Mononuclear phagocytes (MPs; dendritic cell, monocyte, and tissue macrophage) are among the first lines of host defense against an invading pathogen and represent the most critical cell type that serves to contain or eliminate infection (1, 2). What governs initial clearance of any organism is innate immunity mediated in part through monocyte-macrophage differentiation and activation. In this regard, a body of literature demonstrates that MPs are the principal elements in clearance and inactivation of many microbial pathogens while acting, paradoxically and in parallel, as a major target and cell reservoir for persistent infection (3). Such a paradox is underscored by mycobacterial infections and in particular tuberculosis, which shows the uncanny ability to persist in MP lineage cells for periods measured in decades (4, 5). Eradicating this nidus of infection, in the human host, remains a global health problem. The significance cannot be understated as tuberculosis is responsible for more deaths in the developing world than can be attributed to any other single infectious organism (6). The pathogenesis of this disease starts from the respiratory route with aerosol being the main portal of microbial dispersion (7). Once the organism lodges in the lung, mycobacterium encounters the MP, and a variety of pathological outcomes follow dependent on microbial evolution and complex immune responses that occur subsequently (8). These include the engagement of surface receptors (specific for binding and pathogen entry), phagosome-lysosome fusion, and direct mycobacterial killing. Only later in the course of an immune response do adaptive immune mechanisms become operative and involve secretion of pro-inflammatory and regulatory cytokines resulting from presentation of antigens to T cells. Clearance follows as a terminal outcome.

Redox metabolism is critical for innate immune function and tissue homeostasis (9). In the past decade its role in critical MP processes, in microbial surveillance, and in affecting the tempo of inflammatory degenerative disorders has been realized (3, 10). Nonetheless, a missing piece to the puzzle linking redox metabolism and disease is how it can be harnessed for therapeutic benefit in favor of the host. We reasoned that since MPs are the first line of defense against invading microbes, the conductor of final immune outcomes of host cell-pathogen interactions, and instigators of inflammatory activities, redox pathways could influence a range of macrophage functions that affect the outcome of pathogen-cell interactions (10). To explore this possibility common monocyte-macrophage activities including cell differentiation, immune activation, and intracellular pathogen killing were investigated for their links to...
redox metabolism. Macrophages produce prooxidants in abundance (9). GSH is a major intracellular antioxidant whose biosynthesis is limited by cysteine (11). The latter can be procured via transport or synthesized via transsulfuration in a pathway that converts the essential amino acid, methionine, to cysteine (Fig. 1). An important junction intermediate in this metabolism is homocysteine, which is either diverted to cysteine or recycled via transmethylation to methionine in a cycle that also furnishes S-adenosylmethionine (AdoMet), a major methyl group donor. The enzymes at the homocysteine junction that control its intracellular flux are methionine synthase (MS) and cystathionine β-synthase (CBS). The transsulfuration pathway is a quantitatively significant supplier of cysteine utilized for GSH synthesis (12, 13). Diminution of the intracellular GSH pool size decreases cell survival, enhances HIV and mycobacterial replication, and increases sensitivity to tumor necrosis factor α-induced cell death (14, 15). Interactions between microbes and MPs are the net result of a complex series of intracellular redox events that determine whether the pathogen evades or succumbs to the immune response (9). However, despite the physiologically important ramifications of redox homeostasis to MP function and immune modulation, fundamental questions regarding the mechanism of redox regulation in these cells remain unanswered.

In this study, we report that both the transmethylation and transsulfuration pathways are strongly induced during differentiation of monocytes and that this paralleled increases in the pathway-associated metabolites, AdoMet and GSH. Activation of monocytes or macrophages with lipopolysaccharide (LPS) or infection with Mycobacterium smegmatis diminished expression of CBS and MS but increased cysteine and GSH levels 300% and ~100%, respectively, compared with untreated controls. Pharmacological blockade of the transsulfuration pathway by propargylglycine (PPG), diminished the efficiency of bacterial clearance, while supplementation with N-acetylcysteine (NAC), a cysteine prodrug, increased the efficiency of bacterial killing by 3-fold. Furthermore, PPG inhibited while NAC enhanced phagolysosomal fusion revealing a mechanistic link between cysteine status and bacteriocidal activities. Modulation of the transsulfuration pathway during differentiation, activation, and infection represents a heretofore unrecognized strategy deployed by macrophages and represents a potential target for therapeutic intervention.

