated enhancement of GSH efflux contributes to a depletion of the tripeptide during this infection. Alternatively, lowering intracellular GSH levels (again perhaps by increasing efflux) could represent an innate response of lymphocytes to viral infection. The probability of the host cell undergoing apoptotic death following stimulation of the FAS/APO-1 receptor would thereby be increased and overall viral replication in the infected organism lowered.

In summary, GSH levels have been observed to fall coincident with the onset of apoptosis in different systems. We show that in a human T cell anti-Fas/APO-1 system accelerated GSH efflux is the major cause of GSH loss during apoptosis. Furthermore, we propose that a plasma membrane GSH transporter with properties resembling the hepatocyte canalicular membrane low affinity/high capacity GSH transporter is the channel responsible for this phenomenon. Further understanding of the mechanism and physiological significance of this event will contribute to the biochemical understanding of apoptotic cell death.

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