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Role of Oxidants in Microbial Pathophysiology

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INTRODUCTION

Reactive oxygen species have been increasingly implicated as playing a central role in the pathophysiology of clinical infections. More specifically, superoxide, hydrogen peroxide, hydroxyl radical, hypohalous acid, and recently, nitric oxide are thought to contribute to these processes. These compounds exhibit a broad spectrum of biotoxicity and are crucial to host defense for the optimal microbicidal activity of neutrophils and other phagocytes (148, 209, 216, 313). In response, microorganisms have developed complex strategies not only to avoid contact with phagocyte-derived oxidants but also to defend themselves from injury once oxidants are encountered. Host cells have developed similar adaptations to protect themselves against a deleterious consequence of oxidant exposure, inflammatory tissue injury (209, 313). This review will discuss the formation of oxidants in vivo and their central role in the complex interplay between microbial invasion and host defense.

GENERATION AND TOXICITY OF SELECTED OXIDANTS IN BIOLOGIC SYSTEMS

Many biochemical reactions vital to normal aerobic metabolism of human and microbial cells require the transfer of four electrons to molecular oxygen to form H_2O. Under most circumstances, this transfer occurs simultaneously without the formation of other intermediates. However, molecular oxygen does have the capacity to undergo sequential univalent reduction to form other oxygen intermediates with different toxicities prior to the generation of H_2O.

The addition of one electron to O_2 yields the superoxide radical ($^{\cdot}O_2^-$), which at physiologic pH rapidly reduces itself (dismutes, $k \approx 2 \times 10^5\ M^{-1}\ s^{-1}$) to form the divalent oxygen reduction product, hydrogen peroxide (H_2O_2). Trivalent oxygen reduction in vitro occurs via the reaction of H_2O_2 with $^{\cdot}O_2^-$ to produce the hydroxyl radical ($^{\cdot}OH$). However, at physiologic pH, this reaction is of little biologic importance unless a transition metal catalyst (e.g., Fe^{3+}) is present to enhance the reaction rate, yielding $^{\cdot}OH$ via the Haber-Weiss reaction (123) (Table 1). As discussed below, not all iron complexes can serve as a catalyst in this reaction (125). Besides $^{\cdot}OH$ formation, experimentally induced interactions between H_2O_2 and iron chelates may also lead to the production of the reactive iron peroxocomplex and ferryl ion (268, 321). However, their role in human and microbial physiology is largely unknown.

Although most investigations have focused on $^{\cdot}OH$ formation via the Haber-Weiss mechanism, evidence also exists for the formation of $^{\cdot}OH$ from $^{\cdot}O_2^-$-mediated reduction of hypochlorous acid (HOCl) (51, 189, 232, 250). A potent oxidant in itself, HOCl is generated by the interaction of H_2O_2 with phagocyte-derived peroxidases (148).

Recently, intense investigation has been directed at another oxidant species, nitric oxide ($NO^{\cdot}$). $NO^{\cdot}$ is not a classic product of O_2 reduction; instead, its formation in mammalian cells is dependent on a group of enzymes termed nitric oxide synthases (NOS) (216, 224). These enzymes oxidize L-arginine to L-citrulline and $NO^{\cdot}$. Although several related NOS isoforms have been isolated, they are divided into two categories, constitutive and inducible, based on differences in regulation and activities. Constitutive isoforms (cNOS) are found in neuronal

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TABLE 1. Chemical reactions involving reactive oxygen species

Reaction	Formula[a]
Haber-Weiss reaction	$\cdot O_2^- + Fe^{3+} \rightarrow O_2 + Fe^{2+}$ $H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$ $\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$
Myelo (eosinophil) peroxidase	$H_2O_2 + HX \rightarrow HOX + H_2O$
Nitric oxide synthase	L-Arginine → L-citrulline + NO˙
Peroxynitrite formation/ decomposition	$NO\cdot + \cdot O_2^- \rightarrow ONOO^-$ $ONOO^- + H^+ \rightarrow ONOOH$ $ONOOH \rightarrow \cdot OH + NO\cdot$
GSH	
Peroxidase	$2\ GSH + H_2O_2 \rightarrow G\text{-}S\text{-}S\text{-}G + 2H_2O$
Reductase	G-S-S-G + 2NADPH → 2GSH + 2NADP
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
SOD	$2\cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