**EXPERIMENTAL PROCEDURES**

Preparation of Monocytes and Culture—Peripheral blood mononuclear cells were isolated from HIV-1- and -2 and hepatitis seronegative human donors by leukopheresis and purified by countercurrent centrifugal elutriation (16). Purified monocytes (1 × 10⁶ cells/ml) were cultured in media-A (Dulbecco’s modified Eagle’s medium (Invitrogen)) supplemented with 10% heat-inactivated pooled human serum, 2 mM glutamine, 50 μg/ml gentamicin (Sigma), 10 μg/ml ciprofloxacin (Sigma) and 1000 units/ml highly purified recombinant human macrophage colony-stimulating factor (a generous gift from Wyeth, Inc., Cambridge, MA). Cells were maintained at 37 °C with 5% CO₂. Since detachment of a minority of cells (<10%) was observed, floating and adherent cells were combined during harvesting in all experiments.

Fluorimetric Assay Using Cathepsin B—Cells were incubated in the presence or absence of 2.5 mM PPG for 24 h. Following incubation, cells were washed twice with PBS and stained with 26X MR-Cathepsin B for 1 h as per the manufacturer’s protocol (Immunochemistry Technologies). Labeled cells were detached by gentle pipetting on ice and transferred to a 96-well plate, and fluorescence was measured on a fluorimeter equipped with an excitation filter of 550 nm (540–560 nm) and emission filter of 600 nm (580–620 nm). The number of cells in each sample was determined by labeling nuclei with Hoechst stain (excitation 365 nm, emission 480 nm) and expressing the results after normalization (F₆₀₀ nm/F₄₈₀ nm).

Western Blot Analysis— Cultured cells were harvested at 24 h intervals over a 7-day period. LPS, when used, was administered as a bolus to a final concentration of 10 ng/ml at day 0. Cells were washed three times with cold PBS, resuspended in PBS, and then harvested and lysed by suspending in lysis buffer as described previously (17, 18). MS, CBS, and actin levels in cell extracts were determined using the respective primary and secondary antibodies and detected using the chemiluminescent horseradish peroxidase system (Sigma) as described previously (17, 18). The intensity of protein bands was quantified using the Quantity One software (Bio-Rad) and normalized to the actin level in the same sample.

Northern Blot Analysis—RNA was isolated using an RNAqueous kit (Ambion), and 5–7 μg of total RNA from each sample was used. Probes for CBS and MS and the procedure used for Northern blot analysis have been described previously (19, 20). Densitometric analysis was performed using Quantity One software. To account for variations in loading, band intensities were normalized to the 18 S rRNA in each sample, as visualized by ethidium bromide staining.

Metabolite Analysis—AdoMet, cysteine, GSH, and GSSG were separated from cells extract and quantified by high performance liquid chromatography as described previously (21). When [³⁵S]methionine was used to label cells, incorporation of radiolabel was determined by measuring radioactivity in the
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chromatographic fractions corresponding to the specific thiol compounds. The concentration of each metabolite was determined using a calibration curve generated for each compound. Results were normalized to protein concentration in each sample.

Phospholipid Methylation—Every 24 h between day 0 and 6 before harvesting, cultured cells were washed with Hanks’ balanced salt solution and incubated for 1 h in Hanks’ balanced salt solution containing 1 μCi/ml [14C]formate (Amersham Biosciences). The reaction was terminated, cells were harvested, and phospholipids were isolated by following a procedure described previously (22). Radioactivity recorded as disintegrations/min was normalized to the protein concentration in each sample.

Infection of Human Monocytes and Macrophages with M. smegmatis—M. smegmatis mc² was grown on Middle-brook 7H9 broth (BD Biosciences) supplemented with 10% albumin, dextrose, and catalase (ADC), and the titer was determined before infection by growing the bacteria on 7H10 plates supplemented with 10% ADC. Cells were infected at either day 1 or 7 in culture in a 24-well plate. Before infection, the cell culture medium was changed with fresh media-A lacking antibiotics. Cells were then infected for 3 h with M. smegmatis at a multiplicity of infection of 1. Following infection, non-phagocytosed bacilli were removed by washing cells three times with Dulbecco’s modified Eagle’s medium before resuspending in media-A without antibiotics, followed by incubation with the desired additives (1 mM NAC, 10 ng/ml LPS, 2.5 mM PPG or both NAC and PPG) for 24 h as indicated in the figure legends. The colony-forming unit (CFU) assay was performed as described previously (23). In brief, 24 h after incubation, cells were lysed using ice-cold sterile PBS containing 0.1% saponin and serially diluted in PBS containing 0.01% Tween 80 (Sigma) and plated in triplicate on Middle-brook 7H10 agar. M. smegmatis colonies were counted after growth for 3 days at 37 °C.

