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The Phagocytosis-Associated Respiratory Burst in Human Monocytes Is Associated with Increased Uptake of Glutathione

Tamas Seres,* Roy G. Knickelbein,* Joseph B. Warshaw,* and Richard B. Johnston, Jr.2†

During the phagocytic respiratory burst, oxygen is converted to potent cytotoxic oxidants. Monocytes and macrophages are potentially long-lived, and we have hypothesized that protective mechanisms against oxidant stress are varied and fully expressed in these cells. We report here that the respiratory burst in monocytes is accompanied by an increase in the uptake of [35S]glutathione (GSH) after 20–30 min to levels up to 10-fold greater than those at baseline. By 30 min, 49% of the cell-associated radioactivity was in the cytosol, 41% was in membrane, and 10% was associated with the nuclear fraction. GSH uptake was inhibited by catalase, which removes hydrogen peroxide (H2O2), and micromolar H2O2 stimulated GSH uptake effectively in monocytes and also lymphocytes. Oxidation of GSH to glutathione disulfide with H2O2 and glutathione peroxidase prevented uptake. Acivin, which inhibits GSH breakdown by γ-glutamyl transpeptidase (GGT), had no effect on the enhanced uptake seen during the respiratory burst. Uptake of cysteine or cystine, possible products of GGT activity, stayed the same or decreased during the respiratory burst. These results suggest that a GGT-independent mechanism is responsible for the enhanced GSH uptake seen during the respiratory burst. We describe here a sodium-independent, methionine-inhibitable transport system with a Km (8.5 μM) for GSH approximating the plasma GSH concentration. These results suggest that monocytes have a specific GSH transporter that is triggered by the release of H2O2 during the respiratory burst and that induces the uptake of GSH into the cell. Such a mechanism has the potential to protect the phagocyte against oxidant damage.

Elimination of invading micro-organisms by neutrophils, monocytes, and macrophages depends heavily on the generation of reactive oxygen species during the phagocytosis-associated respiratory burst. The NADPH oxidase that mediates this process assembles in the plasma membrane, and toxic oxidants are released to the inside and outside of the cell (1). Thus, the oxidants created in this process, particularly superoxide anion (O2·−), hydrogen peroxide (H2O2), hypochlorous acid, and hydroxyl radical, carry the potential to damage the phagocytes themselves as well as other cells at sites of inflammation (2).

The intracellular reducing potential supplied by the thiol tripeptide glutathione (γ-glutamyl-l-cysteinylglycine (GSH)1) is believed to play an essential role in protecting cells against oxidant damage (3). We have published data indicating that GSH also serves as the key component in the respiratory burst-driven formation of cellular protein-mixed disulfides, a process termed S-thiolation (4–7). This reversible process can modify enzyme function and could protect protein sulphydryl groups from irreversible oxidative damage (4, 5). The importance of GSH to cell integrity is emphasized by experiments in which inhibition of GSH synthesis by l-buthionine-(S,R)-sulfoximine in adult mice or newborn rats led to mitochondrial degeneration, multiorgan failure, and death (3, 8).

Most cell types can metabolize extracellular GSH by an ectoenzyme, γ-glutamyl transpeptidase (GGT), which transfers glutamate to an amino acid acceptor. Cysteinylglycine is then hydrolyzed, and the amino acids are internalized independently (9, 10). The rate of transport and the intracellular availability of cysteine/cystine appear to control the rate of GSH synthesis (9–12). Transport of these amino acids is accomplished by well-characterized amino acid transporters (13, 14). More recently, distinct transport systems for GSH have been described on the canalicular and sinusoidal membranes of rat and human liver, and analysis of cell lines and tissues suggests that a transport mechanism also exists on certain other cell types (15–18).

The studies reported here were framed on the hypothesis that stimulation of the phagocytic respiratory burst induces increased uptake of cysteine, cystine, or GSH, individually or in combination. The results indicate that activation of the respiratory burst in human monocytes or exposure of the cells to H2O2 decreases the uptake of cysteine, does not affect cystine uptake, and induces a prominent increase in uptake of GSH.