[a] X, halide.

and endothelial cells. cNOS activity responds to changes in intracellular calcium concentration via calcium-calmodulin binding. This results in the intermittent production of small amounts of NO˙ necessary for physiologic processes such as neurotransmission and blood pressure regulation. By using spin-trapping techniques, brain NOS has also been demonstrated to generate $\cdot O_2^-$ in a calcium-calmodulin-dependent manner (243). The inducible NOS isoform (iNOS) is expressed in many cell types, including hepatocytes, respiratory epithelium, and macrophages. Its activity is independent of fluctuations in the intracellular calcium concentration. Factors known to modulate iNOS levels include a number of cytokines, microorganisms, and microbial products, consistent with the importance of iNOS activity in host defense and inflammation. Many bacterial species are also capable of generating NO˙ under conditions of low oxygen tension via nitrite reductases (334). Once formed, NO˙ has the ability to act as an oxidizing agent alone or interact with $\cdot O_2^-$ to generate peroxynitrite ($ONOO^-$) ($k \approx 6.7 \times 10^9\ M^{-1}\ s^{-1}$) (245, 247, 284) and ultimately ˙OH via peroxynitrate formation and decomposition (Table 1) (19). Although a transition metal catalyst is not required in this system, thermodynamic and kinetic considerations may not favor the formation of ˙OH via this reaction in vivo (170).

Superoxide and Hydrogen Peroxide

Superoxide is a moderately reactive compound capable of acting as an oxidant or reductant in biologic systems. This relative inactivity allows $\cdot O_2^-$ to diffuse for considerable distances before it exerts its toxic effects. Extracellularly generated $\cdot O_2^-$ can gain access to intracellular targets via cellular anion channels (264). These targets include bacterial enzymes, particularly those involved in biosynthesis of branched amino acids (e.g., α,β-dihydroxyisovalerate dehydratase and NADH-bound lactic dehydrogenase) (122, 174). Several *Escherichia coli* (and mammalian) dehydratases containing [4Fe-4S] clusters are particularly susceptible to inactivation by $\cdot O_2^-$, including aconitase, 6-phosphogluconate dehydratase, α,β-dihydroxyacid dehydratase, and fumarases A and B (101, 108, 109, 111, 183). Aconitase has also been shown to be inactivated by $ONOO^-$ but not NO˙ (57, 139). These enzymes are unique in that they can subsequently undergo reactivation by an iron-dependent mechanism (110). It is postulated that the inactivation occurs at an early stage of oxidative stress, such that aconitases function as "circuit breakers," halting the production of toxic $\cdot O_2^-$ by temporarily shutting down cellular oxidative metabolism (109). Once the stress has passed, the dehydratases can be reactivated by intracellular iron and thiols rather than having to be synthesized de novo (109).

In environments of low pH, such as at sites of inflammation or inside the phagosome, $\cdot O_2^-$ becomes protonated to form $HO_2\cdot$. Because of its neutral charge, $HO_2\cdot$ is more membrane permeable and more likely to react with itself to form H_2O_2. Additional toxicity of $\cdot O_2^-$ in biologic systems is likely to occur via its participation in the Haber-Weiss reaction in the presence of catalytically active iron (123).