For metabolite analysis, following infection and removal of non-phagocytosed bacilli (as described above), cells were incubated with [1-35S]methionine to a final concentration of 2 μCi/ml in the presence or absence of 2.5 mM PPG or 3 or 8 h. Cells were harvested and analyzed for thiols as described above.

Addition of NAC, PPG, or LPS did not affect the viability of either the cells (determined by the methylthiazoIyldiphenyltetrazolium bromide assay (24)) and/or the bacteria (determined at 600 nm) (data not shown). The efficiency of phagocytosis of mycobacteria was evaluated by performing the CFU assay on media collected 3 h following infection. Approximately 90% of mycobacteria were phagocytosed in control and treated cells (data not shown).

Confocal Microscopy—The degree of maturation of M. smegmatis-containing phagosomes was assessed using confocal microscopy by colocalization of bacilli stained with auramine and lysosomes stained with the acidophilic dye, Lysotracker Red (Molecular Probes). Following infection and removal of non-phagocytosed bacilli (as described above), macrophages were incubated in media-A lacking antibiotics and stimulated with different additives for 24 h as indicated in the figure legends. At 24 h postincubation, Lysotracker Red in media-A containing 20 mM HEPES (pH 7.4) was added at a 1:3000 dilution and incubation was continued for 1 h at 37 °C. Cells were washed to remove unincorporated dye, and samples were prepared for microscopy following the protocol previously described (21). Confocal fluorescence microscopy was performed on an FN 500 Olympus confocal microscope. Dual display images were collected sequentially at a magnification of ×100 using an argon laser (excitation, 488 nm; emission, 522 nm) for detection of auramine fluorescence and a helium-neon laser (excitation, 543 nm; emission, 598 nm) for detection of Lysotracker Red. Neither PPG nor NAC directly affected the fluorescence of auramine or Lysotracker Red (data not shown).

RESULTS

MS and CBS Expression Are Up-regulated during Monocyte-Macrophage Differentiation—Cultivation of monocytes leads to differentiation into macrophage-like cells, which is accompanied by marked morphological and physiological changes (25, 26). In this study, we followed the activity of cathepsin B, a lysosomal protease, which increased linearly during the first 5 days of monocyte cultivation (data not shown). Changes in the homocysteine junction enzymes, MS and CBS, were assessed during monocyte-macrophage differentiation. Western blot analysis revealed that both enzymes were undetectable in monocytes but were induced during differentiation (Fig. 2A). MS could be visualized starting from day 2 and continued to increase over a 7-day period. CBS was also detected from day 2 and reached a steady-state level by day 3. A quantitative analysis of the time-dependent increase in MS and CBS expression is shown in Fig. 2B.

Northern blot analysis revealed that while increased expression of CBS protein is correlated with changes in its mRNA level, changes in MS were not (Fig. 2, C and D). Furthermore, CBS mRNA levels decreased after day 6, although protein levels were unchanged. Loss of correlation between mRNA and protein levels at later time points is likely related to the stability of CBS, which is reported to have a half-life of 49 h in liver cells (19).

Changes in Pathway-relevant Metabolites during Monocyte Differentiation—The effect of CBS and MS induction on the intracellular pool of AdoMet, cysteine, and GSH was determined (Fig. 3). Intracellular AdoMet level increased 150% from 0.2 ± 0.1 μmol/g of protein (on day 0) to 0.5 ± 0.15 μmol/g of protein (on day 6) in culture. This was accompanied by a 150% increase in phospholipid methylation measured by incorporation of radiolabel from [14C]formate into cell lipids (Fig. 3B). Under these conditions, the radiolabel in AdoMet is derived from methionine formed via the transmethylation reaction catalyzed by MS (Fig. 1). Hence increased phospholipid methylation is a reflection of increased incorporation of methyl groups derived via the MS reaction into lipids.