Materials and Methods

Preparation of monocytes and lymphocytes

Mononuclear leukocytes were separated from heparinized blood of normal donors using lymphocyte separation medium (Organon Teknika, Durham, NC). Monocytes were washed and resuspended in DMEM (Life Technologies, Grand Island, NY), and 4 × 10⁶ cells were added to each 35-mm-diameter tissue culture dish. The cells were allowed to adhere for 2 h at 37°C in a humidified chamber containing 5% CO2. The adherent cell population contained at least 82–89% monocytes by microscopic examination after staining with Wright-Giemsa or esterase stains (Sigma, St. Louis, MO); other cells appeared to be lymphocytes. More than 98% of the...
The study of amino acid and GSH uptake

In control experiments we studied the effect on GSH uptake of inhibiting the PMA-stimulated respiratory burst with diphenyleneiodonium (DPI) (21, 22), DPI (ICN, Costa Mesa, CA) dissolved in DMSO (21) was added to a volume of 2 μl to render the cells sensitive to a final concentration of 0.1–2 μM. DPI was preincubated with the cells in Na+-HEPES buffer for 10 min. PMA (500 ng/ml) was added for an additional 10 min (O2− release) or 30 min (GSH uptake). DMSO alone (2 μl) had no effect on GSH uptake or O2− release. Monocyte viability (methylene blue exclusion) was 99% in buffer and 98% after incubation for 30 min in 2 μM DPI.

Subcellular localization of cell-associated radioactivity

We studied the subcellular localization of the respiratory burst-stimulated cell-associated 35S-labeled thiol by separating the cells into membrane, nuclear, and cytosolic fractions. After incubation of the cells with 131I-GSH and stimulation with PMA as described, the monocytes were scraped from the dishes and sonicated for 60 s (six 10-s bursts) on ice. An aliquot was taken to determine total counts per minute. Cell debris and nuclear material were pelleted from the remaining homogenate, using an Eppendorf centrifuge at 1,000 × g for 10 min. The supernatant was saved, and the pellet was further fractionated using Opti Prep, as described in the manufacturer’s booklet (Nycopord Pharma, Oslo, Norway). To isolate nuclei, the 1,000 × g pellet was homogenized in diluent A (8% w/v) sucrose, 25 mM KCl, 5 mM MgCl2, and 20 mM Tris, pH 7.8, and a second centrifugation was performed at 1,000 × g for 10 min. The pellet was layered on the 25/30/35% (w/v) discontinuous gradient of Opti Prep and centrifuged at 10,000 × g for 20 min. The other monolayer was collected from a distinct band at the 30/35% interface. The supernatant, saved after the first centrifugation at 1,000 × g, was centrifuged at 100,000 × g for 30 min, and the radioactivity of the membrane fraction (pellet) was measured by directly resuspending it in scintillation fluid. The radioactivity remaining in the supernatant represented the nonmembrane-bound cytosolic thiol. This supernatant was treated with 10% (final concentration) TCA, then centrifuged at 14,000 × g for 10 min to precipitate soluble proteins and any protein-bound thiol. Radioactivity in the TCA supernatant represented free thiol.

Each cell fraction was studied to verify the success of the separation procedure. We found 93% (mean; n = 3) of the total activity of GGT in the membrane fraction, consistent with the known localization of this enzyme (19, 23). In contrast, 99% (mean; n = 3) of the total activity of glucose-6-phosphate dehydrogenase (19) was located in the cytosolic fraction.

Release of radioactivity from monocytes

Monocytes were incubated with L-[35S]GSH with or without stimulation with PMA for 30 min as described above. The reaction was stopped by removing the medium and washing the cells four times. The cells were then incubated for 10 min at 37°C in HEPES buffer with or without 1 mM diithioerythritol (DTE) to release plasma membrane protein-bound radioactivity by reducing disulfide bonds. Radioactivity released into the extracellular medium and that which remained associated with the washed adherent cells was determined. The uptake and release of GSH was presented as picomoles per milligram of protein, as described above.

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The relationship between the PMA-induced respiratory burst and GSH uptake was also studied after inhibition of the respiratory burst by preincubation of the monocytes for 10 min with DPI (21, 22). Release of $O_2^-$ and uptake of GSH were inhibited to an equivalent extent over a range of DPI concentrations of 0.2–2 μM (Fig. 2C). Preincubation for 10 min with 1 μM DPI had no effect on the increased GSH uptake stimulated by 0.5 mM H$_2$O$_2$ (without DPI; $p = 0.04$) and a significant increase in the uptake of GSH (with DPI; $p < 0.001$); the reduction in Cys$_2$ uptake was not significant.