Hydrogen peroxide is a more reactive oxidant than $\cdot O_2^-$, and readily diffuses across cell membranes. Potential sources of H_2O_2-mediated damage of cellular constituents include the oxidation of cellular membranes and enzymes, DNA damage and mutagenesis, and the inhibition of membrane transport processes (313). Imlay and Linn have described in greater detail the mechanisms of H_2O_2-mediated damage. They demonstrated that killing of *E. coli* by H_2O_2 is bimodal in that low (1 to 3 mM) and high (>20 mM) concentrations of H_2O_2 are more lethal than intermediate concentrations (153). Mode 1 (low H_2O_2 concentration) killing has been attributed to DNA damage mediated by the interaction of H_2O_2 with Fe^{2+} to form the toxic ferryl radical (149), an intermediate product in the formation of ˙OH. Exposure of *E. coli* to these low concentrations of H_2O_2 induces a protective response which confers increased resistance to subsequent H_2O_2 exposures by an enhanced ability to carry out recombinational DNA repair (154). Mode 2 killing, which does not require iron or an electron source to occur, is not due to DNA damage but may involve the oxidation of a separate cellular target (137). Recent data by Pacelli et al. demonstrate that NO˙ potentiates H_2O_2-induced killing of *E. coli* (235). This suggests that macrophage-derived NO˙, in addition to its own cytotoxic effects, may interact with H_2O_2 to enhance microbicidal activity at sites of infection (235).

Hydroxyl Radical

In many cases where $\cdot O_2^-$ and/or H_2O_2 is implicated in cell injury, it is unclear whether the process is mediated by these compounds or whether they simply serve as precursors for another, more potent oxidant species (e.g., ˙OH), which is truly mediating the injury. Studies using more sensitive free radical detection systems implicate ˙OH in the oxidation of a large number of biomolecules including proteins, DNA, and lipids, as a result of their initial exposure to $\cdot O_2^-$ and/or H_2O_2. Owing to its high reactivity, ˙OH is diffusion limited such that once formed in a biologic system, it is likely to travel only very short distances before it encounters an oxidizable substrate. This property dictates that ˙OH must be generated in close proximity to a critical cellular target molecule in order for it to mediate injury directly (77).

A mechanism by which ˙OH and other oxidants may cause cell injury at sites distant from their formation is via the initiation of a free radical cascade (43, 313). Oxidation of unsaturated fatty acids within a lipid membrane can produce peroxyl radical, which in turn can react with other nearby lipid molecules to generate additional lipid radicals. These new lipid radicals can then react with other unsaturated lipids, thereby setting up a free radical chain reaction (43). This reaction

eventually results in the oxidation of biomolecules at sites considerably distant from where the initial free radical reaction occurred (43).

Sources of Iron Available for Hydroxyl Radical Generation In Vivo

Since $\cdot OH$ formation from $\cdot O_2^-$ and H_2O_2 under physiologic conditions requires the presence of a transition metal catalyst, there has been considerable interest in determining which iron chelates potentially present in vivo could serve as $\cdot OH$ catalysts. In humans, intracellular iron is predominantly complexed to ferritin in a relatively noncatalytic form (301). Likewise, almost all extracellular iron is tightly bound to host binding proteins (transferrin and lactoferrin) in forms unable to catalyze $\cdot OH$ formation (7, 9, 42, 45, 320). In fact, there are strong data suggesting that lactoferrin serves as an antioxidant (34, 39, 40, 71, 215). Neutrophil lactoferrin may function to trap iron from ingested microorganisms (215). In phagocytes that do not contain lactoferrin (i.e., monocytes and macrophages), a specific surface receptor binds exogenous lactoferrin (20, 24, 50, 213, 326). Monocytes/macrophages previously incubated with lactoferrin are less susceptible to iron-dependent peroxidation of their membranes (40). Thus, via its interaction with phagocytes, lactoferrin may prevent iron-catalyzed oxidant formation, thereby limiting inflammatory tissue injury. This would complement the ability of the protein to limit the availability of iron for microbial growth (88, 99). Lactoferrin also binds lipopolysaccharide, an important compound mediating toxicity in sepsis. Although this interaction has no effect on the ability of lactoferrin to inhibit the Haber-Weiss reaction, it does disrupt lipopolysaccharide priming of phagocytes for $\cdot O_2^-$ production (71).