Intracellular GSH concentration also increased 170% from 26 ± 6 μmol/g of protein (on day 0) to 69 ± 8 μmol/g of protein (on day 4), during monocyte differentiation, whereas cysteine increased transiently at day 1 and remained at initial levels (~11 μmol/g of protein) thereafter (Fig. 3C). The [GSH]/[GSSG] ratio, an index of the intracellular redox status, exhibited a sharp increase from 30 ± 13 on days 0–1 to 170 ± 36 on day 2 and remained high thereafter (Fig. 3D).
transsulfuration flux. To assess the contribution of the endogenous transsulfuration flux, the intracellular GSH was examined. No significant change in either metabolite was observed in infected monocytes (day 1) versus uninfected controls (data not shown). In contrast, a 100 and 33% higher concentration of cysteine and GSH was observed in infected macrophages (day 7) compared with uninfected controls.

**Host Transsulfuration Pathway Is Activated during Mycobacterial Infection**—Next, the effect of *M. smegmatis* infection on cysteine and GSH was examined. No significant change in either metabolite was observed in infected monocytes (day 1) versus uninfected controls consistent with the presence of very low levels of CBS in cells at this stage (data not shown). In contrast, a 100 and 33% higher concentration of cysteine and GSH was observed in infected macrophages (day 7) compared with uninfected control (Fig. 5, A and B). In principle, the observed increase in cysteine could result from stimulation of cystine uptake and/or activation of the transsulfuration flux. To assess the contribution of the endogenous pathway to accumulation of cysteine, infected cells were exposed to PPG, a suicide inactivator of cystathionine γ-lyase (Fig. 1). Cysteine and GSH accumulation was inhibi-
human monocytes and macrophages were infected with *M. smegmatis*, and intracellular mycobacterial viability was monitored using the CFU assay after 24 h (Fig. 6). Addition of PPG to monocytes (day 1) did not have a significant effect on intracellular mycobacterial viability compared with untreated controls (Fig. 6A). However, when macrophages (day 7) were employed, PPG treatment increased bacterial viability ~2-fold (Fig. 6B). Interestingly, circumvention of the inhibition imposed by PPG with NAC enhanced intracellular clearance of mycobacteria 3-fold compared with untreated controls. The effect of PPG was negated by NAC when the two compounds were coadministered.

**Blockade of Transsulfuration Arrests Phagosome-Lysosome Fusion**—Since phagolysosome biogenesis is an important microbicidal mechanism in macrophages, we hypothesized that the inhibitory effect of PPG on macrophage immunity may affect maturation of phagosomes containing *M. smegmatis*. To test this hypothesis, the role of the transsulfuration pathway on the degree of maturation of *M. smegmatis*-containing phagosomes was analyzed by the colocalization of bacilli and lysosomes (Fig. 7A). Following PPG treatment, more *M. smegmatis* bacilli appeared green, revealing that they were not harbored inside the red-staining acidic vesicles. This result is consistent with the CFU data in Fig. 6 indicating that PPG impairs the innate ability of the macrophage to clear intracellular replicating bacteria. Treatment

![Image](Image 60x341 to 396x733)

**FIGURE 4. Effect of LPS on MS, CBS, and pathway metabolites.** Human monocytes were incubated with or without a bolus treatment of LPS (10 ng/ml) and harvested at 24-h intervals as described under “Experimental Procedures.” A, MS, CBS, and actin were detected in cell extracts by Western analysis and are representative of four independent experiments. B, quantitative analysis of changes in CBS and MS protein concentration after LPS treatment normalized to the intensity of actin in each lane. MS and CBS levels were significantly diminished (~70% of control (*n* = 4)), respectively, during days 1–5 days following LPS addition. C and D, LPS treatment decreased intracellular AdoMet levels by ~30% (*n* = 5; *, *p* ≤ 0.01) (C) and increased cysteine levels by ~300% (*n* = 6; *, *p* ≤ 0.001). D, compared with untreated controls. Intracellular GSH concentration increased ~30% at day 1, returned to steady-state levels and then increased ~100% at 7 (*n* = 6; *, *p* ≤ 0.04). Data are represented as mean ± S.D., and *n* refers to the number of independent experiments performed on cells from different donors; ns is not significant. d, day.