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Both Na$^+$-dependent and Na$^+$-independent mechanisms have been described for cystine, cysteine, and GSH uptake. To explore these mechanisms in monocytes, uptake was measured in HEPES buffer in which sodium chloride was replaced with choline chloride. The effect of stimulating the respiratory burst with PMA was similar to the effect in Na$^+$-HEPES buffer; there was a decrease in uptake of cysteine (44%), a minimal effect on cystine uptake, and a stimulation of GSH uptake by 4.9-fold during the respiratory burst. Almost all (93%) the GSH uptake was sodium independent (Fig. 3, compared with Fig. 1). Under basal (unstimulated) conditions, about 60% of GSH uptake was Na$^+$ independent.

Transporters for cysteine, cystine, or both can be differentiated by their substrate and inhibitor specificity and whether Na$^+$ is necessary for transport activity. We explored cystine and cysteine uptake (5 min, 37°C) in monocytes under basal culture conditions by determining transport in the presence of these selective inhibitors (14). Cystine transport was predominantly (72%) Na$^+$ independent (Figs. 1 and 3). This transport was inhibited 43 ± 13% (mean ± SEM; $n = 4$) by quisqualate, a specific inhibitor of system x$_c$, a glutamate/cystine exchanger. Sodium-dependent cystine

**Results**

**Thiol uptake in human monocytes during the respiratory burst**

Under basal culture conditions, in Na$^+$-HEPES buffer the uptake of cysteine over 30 min was 4.3-fold higher than that of cystine and 10.3-fold higher than that of GSH (Fig. 1). The combined cysteine and cysteine uptake represented 93% of the thiol taken up in these experiments. In the presence of PMA cysteine uptake decreased by 70%, whereas cystine uptake was not significantly altered. In contrast, GSH uptake was increased 3.1-fold (Fig. 1).

Total thiol uptake decreased during the respiratory burst because of the marked decrease in cysteine uptake.

**Data presentation**

All data shown are the mean ± SEM of $n$ experiments, each performed in duplicate or triplicate. Statistical significance was determined using Student’s $t$ test unless otherwise specified.
transport was inhibited 83 ± 12% (n = 4) by l-aspartic acid-β-hydroxamate, a specific inhibitor of system XAG, which also transports aspartate and glutamate. Cysteine transport was only 24% Na+-independent (Figs. 1 and 3). Most (82 ± 2%; n = 4) of the Na+-dependent transport was inhibited by serine, implicating system ASC. The Na+-independent cysteine transport was inhibited by 2-aminobicyclo-[2.2.1]-heptane-2-carboxylic acid (46 ± 3%), indicating the participation of system L, a transporter that carries a number of amino acids, including cysteine (14).

Characterization of Na+-independent GSH uptake

The effect of the respiratory burst on uptake of GSH in Na+-free buffer was studied during phagocytosis of a serum-opsonized particle (OZ) (20). The relationship between the OZ-stimulated respiratory burst and GSH uptake as a function of time (Fig. 4) was similar to that shown with PMA as stimulus in Na+-HEPES buffer (Fig. 2A). The characteristically slower rate of O2 release with OZ compared with that using PMA (20) was associated with a longer interval before decline in GSH uptake (30 min with OZ, Fig. 4; 20 min with PMA, Fig. 2A). In these experiments GSH uptake during the respiratory burst was 10.2 times higher than uptake under basal conditions (control).

During the respiratory burst, superoxide anion is converted to the more stable and membrane-permeable oxidant, H2O2. Uptake of GSH during the PMA-induced respiratory burst was consistently (but not statistically significantly) further enhanced by SOD, which accelerates H2O2 production (Fig. 5). In contrast, catalase, which removes H2O2, reduced GSH transport during the respiratory burst almost to the level of the control (no PMA).

