Glucose 6-Phosphate Dehydrogenase Is Required for *Salmonella typhimurium* Virulence and Resistance to Reactive Oxygen and Nitrogen Intermediates

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*Salmonella typhimurium zwf* mutants lacking glucose 6-phosphate dehydrogenase (G6PD) activity have increased susceptibility to reactive oxygen and nitrogen intermediates as well as attenuated virulence in mice. Abrogation of the phagocyte respiratory burst oxidase during experimental infection with zwf mutant strain 14028s of *Salmo- nella* causes a prompt restoration of virulence, while inhibition of inducible nitric oxide synthase results in delayed lethality. These observations suggest that G6PD-dependent bacterial antioxidant defenses play an important pathogenic role during early salmonellosis and additionally may help to antagonize NO-dependent antimicrobial mechanisms later in the course of infection.

Glucose 6-phosphate dehydrogenase (G6PD) encoded by the zwf gene catalyzes the first enzymatic step in the pentose phosphate cycle. This pathway provides ribose for nucleoside synthesis and reducing equivalents in the form of NADPH for reductive biosynthetic reactions and maintenance of the cellular redox state. Notably, NADPH is the electron source for several reductases that repair oxidative damage and regenerate antioxidant species, including glutathione reductase, thioredoxin reductase, and methionine sulfoxide reductase (10, 25).

In *Escherichia coli*, zwf expression is subject to at least three forms of regulation: growth rate-dependent regulation (22, 26), induction by the MarA (multiple antibiotic resistance) regulon (16), and induction by the SoxRS (superoxide radical response) regulon (11, 17). SoxRS can augment zwf expression during specific conditions of oxidative stress but is not required for basal levels of expression (11). A chromosomal deletion encompassing the zwf and edd genes has been correlated with increased susceptibility of *E. coli* to redox-cycling agents, nitric oxide gas, and killing by murine macrophages (15, 19), suggesting that G6PD is both induced by and involved in resistance to the antimicrobial activity of phagocyte-derived reactive oxygen and nitrogen species.

The present study examines the function of G6PD in *Salmonella typhimurium*, a pathogenic bacterium specifically adapted to survival within phagocytic cells (12, 13, 21). Phagocyte-derived oxygen and nitrogen intermediates have been strongly implicated in host defense against salmonellosis (2, 5, 6, 18), although essential antioxidant and antinitrosative defenses of *Salmonella* have been incompletely defined. The transcriptional regulator SoxS was recently found to be nonessential for survival of *Salmonella* within phagocytic cells (9), in contrast to observations for *E. coli* (19). We constructed and phenotypically characterized a zwf mutant *S. typhimurium* strain to examine the specific role of G6PD in *Salmonella* virulence.

*Salmonella* mutants with interruptions of the zwf gene were constructed by two approaches. First, oligonucleotide primers corresponding to nucleotides 525 to 551 and 964 to 988 of the published *E. coli* zwf sequence (23) were used to amplify an internal fragment of the zwf gene from *S. typhimurium* ATCC 14028s (12) genomic DNA. The sequenced fragment, which is 87% identical to the corresponding region of the *E. coli* gene, was ligated into the suicide vector pRR10[ΔtrfA 250V] (8). Conjugation of this plasmid from *E. coli* S17-1 (24) into *S. typhimurium* ATCC 14028s produced *S. typhimurium* BL850 carrying a zwf mutation. Interruption of zwf was confirmed by Southern blotting and a biochemical assay of G6PD activity (14). The zwf mutant lacked detectable G6PD, which was restored by introduction of the cloned *E. coli zwf* gene on plasmid pDR17 (23) in trans (data not shown). Moreover, bacteriophage P22-mediated transduction of a pgi::Tn5 mutation from *S. typhimurium* CH1021 into the zwf mutant resulted in a strain unable to grow on minimal medium with glucose. An additional zwf mutant derivative of *S. typhimurium* 14028s was obtained by transduction of zwf::Tn10 from *S. typhimurium* LT2-derivative DM653 (7), producing strain BL851.

Susceptibility to hydrogen peroxide (H$_2$O$_2$) or S-nitrosoglutathione (GSNO) was determined by a disk diffusion method (4). Fifteen microliters of 3% H$_2$O$_2$ or 500 mM GSNO was added to a 0.25-in. paper disk placed over a lawn of $10^6$ bacteria on M9 minimal agar with 0.2% glucose. The zone of inhibition after overnight incubation is a measure of susceptibility. We were able to confirm increased susceptibility of *E. coli* HB351 (Δ[edd-zwf]/22) (1) to reactive oxygen or nitrogen intermediates in comparison to wild-type parental strain *E. coli* W3110 (Fig. 1). However, although the zwf gene on plasmid pDR17 (23) restored wild-type levels of resistance to H$_2$O$_2$, pDR17 failed to restore HB351 resistance to GSNO. This suggests that phenotypic analyses of this *E. coli* strain should be interpreted with caution; it is likely that loci in addition to zwf contribute to the increased susceptibility of HB351 to re-
active nitrogen intermediates. Plating of *E. coli* DR612 (pgi zwf) (23) carrying pDR17 on gluconate-bromthymol blue medium (27) confirmed expression of zwf from plasmid pDR17 (not shown). The *S. typhimurium zwf* mutant strains BL850 and BL851 were also found to be hypersusceptible to H$_2$O$_2$ and GSNO, but introduction of the cloned zwf gene was able to restore wild-type resistance levels to both compounds (Fig. 1), in contrast to the *E. coli* mutant.