The contribution of transsulfuration to GSH synthesis was further investigated by labeling infected cells with [35S]-methionine and monitoring incorporation of radiolabel into GSH in the presence or absence of PPG. GSH labeling was inhibited ~50% at 3 and 8 h following PPG treatment of uninfected cells (Fig. 5C) and paralleled a decrease in cysteine concentrations (Fig. 5A). Under conditions of infection, an ~40% higher radiolabel incorporation was observed in GSH, and this was inhibited ~50% after 8 h in the presence of PPG.

**Transsulfuration Pathway Blockade Impairs Host-mediated Intracellular Killing of Mycobacteria**—To assess the physiological significance of the increase in transsulfuration flux to infection, with NAC significantly increased acidification of *M. smegmatis*-containing phagosomes, consistent with formation of endosomes. The yellow color indicated colocalization of green bacilli in acidic vesicles. Quantitative analysis of *M. smegmatis* residing in acidic phagosomes versus total intracellular mycobacteria confirmed the increased incarceration of bacteria in late endosomes in NAC-treated cells (Fig. 7B).

**DISCUSSION**

MP are the first lines of host defense against microbial pathogens acting as phagocytic cells, function to clear debris and pathogens, secrete bioactive molecules, and regulate adaptive immunity. In these capacities MPs protect the host against environmental, endogenous, microbial, and immune-based injuries. Although oxidation-reduction reactions are central to

![Image](Image 351x26 to 378x38)
Sulfur transactions are central to the pathogenic success of microbes as key sulfur-containing compounds determine initiation of protein synthesis (N-formylmethionine), methylation reactions (AdoMet), lipid biosynthesis (CoA), and redox homeostasis (mycothiol in Mycobacterium). In fact, sulfur metabolism has been implicated in mycobacterial virulence, antioxidant defense, and antibiotic resistance (27, 28). The high proportion of genes encoding lipid biosynthetic functions that generate the diverse set of lipids found in mycobacteria suggest that sulfur acquisition from the host is critically important for microbial growth in the infected host. Mycobacteria obtain sulfur either via uptake of sulfate (which is assimilated into cysteine or homocysteine (Fig. 1) or methionine, with the latter being the preferred sulfur source in the host (29). Methionine is subsequently converted to cysteine via the transsulfuration pathway. Thus, while loss of a functional sulfate transporter in Mycobacterium tuberculosis does not hinder the ability of the mutant to survive in the host versus the wild type bacterium, the inability to convert methionine to cysteine attenuates its pathogenicity (30). Interestingly, unlike the host, Mycobacterium does not accumulate cysteine to detectable levels. Excess cysteine is apparently eliminated by an active cysteine desulphydrase, suggesting that cysteine may be toxic (29). The metabolic interplay between the host and the sulfur needs of pathogen is therefore likely to play an important role in modulating the outcome of their interaction.

Coordinate changes in redox status and cellular developmental stages, viz. proliferation, differentiation, and apoptosis are known to occur (31), and several studies have examined changes in GSH levels during differentiation of monocytes into macrophages and during infection (15, 32, 33). As reported previously (33), we also observed a low [GSH]/[GSSG] ratio in monocytes indicating a more oxidizing environment compared with cells after day 2, when a sharp increase in the ratio was seen (Fig. 3D). This coincided with a 150% increase in GSH concentration (Fig. 3C) and with the induction of CBS expression (Fig. 2A). CBS catalyzes the committing step in the transsulfuration pathway and provides an endogenous route for the synthesis of cysteine, needed for GSH biosynthesis. In contrast to GSH, the cysteine pool size remained relatively constant, suggesting that increased synthesis of cysteine was balanced by enhanced consumption. In concert with changes in CBS, expression of MS was induced during differentiation and this was accompanied by an increase in AdoMet concentration and phospholipid methylation (Fig. 2).

Monocyte-macrophage activation after bolus stimulation by LPS had a profound impact on expression of CBS and MS over a period of 5 days (Fig. 4). Beyond that time, LPS ceased to have an effect. LPS is known to induce reactive oxygen species production, which in turn modulates flux of homocysteine via competing pathways. Hence, transmethylation is depressed

### FIGURE 5. Influence of bacterial infection and PPG on transsulfuration pathway in macrophages

Human monocytes (A) and macrophages (B) were infected with *M. smegmatis* at an multiplicity of infection of 1 for 3 h and then incubated in the presence or absence of 10 ng/ml LPS, 2.5 mM PPG, 1 mM NAC or both PPG and NAC for 24 h. Following incubation, cells were lysed, and the CFU assay was performed as described. Data are the mean ± S.D. of three independent experiments performed on cells from different donors. *, p = 0.04 and **, p = 0.05 in comparison with control; ns = not significant.