These results suggested that among the oxygen derivatives of the respiratory burst, H2O2 plays a particularly important role in inducing GSH uptake. In support of this conclusion, H2O2 in micromolar concentrations induced a concentration-dependent enhancement of GSH transport to levels over 7 times greater than those at baseline (Fig. 6). In experiments not shown the time course of this effect was similar to that seen with the PMA-induced respiratory burst, with GSH uptake reaching a maximum after 15 min of exposure, then decreasing slowly (by ~15%) by 30 min (n = 3). The highly reactive H2O2 derivative sodium hypochlorite (NaOCl; Sigma; 0.05 mM) did not enhance GSH uptake significantly during a 30-min incubation: NaOCl, 35.1 ± 6.5 pmol/mg; 0.05 mM H2O2, 214 ± 28 pmol/mg; control, 27.5 ± 6.6 pmol/mg (n = 3).

Because the GSH used for uptake studies was radiolabeled on the cysteinyl moiety, it was necessary to demonstrate that we were measuring the uptake of intact GSH and not cysteinylglycine or
Results are mean ± SEM of three experiments performed in duplicate. Measured radioactivity was significantly higher in each of the three fractions from cells exposed to PMA (p < 0.005 to p < 0.05).

cysteine released by the action of GGT, then dipeptidase. Therefore, cells were preincubated for 10 min with acivicin, an irreversible inhibitor of GGT, before exposure to PMA. As shown in Fig. 7, acivicin preincubation resulted in a modest (14%) decrease in GSH uptake in unstimulated cells, but no change in the enhancement associated with the respiratory burst, suggesting that GSH is being transported in intact form, and that GGT is not involved in the increased GSH uptake seen during the respiratory burst.

We explored the possibility that H2O2 stimulated GSH uptake by oxidizing GSH to GSSG, which, in turn, was taken up preferentially. When uptake was studied in the presence of glutathione peroxidase (1 U/ml) and H2O2 (10 μM; 30 min) to convert GSH to GSSG, uptake of [35S]GSH was less than uptake at baseline (no stimulus or H2O2 added; 9 ± 1 pmol/mg protein vs baseline, 20 ± 3 pmol/mg; mean ± SEM; n = 3). In these experiments, H2O2 alone enhanced uptake of GSH, as shown in Fig. 6, and glutathione peroxidase alone had no appreciable effect. A similar effect (mean 70% reduction below baseline; n = 3) was noted when the GSH was preincubated with the strong oxidant diamide (50 μM).

Distribution of GSH taken up by human monocytes during oxidant stress

We explored the cellular distribution of radioactivity in monocytes after a 30-min incubation in Na+-free HEPES buffer with [35S]GSH under basal conditions or stimulated with PMA (Fig. 8).

In unstimulated cells, most (78%) of the cell-associated radioactivity was in the cytosol, with 18% being in the membrane and 4% in the nuclear fraction (n = 3). After stimulation with PMA, there was an almost 6-fold increase in total radioactivity. The cellular distribution of radioactivity was different from that under basal conditions, with equivalent levels being achieved in the cytosol and membrane fractions (49 and 41% of the total, respectively) and a smaller amount localizing in the nuclear fraction (10%; Fig. 8).

Thiol in the cytosol under basal conditions and after PMA stimulation was further separated into TCA-soluble and -precipitable fractions; the respiratory burst increased the level of cytosolic free GSH by 2.1-fold and cytosolic protein-bound GSH by 3.4-fold (mean; n = 3), in agreement with our previous studies showing avid binding of GSH to cytosolic proteins during the respiratory burst (4–7).

In an attempt to probe further the differentiation of internalized thiols from extracellular binding, we allowed GSH to associate with the cell for 30 min in basal or PMA-stimulated conditions. After washing the cells as usual, the ability of 1 mM DTE to reduce disulfide bonds and release radioactivity into the supernatant during a 10-min incubation was taken as a measure of extracellularly bound GSH (Table I). The total cell-associated radioactivity before the incubation with buffer or DTE was increased about 7-fold during the respiratory burst. There was only a minor (3%) difference in the amount of radioactivity released into the supernatant of stimulated cells by buffer compared with DTE, in agreement with the concept that cell-associated radioactivity represents internalized thiol.

Table I as well as Figs. 2A and 4 show that there was gradual efflux of radiolabel after peak uptake had occurred. Thus, uptake (accumulation of radiolabel) is a net value, although efflux appears to be small during the initial 30–40 min.

