The Journal of Clinical Investigation

RecA versus catalase: who's on first?

D J Hassett, M S Cohen

J Clin Invest. 1995;95(3):924-925. https://doi.org/10.1172/JCI117799.

Research Article





Editorial RecA versus Catalase: Who's on First?

Phagocytic cells possess a complex and unique system for the generation of superoxide anion (O₂⁻). However, due to the acidic nature of the phagosomal vacuole, O₂⁻ is rapidly converted to hydrogen peroxide (H₂O₂) by spontaneous dismutation. These reactive oxygen reduction species are of well documented importance in the microbicidal and inflammatory response (1). H₂O₂ is believed to be particularly important because (a) it can traverse bacterial membranes to gain access to an intracellular milieu where it can cause DNA damage and oxidation of respiratory chain components and (b) H₂O₂ can lead to formation of more reactive "down-stream" oxidative species: H₂O₂ can react with Fe²⁺ or other reduced transition metals (e.g., Cu⁺) in a Fenton reaction to form the hydroxyl radical (HO'); H₂O₂ can react with myeloperoxidase (or other peroxidases) in the presence of a halide such as chloride to form OCl⁻; OCl⁻ derived from H₂O₂ can react with O₂⁻ to form $HO^{\bullet}(2)$. Last, H_2O_2 can react with HOCl to form the highly reactive singlet oxygen (¹O₂). Therefore, H₂O₂ is likely a critical threat to microbial survival.

How do microbes (and by extrapolation humans) reduce the stress imposed by exposure to H₂O₂? Two fundamental mechanisms have been extensively studied. First, the heme enzyme catalase catabolizes H₂O₂ to oxygen and water. Some (but not all) microbes have both cytoplasmic and periplasmic catalase isoenzymes encoded by separate genes and regulated by positive regulatory loci such as oxyR. Second, microbes are able to utilize several DNA repair systems in response to H₂O₂mediated DNA damage. While many DNA repair systems have been described, only a few have been closely linked to oxidative stress. These include (but are not limited to) those involved in recombination repair (recA, -BC, recF, recN), excision repair (xth, exonuclease III), and DNA polymerization/3 $'\rightarrow 5'$ exonuclease (polA1) (3, 4).

How can we compare the relative importance of catalase and DNA repair systems during exposure to H₂O₂? First, investigators have looked at the effects of exogenous H₂O₂ in studies conducted in vitro. In seminal work by Imlay and Linn (3, 4), E. coli demonstrated a bimodal sensitivity to H₂O₂ such that exaggerated microbial killing was observed at very low (1-3 mM, mode I) or very high (> 30 mM, mode II) H₂O₂ concentrations. Mutations in DNA repair genes enhanced mode I killing of E. coli by 2-4 logs and mode II killing by 1-3 logs. Catalase deficiency increased mode I killing by $\sim 1.5 \log s$ and mode II killing by 1 log (4). These latter observations must be further interpreted in light of the more recent work of Ma and Eaton (5) who have noted the greatest protection of catalase at higher bacterial density. These investigators concluded that at increased cellular density, H₂O₂ has poor access to individual cells. However, during bacterial growth as colonies on nutrient agar, the edge of each colony is actively growing while those on the interior are in stationary or death phase. Most bacteria in late log or stationary phase express their highest catalase activity and therefore demonstrate greatest resistance to H₂O₂.

Provocative observations with bacteria grown in vitro in the

form of colonies often receive further consideration in more physiological models using mammalian cells or whole animals. In this issue of *The Journal*, Buchmeier et al., (6) used mutants of S. typhimurium to examine the contributions of recA and catalase to virulence. The results seem to demonstrate greater importance for recA than catalase during macrophage phagocytosis and growth of S. typhimurium in vivo. Indeed, because a recA mutant (6, 7) (but not a catalase-deficient mutant) demonstrated reduced survival in BALB/c mice, the authors concluded that S. typhimurium was likely exposed to low concentrations of H₂O₂ in vivo and grew at low cell density. Otherwise, it might be assumed, catalase deficiency would have proven of greater importance.

While provocative, these ideas deserve further scrutiny. First, microbial catalase is not necessarily unimportant during phagocytosis or growth in vivo. Many catalase deficient microbes (e.g., S. aureus, E. coli, N. gonorrhoeae) demonstrate increased susceptibility to neutrophil attack. Indeed, during phagocytosis of catalase-deficient E. coli, a greater production of the highly destructive HO can be demonstrated (8). Catalase deficiency may also lead to reduced virulence in some animal or tissue culture models (9). Second, recA (and other virulence genes studied) often serve functions beyond DNA repair evoked by oxidative stresses such as H₂O₂. Depending on the bacterial species, recA has been involved in competence for transformation, pilin antigenic variation, expression of bacterial chemoattractant formyl-methionyl-leucylphenylalanine, and other functions. Therefore, the assumption that recA sensitivity to H₂O₂ actually defined the degree of oxidative stress experienced by S. typhimurium in vivo may not ultimately prove correct. In addition, a recA mutation does not necessarily assure reduced bacterial virulence. For example, recA mutants of Brucella abortis (a well studied intracellular pathogen) demonstrated persistent infection in BALB/c mice (10).