### FIGURE 6. Inhibition of the transsulfuration pathway inhibits bacterial clearance

A. *Control*  
B. *Control + PPG*  
C. *Infected*  
D. *Infected + PPG*

Monocytes (Day-1) and Macrophage (Day-7) viability (% of control) and intracellular mycobacterial viability (% of control) in control cells. All experiments were performed in triplicates on cells from different donors. *, p = 0.001 and **, p = 0.02 in comparison with control; ns = not significant.

The response of macrophages to pathogens and reactive oxygen and nitrogen species are engaged in the process (9), fundamental questions remain regarding how MPs modulate redox metabolic pathways during cellular differentiation, activation, and microbial killing.

### Transsulfuration and Immunity

Sulfur transactions are central to the pathogenic success of microbes as key sulfur-containing compounds determine initiation of protein synthesis (N-formylmethionine), methylation reactions (AdoMet), lipid biosynthesis (CoA), and redox homeostasis (mycothiol in Mycobacterium). In fact, sulfur metabolism has been implicated in mycobacterial virulence, antioxidant defense, and antibiotic resistance (27, 28). The high proportion of genes encoding lipid biosynthetic functions that generate the diverse set of lipids found in mycobacteria suggest that sulfur acquisition from the host is critically important for microbial growth in the infected host. Mycobacteria obtain sulfur either via uptake of sulfate (which is assimilated into cysteine or homocysteine (Fig. 1) or methionine, with the latter being the preferred sulfur source in the host (29). Methionine is subsequently converted to cysteine via the transsulfuration pathway. Thus, while loss of a functional sulfate transporter in *Mycobacterium tuberculosis* does not hinder the ability of the mutant to survive in the host versus the wild type bacterium, the inability to convert methionine to cysteine attenuates its pathogenicity (30). Interestingly, unlike the host, *Mycobacterium* does not accumulate cysteine to detectable levels. Excess cysteine is apparently eliminated by an active cysteine desulphydrase, suggesting that cysteine may be toxic (29). The metabolic interplay between the host and the sulfur needs of pathogen is therefore likely to play an important role in modulating the outcome of their interaction.

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Monocyte-macrophage activation after bolus stimulation by LPS had a profound impact on expression of CBS and MS over a period of 5 days (Fig. 4). Beyond that time, LPS ceased to have an effect. LPS is known to induce reactive oxygen species production, which in turn modulates flux of homocysteine via competing pathways. Hence, transmethylation is depressed
whereas transsulfuration is enhanced under oxidative stress conditions (13). In accordance with this regulation, AdoMet levels were slightly diminished in response to LPS activation of monocyte-macrophages whereas cysteine levels showed a dramatic 300% increase (Fig. 4). LPS is reported to stimulate cysteine transport by induction of the xc/glutamate/cystine antiporter in mouse peritoneal macrophages (34). Thus, a combination of increased synthesis and enhanced transport probably contributes to intracellular cysteine accumulation in LPS-treated cells.

In contrast to cysteine, GSH exhibited a more complex response and following a transient ~30% increase in the first day returned to steady-state levels and increased ~100% thereafter between days 5 and 7. Hence, the kinetics of LPS-induced increase in cysteine appear to be distinct from the kinetics of change in GSH, suggesting that accumulation of cysteine may not serve to simply boost the GSH pool of the host. Unlike liver cells, where the intracellular cysteine (~100 μM) and GSH (~6–7 mM) pool sizes are dramatically different (13, 17), a 7-fold difference in the relative pool sizes of these sulfur metabolites is seen in macrophages. Hence, while cysteine is limiting for GSH synthesis in hepatocytes (11), that may not be the case in macrophages.