Kinetics of GSH uptake

If activation of a transport protein were responsible for the increase in GSH entering the cell during the respiratory burst or exposure to H2O2, then uptake might be expected to follow Michaelis-Menten kinetics. Consistent with this premise, uptake was found to be a saturable event, with a similar high affinity (Km) for GSH in both the presence and the absence of added H2O2 (Table II). The Km demonstrated was in the range of the concentration of GSH in plasma under physiologic conditions (15, 24, 25). In contrast, the Vmax was increased 11-fold in the presence of H2O2.

![Graph showing uptake and distribution of GSH in monocytes during the respiratory burst.](http://www.jimmunol.org/)

**FIGURE 8.** Uptake and distribution of GSH in monocytes during the respiratory burst. Monocytes were preincubated with l-[35S]GSH in Na+-free HEPES buffer with or without PMA for 30 min. The reaction was stopped by scraping the monocytes from the dishes and sonicating them in Eppendorf tubes for 60 s (six 10-s bursts) on ice. An aliquot was taken to determine total counts per minute. The cell debris and the nuclear material were centrifuged at 1,000 g for 10 min. The supernatant was separated into the membrane fraction, obtained as the pellet after centrifugation at 100,000 g for 30 min, and the supernatant, which represented the cytosolic fraction. Nucleus-associated GSH was separated from the cell debris as described in Materials and Methods. Results represent the mean ± SEM, for at least three different monocyte preparations. Uptake of GSH was studied in the absence of Na+. Uptake was determined for at least five different substrate concentrations. Km was calculated from Lineweaver-Burk plots, and Vmax was calculated by using the Dixon plot. BSP, Bromsulfophthalein.

| Table II. Effect of H2O2 and transport inhibitors on GSH uptake in human monocytesa |
|---------------------------------|-----------------|
| GSH (-)                        | H2O2 (+)        |
| Km (μM)                        |                 |
| 6.1 ± 0.3                      | 8.5 ± 2.8       |
| Vmax (pmol/mg/min)             |                 |
| GSH                            |                 |
| 3.8 ± 1.8                      | 42 ± 9.0        |
| K (μM)                         |                 |
| GSSG                           |                 |
| 111.8 ± 45.5                   | 53.6 ± 12.3     |
| Methionine                     |                 |
| –                              | 18.5 ± 4.5      |
| BSP                            |                 |
| –                              | 77.5 ± 1.5      |

a Results are means ± SEM for transport studied in at least three different monocyte preparations. Uptake of GSH was studied in the absence of Na+. Uptake was determined for at least five different substrate concentrations. Km was calculated from Lineweaver-Burk plots, and Vmax was calculated by using the Dixon plot. BSP, Bromsulfophthalein.

*PMA was used to stimulate the respiratory burst in monocytes for 30 min in the presence of radiolabeled GSH, as described in Materials and Methods. After washing four times, the cells were exposed to HEPES buffer with or without DTE. 1 mM for 10 min at 37°C, and radioactivity was measured in the supernatant and in adherent cells. Results are mean ± SEM, n = 3.

**Table I.** Estimation of thiol bound to the outside surface of the plasma membrane using disulfide reducing agent DTEa

<table>
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<th>Thiol Content (pmol/mg)</th>
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<td>Unstimulated</td>
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INCREASED GSH UPTAKE IN MONOCYTE RESPIRATORY BURST

The role of H\textsubscript{2}O\textsubscript{2} or the respiratory burst induced by PMA in GSH uptake by lymphocytes (Fig. 9). PMA did not induce superoxide anion release in our lymphocyte preparations; therefore, the direct effect of PMA on GSH uptake could be evaluated. Hydrogen peroxide caused a significant increase in GSH uptake in both purified monocytes (p < 0.002) and purified lymphocytes (p < 0.02). In contrast, the respiratory burst induced by PMA caused a 4-fold increase in GSH uptake in monocytes, but PMA had no effect on GSH uptake by lymphocytes.