In contrast, in vitro data suggest that modification of some host iron-chelating proteins by proteases or $\cdot O_2^-$ can result in the generation of products capable of catalyzing $\cdot OH$ formation (23, 33). The *Pseudomonas aeruginosa* secretory product *Pseudomonas* elastase and other host-derived proteases present at sites of inflammation are known to cleave transferrin and lactoferrin into lower-molecular-weight iron chelates (27, 32, 84, 90, 91, 254). *Pseudomonas* elastase-cleaved transferrin and, to a lesser extent, lactoferrin are capable of catalyzing $\cdot OH$ formation when a source of $\cdot O_2^-$ and H_2O_2 is concurrently present (33, 208, 211). Additional studies have demonstrated the ability of *Pseudomonas* elastase and other protease-cleaved transferrin to enhance oxidant-mediated porcine pulmonary artery endothelial cell injury via $\cdot OH$ generation in an in vitro model (208). Evidence supporting the potential clinical relevance of these findings has been obtained by the detection of transferrin cleavage products in bronchoalveolar lavage specimens from *P. aeruginosa*-infected cystic fibrosis patients but not in those from normal individuals (35).

As microorganisms require iron for growth and replication, their mechanisms of iron acquisition and storage have evolved to fulfill these needs. Intracellular bacterial iron is primarily complexed to ferritin-like iron storage proteins (229, 290). To acquire iron from the extracellular environment, aerobic and facultative anaerobic bacteria, as well as fungi, synthesize diverse low-molecular-weight Fe(III)-scavenging ligands collectively termed siderophores (229). These compounds possess a high affinity for iron, which is probably important at sites of infection (e.g., the airway), where the availability of iron for bacteria is extremely limited due to competition from host iron-binding proteins. As an example, to be able to compete for iron effectively, *P. aeruginosa* synthesizes and secretes two types of siderophores: pyochelin and pyoverdin (74, 75). Studies from our laboratory suggest that pyochelin may play an important role not only in iron acquisition but also in *P. aeruginosa*-associated inflammatory tissue injury (38). The environment at sites of *P. aeruginosa* infections is replete with $\cdot O_2^-$ and H_2O_2 generated by local phagocytes and via the redox action of pyocyanin on target cells (discussed below). Ferripyochelin can act as a catalytically active iron chelate in the formation of $\cdot OH$ (37) and can enhance oxidant-mediated in vitro porcine pulmonary artery endothelial (38) and epithelial (36) cell injury. Therefore, although the production of siderophores by *P. aeruginosa* is an adaptive mechanism for obtaining necessary iron under stressful conditions, the same compounds may also potentiate oxidant-mediated tissue injury via the catalysis of $\cdot OH$. A similar role for pyoverdin has not been found (69). Nevertheless, it is possible that siderophores produced by other organisms play a similar role, but there are currently no available data to substantiate this.

Additional potential sources of catalytically active iron related to host-microbe interactions in vivo include iron released from hemoglobin through the action of the bacterial toxin hemolysin, host cell exposure to bacterially derived iron reduction compounds such as pyocyanin (discussed in a later section), and/or the release of intracellular iron from damaged mammalian or bacterial cells into the microenvironment. Regardless of their source, it is necessary that extracellularly generated iron catalysts remain in close proximity to the cell in order to facilitate $\cdot OH$-mediated injury, given the limited diffusibility of $\cdot OH$ (116).

Myeloperoxidase-Derived Oxidants

Coincident with their production of $\cdot O_2^-$ and H_2O_2, stimulated human phagocytes release one of two distinct peroxidases from their cytoplasmic granules. In the case of neutrophils and monocytes, this enzyme is myeloperoxidase (MPO), whereas for eosinophils it is eosinophil peroxidase (EPO) (148). The interaction of MPO and EPO with H_2O_2 forms hypohalous acids (HOX, where X = halide). It is generally thought that macrophages lack either enzyme (169); however, recent data suggest that this may not be universally true (79). Myeloperoxidase is a glycoprotein (molecular weight, 150) consisting of a pair of glycosylated heavy (α)-light (β) protomers, each of which contains an iron atom (225). EPO is an $\alpha\beta$ glycoprotein, similar in structure to hemi-MPO (148). These enzymes are cationic, thus allowing them to stick to cell surfaces and perhaps enhancing their potential for cell injury by increasing the local concentration of hypohalous acid at the target cell membrane (184, 212, 251, 277, 325).