The virulence of zwf mutant *S. typhimurium* was determined by intraperitoneal inoculation of $1 \times 10^3$ to $2 \times 10^5$ organisms into 6-week-old female C57BL/6 (ItyS) mice. By this route of infection, zwf mutant *S. typhimurium* BL850 was found to be avirulent (Fig. 2). Genetic abrogation of the NADPH phagocyte oxidase in congenic C57BL/6-derived gp91 phox knockout (KO) mice (20) restored 100% mortality following intraperitoneal challenge (mean time to death, 4.4 days). Administration of 2.5% (wt/vol) aminoguanidine (3, 6), an inhibitor of inducible nitric oxide synthase, also restored virulence to zwf mutant *S. typhimurium* BL850, but mortality occurred significantly later (mean time to death, 17 days). No deaths occurred

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**FIG. 1.** Susceptibility of *E. coli* and *S. typhimurium* strains to hydrogen peroxide (A) and GSNO (B). Susceptibility was determined by a disk diffusion method (4); the zone of inhibition is a measure of susceptibility. pDR17 carries the *E. coli zwf* gene. Complementation with the cloned zwf gene restores hydrogen peroxide (H$_2$O$_2$) resistance to all strains and GSNO resistance to the *S. typhimurium zwf* mutant strains but fails to restore GSNO resistance to *E. coli* HB351 (23).
FIG. 2. Virulence of wild-type or zwf mutant S. typhimurium in mice. Mortality was determined following intraperitoneal inoculation of 1 × 10^7 to 2 × 10^8 wild-type (WT) or zwf mutant (zwf) organisms in C57BL/6 mice (BL6), congenic phox KO mice (phox), or C57BL/6 mice with 2.5% aminoguanidine added to their drinking water (AG). The results of a single experiment are shown (n = 5 mice per group). The virulence study was performed twice, with essentially identical results.

in C57BL/6 or congenic gp91 phox KO mice receiving intra-
peritoneal injections of phosphate-buffered saline alone.

This represents the first demonstration of an essential role of
Glucose-6-phosphate dehydrogenase level in

6-phosphogluconate dehydrogenase level in Escherichia coli K-12: β-galactosida-


tion-deficient mutants of Salmonella typhimurium are avirulent and sensitive to the oxidative burst of macrophages. Mol. Microbiol. 7:933–936.

nitric oxide synthase inhibitors on murine infection with Mycobacterium


1995. Genetic and redox determinants of nitric oxide cytotoxicity in Sal-

Periplasmic superoxide dismutase protects Salmonella from products of
USA 94:13997–14001.

Homocysteine antagonism of nitric oxide-related cytoxygen in Salmonella

7. Engerlade, J. L., and D. M. Downes. 1996. Involvement of the oxidative
pentose phosphate pathway in thiamine biosynthesis in Salmonella typhi-

Harwood, and D. G. Guiney. 1992. The alternative σ factor KatF (RpoS)

lator SoxS is required for resistance of Salmonella typhimurium to pararact


coli zwf “soxbox,” the DNA binding site for SoxS-mediated induction of

177:1742–1750.

Salmonella typhimurium that cannot survive within the macrophage are


olism in an Escherichia coli mutant lacking phosphoglucone isomerase. J.

1990. Positive control of a global antioxidant defense regulon activated by
87:6181–6185.

Wolf, Jr. 1995. Purification and regulatory properties of MarA protein, a
transcriptional activator of Escherichia coli multiple antibiotic and superox-

Escherichia coli. Purification and interaction with DNA. J. Biol. Chem. 269:
18371–18377.

sverity, and prevention of infections in chronic granulomatous disease.

S. typhimurium studied by conditional microscopy. Salmonella typhimurium re-
sides intracellularly inside macrophages and exerts a cytotoxic effect on

analyses of the growth rate-dependent regulation of Escherichia coli zwf.

Escherichia coli K-12 zwf gene encoding glucose 6-phosphate dehydrogenase.

the genetic analysis and manipulation of Rhizobium and other gram-negative

defenses against oxidative stress. Trends Genet. 6:363–368.

alteration of 6-phosphogluconate dehydrogenase and glucose 6-phosphate de-

25. Wolf, R. E., Jr., and F. M. Shea. 1979. Combined use of strain construction and
affinity chromatography in the rapid, high-yield purification of 6-phosphoglu-