At high concentrations (5 mM), GSH is mycobacteriostatic, whereas at similar concentrations, S-nitroso-GSH is bactericidal (35). Our study indicates that mycobacterial infection does not elicit sufficiently high concentrations of GSH to induce bacteriostasis and that a quantitatively significant change occurs instead in the intracellular cysteine pool. The significance of this to the response to infection of the host is presently unclear, and we speculate that it could be toxic to the microbe and/or be important for synthesis of cysteine-rich proteins found in lysosomes and/or for phagolysosomal fusion and bacterial clearance. In this context, it is interesting to note that the cysteine/cystine couple has been proposed to represent a discrete node in the biological redox signaling circuitry that could function as a redox switch independent of the GSH/GSSG and thioredoxin systems (36). Increased biosynthesis of cysteine under conditions that repress CBS expression, viz. LPS treatment (Fig. 4) and mycobacterial infection (data not shown) is paradoxical.

However, the transsulfuration pathway is activated under oxidative conditions (13), and this apparently compensates for the diminished expression of the committing enzyme, CBS. These results suggest that the transsulfuration pathway plays a role in the response of the host cell to infection and that its modulation may influence the outcome of the host-microbe interaction.

To test this hypothesis, the effect of transsulfuration blockade and its circumvention by the cysteine prodrug, NAC, was examined in monocytes and in macrophages infected with M. smegmatis. PPG had no effect on the intracellular viability of M. smegmatis at day 1 (Fig. 6), consistent with the presence of very low levels of CBS and therefore low transsulfuration capacity, at this stage (Fig. 2A). An ~35% increase in intracellular cysteine was observed 24 h after infection of monocytes compared with uninfected controls (data not shown). Addition of NAC decreased bacterial via-
bility by ∼65%, and the effect of NAC and PPG cotreatment was not significantly different from that of NAC alone. LPS reduced intracellular bacterial viability 2-fold and served as a positive control. In contrast, exposure of monocyte-derived macrophages (on day 7) to PPG conferred a marked survival advantage to bacteria within the host, which was negated by coadministration of NAC. In fact, NAC diminished bacterial viability 3-fold (Fig. 6). Under these conditions, a 100% increase in intracellular cysteine and a 33% increase in GSH was observed (Fig. 5). Radiolabeling studies and PPG inhibition revealed that the transsulfuration pathway was a significant source of cysteine accumulated under these conditions. Recently, very high concentrations of NAC (10 mM but curiously not 5 or 15 mM) were reported to elicit growth inhibition of M. tuberculosis in human monocyte-derived macrophages obtained from three out of six individuals (15). In contrast, we obtained consistent results with multiple donors and at a 10-fold lower concentration of NAC.

The extensive endocytic capacity of macrophages is a key component of their response to infectious challenge. Following endocytosis, fusion of phagosomes with lysosomes results in acidification of the microbe-bearing vesicles as a strategy for bacterial lysis and containment. Blockade of the transsulfuration pathway impairs phagolysosomal fusion as revealed by confocal microscopy (Fig. 7). Quantitative analysis of the microscopic data reveals that PPG diminished, whereas NAC increased, the bacterial density in endosomes by ∼30 and 180%, respectively (Fig. 7B). An inverse effect was observed in phagosomes (data not shown).

A limitation of the present study is the use of the avirulent species, M. smegmatis, as a microbial model organism to study host-pathogen interaction. Although used quite extensively due to the advantage of working with a rapidly dividing nonpathogenic model organism, some differences in host responses are known to be elicited by pathogenic versus nonpathogenic bacteria (37–39). For instance, the intraphagosomal concentration of iron increases significantly when M. tuberculosis but not M. smegmatis is ingested by macrophages (38). In addition, the intravacuolar sulfur content is higher in M. tuberculosis- versus M. smegmatis-infected macrophages (38). There also appear to be some differences in sulfur metabolism between the pathogenic and nonpathogenic mycobacteria themselves (40–42). Thus, while the overall thiol composition in the two species is very similar, and the intracellular cysteine content in both M. smegmatis and M. tuberculosis is very low, the cysteine redox ratio is reducing in the former and oxidizing in the latter (41).

In summary, this study reveals an important link between transsulfuration-dependent sulfur metabolism and monocyte differentiation, activation, and intracellular killing of mycobacteria. While previous studies have focused on the role of boosting GSH synthesis under these conditions, our results suggest that accumulation of cysteine may not serve solely to enhance GSH biosynthesis. Inhibition of the transsulfuration pathway diminishes the efficacy of intracellular bacterial clearance, and its activation may represent a strategy for enhancing the innate immune response.

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Acknowledgment—We thank Dr. Joe Zhou in the Microscopy Core Facility (University of Nebraska, Lincoln, NE) for his assistance with the confocal microscopy studies.
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