Hypohalous acids are potent oxidants known to have several cytotoxic effects on mammalian and bacterial cells. Cell membrane integrity may be violated by membrane peroxidation and the oxidation and/or decarboxylation of membrane proteins (2, 322). Likewise, oxidation of components of the bacterial respiratory chain and interference with bacterial DNA-membrane interaction required for bacterial division can disrupt normal cellular metabolism and replication (249, 265). Activated neutrophils and monocytes can also generate cytotoxic chloramines, tyrosyl radical, and $\cdot OH$ via an MPO-dependent pathway (140, 148, 314).

Nitric Oxide

Nitric oxide is cytostatic or cytotoxic for both prokaryotic and eukaryotic cells (105, 216). The primary mechanism of injury involves the interaction of $NO^\cdot$ with iron-containing moieties in key enzymes of the respiratory cycle (e.g., glyceraldehyde-3-phosphate dehydrogenase) and with DNA synthesis

leading to mutagenesis in target cells (216). Nitric oxide also can react with other biomolecules to form new compounds that are also capable of toxicity. For example, the formation of nitrosothiol groups on proteins can lead to the inactivation of enzymes or changes in protein function (216, 261). These groups can react further to cross-link sulfhydryl groups and thus initiate a chain reaction (261). In addition, NO˙ and its derivatives can form toxic alkylating agents by reacting with secondary amines (156).

As shown in Table 1, $ONOO^-$ is generated by the reaction of $^{\cdot}O_2^-$ with NO˙ (19). Its ability to directly oxidize sulfhydryl groups and DNA bases, catalyze iron-independent membrane lipid peroxidation, and react with metals or metalloproteins (e.g., superoxide dismutase [SOD]) to form the toxic nitronium ion (NO^{2-}) has led some investigators to suggest that $ONOO^-$ plays a more important role than its precursor NO˙ in mediating cytotoxicity (19, 144, 155, 247, 248, 287). In addition, evidence suggests that upon protonation, $ONOO^-$ can undergo homolytic cleavage to form ˙OH by an iron-independent mechanism (19); however, the biologic relevance of this reaction has not been definitively addressed.

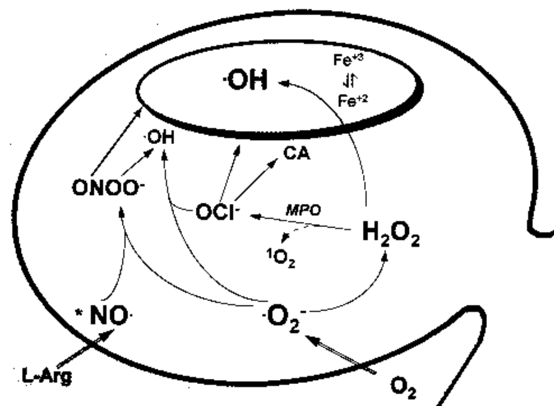

FIG. 1. Overview of intraphagosomal processes leading to oxidant-mediated microbial killings. CA, chloramines. Note that nitric oxide production occurs only in phagocytes with an inducible nitric oxide synthase. Reproduced from reference 209 with permission of the publisher.

SOURCES OF OXIDANTS ENCOUNTERED BY MICROBES IN VIVO

Endogenous Sources

Like eukaryotic cells, aerobic microorganisms are continually exposed to endogenous sources of toxic oxygen species as a consequence of aerobic metabolism (12). As discussed above, this occurs by sequential univalent electron reduction of molecular O_2 to generate such species as $^{\cdot}O_2^-$, H_2O_2, and ˙OH. Under certain conditions, homolytic cleavage of H_2O_2 may also yield ˙OH. These toxic oxygen species also can be generated as by-products of reactions involving glucose oxidase, xanthine oxidase, and thiol groups and flavins (73, 112, 252, 253). Furthermore, microbial exposure to UV or γ irradiation induces $^{\cdot}O_2^-$ production (200). Anaerobic organisms are particularly susceptible to oxidants derived via the above mechanisms, as they often lack the antioxidant defense mechanisms observed in aerobic organisms (discussed below).