Given these caveats, we believe observations about oxidative stress and bacterial virulence are important. First, they help us to better understand the pathogenesis of infectious diseases, and their prevention and treatment. Second, studies of bacterial DNA protection/repair systems can be expected to lend themselves to the understanding of human disease in important (and perhaps unpredictable) ways. For example, a defect in a human mismatch repair (analogous to mutL in bacteria and fungi) has been linked to hereditary colon cancer (11). Reactive oxygen species have already been shown to play a role in several human disease states including inflammation, heart disease, rheumatoid arthritis, ALS (Lou Gehrig's disease), ischemia reperfusion injury, Bloom's and Purtcher's syndromes, adult respiratory distress syndrome, mutations, cancer, and aging (12). It seems likely to us that the balance between DNA repairs systems such as recA and antioxidant enzymes such as catalase, so extensively studied in bacterial systems, will ultimately prove relevant in human disease as well.

Daniel J. Hassett Department of Molecular Genetics, Biochemistry and Microbiology University of Cincinnati College of Medicine

Myron S. Cohen Departments of Medicine and Microbiology and Immunology Division of Infectious Diseases University of North Carolina at Chapel Hill

J. Clin. Invest.

[©] The American Society for Clinical Investigation, Inc. 0021-9738/95/03/0924/02 \$2.00 Volume 95, March 1995, 924-925

References

- 1. Hassett, D. J., and M. S. Cohen. 1989. Bacterial adaptation to oxidative stress: implications for pathogenesis and interaction with phagocytic cells. FASEB (Fed. Am. Soc. Exp. Biol.) J. 3:2574-2582.
- 2. Ramos, C. L., S. Pou, B. E. Britigan, M. S. Cohen, and G. M. Rosen. 1992. Spin trapping evidence for myeloperoxidase-dependent hydroxyl radical formation by human neutrophils and monocytes. *J. Biol. Chem.* 267:8307-8312.
- 3. Imlay, J. A., and S. Linn. 1986. Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. *J. Bacteriol*. 166:519–527
- 4. Imlay, J. A., and S. Linn. 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* 169:2967-2976.
- Ma, M., and J. W. Eaton. 1992. Multicellular oxidant defense in unicellular organisms. Proc. Natl. Acad. Sci. USA. 89:7924-7928.
- 6. Buchmeier, N. A., S. J. Libby, Y. Xu, P. C. Loewen, J. Switala, D. G. Guiney, and F. C. Fang. 1995. DNA repair is more important than catalase for *Salmonella* virulence in mice. *J. Clin. Invest.* 95:1047-1053.
 - 7. Buchmeier, N. A., C. J. Lipps, M. Y. So, and F. Heffron. 1993. Recombina-

- tion-deficient mutants of Salmonella typhimurium are avirulent and sensitive to the oxidative burst of macrophages. Mol. Microbiol. 7:933-936.
- 8. Gunther, M. R., M. Jhing, G. Shetty, and M. S. Cohen. 1994. Use of isogenic mutants of *Escherichia coli* to demonstrate formation of hydroxyl radical during bacterial killing by human neutrophils. *Clin. Res.* 42:2 (Abstr.).
- 9. Mandell, G. L. 1975. Catalase, superoxide dismutase, and virulence of *Staphyloccus aureus*. *In vitro* and *in vivo* studies with emphasis on staphylococcalleukocyte interaction. *J. Clin. Invest.* 55:561–566.
- Tatum, F. M., D. C. Morfitt, and S. M. Halling. 1993. Construction of a Brucella abortus recA mutant and its survival in mice. Microb. Pathogen. 14:177– 185.
- 11. Papadopoulos, N., N. C. Nicolaides, Y.-F. Wei, S. M. Ruben, K. C. Carter, C. A. Rosen, W. A. Haseltine, R. D. Fleishmann, C. M. Fraser, M. D. Adams, J. C. Venter, S. R. Hamilton, G. M. Petersen, P. Watson, H. T. Lynch, P. Peltomaki, J.-P. Mecklin, A. de la Chapelle, K. W. Kinzler, and B. Vogelstein. 1994. Mutation of a mutL homolog in hereditary colon cancer. Science (Wash. DC). 263:1625-1629.
- 12. Halliwell, B., and J. M. C. Gutteridge. 1986. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch. Biochem. Biophys.* 246:501-514.