**Discussion**

During the process of phagocytosis professional phagocytes undergo a vigorous respiratory burst in which they consume oxygen and convert it enzymatically to O\textsubscript{2}\textsuperscript{-}, which interacts to form H\textsubscript{2}O\textsubscript{2}, then hypochlorous acid, hydroxyl radical, and other potent oxidants. It has been recognized that this process is accompanied by activation of the hexose monophosphate shunt and oxidation of GSH to GSSG through the action of GSH peroxidase and H\textsubscript{2}O\textsubscript{2} (26). GSSG is cycled back to GSH by glutathione reductase. Data from a variety of experimental systems indicate that the redox cycling of GSH plays an important role in protecting the cell against oxidant damage (26, 27). We report here that the respiratory burst-induced increase in GSH uptake was inhibited by the strong oxidant diamide and was not increased by hypochlorous acid support the conclusion that the H\textsubscript{2}O\textsubscript{2}-induced cell-associated radioactivity is due primarily to uptake, not oxidant-stimulated mixed disulfide formation with plasma membrane ectoproteins. In addition, incubation of the PMA-stimulated cells with DTE, which would release externally bound thiols, elicited only a few more counts per minute than did buffer alone (Table I).

Uptake of GSH against a concentration gradient would be expected to require active transport. The data reported here support the existence of a Na\textsuperscript{+}-independent GSH transporter on human monocytes. A transport mechanism for GSH has been described in several cell types, including human platelets and canalicular and sinusoidal membranes of rat and human liver (15–18, 32–35). GSH transport in various mammalian cell lines can be bidirectional, at least under certain conditions (33, 35). The Na\textsuperscript{+}-independent activity, redox regulation, and inhibition by methionine and bromsulphthalein that we found with monocyte GSH transport are characteristic of the liver sinusoidal GSH transporter (32, 35, 36), but the K\textsubscript{m} of this hepatic transporter is in the millimolar range, as would be appropriate for its mediating GSH efflux from the liver into the bloodstream. We found monocytes to have a transport mechanism with a considerably higher affinity for GSH (K\textsubscript{m} = 6–9 \textmu M), which was close to the GSH concentration in plasma. A high affinity transporter for GSH has also been described in human platelets (34).

The rate of the respiratory burst-associated uptake of GSH closely approximated the rate of O\textsubscript{2}\textsuperscript{-} release; both peaked at 5 min (Fig. 2B); accumulated GSH uptake after addition of H\textsubscript{2}O\textsubscript{2} peaked at 15 min. SOD, which removes O\textsubscript{2}\textsuperscript{-} by catalyzing its dismutation into H\textsubscript{2}O\textsubscript{2}, increased GSH uptake slightly, whereas catalase reduced the stimulatory effect of PMA by >80% (Fig. 5). H\textsubscript{2}O\textsubscript{2}, which can cross cell plasma membranes relatively efficiently (37), in plasma and are available for transport (24, 25). Cysteine and cystine transport has been shown to be essential for GSH synthesis in lymphocytes, macrophages, and other cells (29, 30).

We report here that under basal conditions human monocytes take up cysteine primarily by a Na\textsuperscript{+}-dependent system (Fig. 1 vs Fig. 3). This system appears to be system ASC, based on the defining inhibition studies (9, 14) as used by us previously with alveolar type II cells (14). System L appeared to play a lesser role in monocyte uptake of cysteine. In contrast, transport of cystine was predominantly (72%) Na\textsuperscript{+} independent and mediated at least in part by system X\textsubscript{c}. In the presence of Na\textsuperscript{+}, cystine was transported primarily by system X\textsubscript{AG}. This finding supports the conclusion of Rimaniol et al. (31) that human mononuclear phagocytes possess a system X\textsubscript{AG}, cystine/glutamate transporter. They have proposed that this transporter might clear neuroexcitatory glutamate in the brain. The changes induced by the respiratory burst in uptake of all three thiols were similar in the presence and the absence of Na\textsuperscript{+} (Figs. 1 and 3).

Our data suggest that the respiratory burst- and H\textsubscript{2}O\textsubscript{2}-induced increase seen in uptake of GSH, which was \textsuperscript{35}S-radiolabeled in the Cys moiety, was due primarily to transport of intact GSH. Preincubation of the cells with acivicin, which inhibits GSH breakdown by \gamma-glutamyl transpeptidase (19), had no effect on the H\textsubscript{2}O\textsubscript{2}-induced GSH uptake (Fig. 7). Uptake of Cys and Cys\textsubscript{2}, possible thiol products of GSH breakdown, was decreased or did not appreciably change during the respiratory burst (Figs. 1 and 3). Oxidation of \textsuperscript{35}S[GSH to \textsuperscript{35}S[GSSG by preincubation with H\textsubscript{2}O\textsubscript{2/} GSH peroxidase or diamide reduced uptake of the thiol to 30–50% of the baseline (no H\textsubscript{2}O\textsubscript{2}) value, in agreement with the higher K\textsubscript{m} for GSSG than for GSH found on kinetic analysis (Table I). In these experiments we cannot rule out a direct effect of the diamide on the cell, but the facts that \textsuperscript{35}S[GSH uptake was inhibited by the strong oxidant diamide and was not increased by hypochlorous acid support the conclusion that the H\textsubscript{2}O\textsubscript{2}-induced cell-associated radioactivity is due primarily to uptake, not oxidant-stimulated mixed disulfide formation with plasma membrane ectoproteins. In addition, incubation of the PMA-stimulated cells with DTE, which would release externally bound thiols, elicited only a few more counts per minute than did buffer alone (Table I).

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stimulated rapid and vigorous GSH uptake when added to the out-
side of the cells in micromolar concentrations. Added hypochlor-
ous acid had a negligible stimulatory effect. Thus, respiratory
burst-associated GSH uptake appears to be driven primarily by 
H$_2$O$_2$.

Small amounts of radiolabeled GSH probably entered in PMA-
induced pinocytic vesicles and OZ-induced phagocytic vacuoles,
but most of the GSH uptake induced by PMA was inhibited by
catalase, which removes H$_2$O$_2$ (Fig. 5), and by DPI, which inhibits
the respiratory burst (Fig. 2C). DPI did not inhibit the increased GSH
uptake induced by H$_2$O$_2$. In addition, PMA stimulated se-
lective uptake of GSH, not cysteine, cystine, or GSSG.

The mechanism by which H$_2$O$_2$ drives such a profound and
rapid uptake of GSH remains to be defined. Rapid modulation of
signal transduction is a likely explanation. The phagocytic respi-
atory burst and other systems of oxidant stress have been related to
modification of signaling in a variety of systems (38–44). H$_2$O$_2$
in particular has been reported to inhibit tyrosine phosphoryl-
ase activity (39–41), to stimulate tyrosine phosphorylation (42, 43), and
to stimulate the activity of mitogen-activated protein kinase (42)
and protein kinase C (44). H$_2$O$_2$ has also been reported to influence
the activation of transcription factors, including NF-kB and AP-1
in mammalian cells (42, 45) and OxyR in Escherichia coli (46),
and to activate RBC KCl cotransport (47).

It is not clear what survival advantage might be offered by this
rapid uptake of GSH and by the shift from uptake primarily of
cysteine/cystine to uptake of GSH. We reported previously that
rapid uptake of GSH and by the shift from uptake primarily of
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Inflammation, and host defense. These include activation in T cells
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protect specific monocyte proteins against oxidant damage.

Sulfides, with a peak at 5 min (4). The de-
rapid decrease in intracellular GSH, with a nadir at 10 min of PMA
stimulation of the respiratory burst in human monocytes induced a
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In addition to protecting against oxidant damage, GSH has been
shown to play a protective role in maintaining the functional integrity of nuclear and mitochond-
dral DNA (48) and in control of apoptosis (49–51). It seems pos-
ible that the amount of GSH taken up during the respiratory burst,
although small relative to the total intracellular GSH concentra-
tion, could appear at the right place in the cell at the right time to
protect specific monocyte proteins against oxidant damage.

In addition to protecting against oxidant damage, GSH has been
reported to play a central role in regulating a large number of biologic systems that are fundamental to the immune response,
inflammation, and host defense. These include activation in T cells
of NF-kB, IL-2-dependent functions including cell proliferation,
and cellular cytotoxicity (52); synthesis of PGE$_2$ and leukotriene C
by macrophages (53); detoxification of xenobiotics through gluta-
thione S-transferase reactions (9); inhibition of apoptosis in mono-
cytes, lymphocytes, and neutrophils (49–51); and replication of
HIV (54). It seems reasonable to speculate that the location and
timing of increased GSH concentration might also be important to
protecting and maintaining these regulatory systems, and thus cell
function.

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thiolation during the respi-
ration burst subsides (4). Thus, S-thiolation represents a form of
redox buffering that could protect proteins against oxidant dena-
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