A number of microorganisms, including *Enterococcus faecalis* (308), *E. coli* (150), *Lactobacillus* spp. (316), *Streptococcus pneumoniae* (316), and a number of *Mycoplasma* spp. (192, 204), also generate extracellular $^{\cdot}O_2^-$ and H_2O_2. Additional studies have shown that these oxidants can exert a number of beneficial and toxic effects on both the host and other microorganisms. For example, H_2O_2-producing *Lactobacillus* spp. inhibit *Neisseria gonorrhoeae* and human immunodeficiency virus (HIV) replication in vitro (167, 332), suggesting a nonspecific antimicrobial defense mechanism resulting from the presence of lactobacilli in the normal vaginal flora. Likewise, in women with bacterial vaginosis, H_2O_2-producing lactobacilli are notably absent from the vaginal flora (89). In contrast, $^{\cdot}O_2^-$ made by *Mycoplasma pneumoniae* can inactivate host cell catalase, resulting in progressive oxidative damage to infected cells in vitro (3). *S. pneumoniae*-derived H_2O_2 may play a role in host cellular injury in pneumococcal pneumonia, as it has been shown to be toxic to rat alveolar epithelial cells in an in vitro model (86). The formation of dental plaque and the subsequent development of gingivitis and periodontitis are also related to the balance of H_2O_2-producing and H_2O_2-degrading organisms in the oral microenvironment (269).

Microorganisms are also continually exposed to endogenously produced NO˙ through denitrification (334). This process is a distinctive mode of respiration that is essential to many forms of bacteria and fungi; it involves the transformation of oxyanions of nitrogen to N_2, mainly under conditions of reduced oxygen tension or strict anaerobiosis. Denitrification is controlled by a number of metalloenzymes, of which nitrite reductase has been identified as the enzyme responsible for the conversion of nitrite to NO˙ (334). Two mutually exclusive nitrite reductases have been identified among denitrifying bacteria: a tetraheme cytochrome *cd* located in the periplasm of gram-negative organisms, and a Cu-containing protein bound to the cytoplasmic membrane of gram-positive organisms (334). The locations of these enzymes may limit the potential toxicity of the endogenously produced NO˙, as NO˙ is subsequently rapidly reduced by a cytoplasmic membrane-associated NO˙ reductase. Recent data also demonstrate the existence of a NOS system in *Nocardia* spp. (64), the first confirmation of such a system in microorganisms. The stoichiometry of products formed with respect to substrates used, cofactor requirements, and inhibition by N^G-nitro-L-arginine were found to be similar to those observed in mammalian NOS (64). There is evidence that erythrocytes infected with *Plasmodium falciparum* may also generate NO˙ via NOS and produce a soluble factor that is able to evoke NO˙ production in host tissues (113). Reports of other microbial NOS are likely to appear in the future.

Exogenous Sources

Phagocyte-derived oxidants and their role in host defense. The primary source of exogenous oxidative stress for pathogenic bacteria during the process of active infection is their attack by host phagocytic cells. Phagocytes utilize the cytotoxic effects of many of the oxidants outlined above as a component of their host defense mechanism (Fig. 1). When a phagocyte encounters a microorganism, the latter is surrounded by a portion of the phagocyte membrane, which then invaginates, forming a discrete phagosome (148). This process leads to increased phagocyte oxygen consumption and initiates a complex biochemical signaling system which activates a unique membrane-associated NADPH-dependent oxidase complex (67). This enzyme univalently reduces O_2 to $^{\cdot}O_2^-$, which is then secreted into the phagosome (67). There, $^{\cdot}O_2^-$ dismutes to H_2O_2. These toxic compounds may also leak extracellularly as the phagosome is closing.

Following phagocytosis, microorganisms are subjected to

further insult as phagocyte primary (or azurophilic) cytoplasmic granules fuse with the phagosome. In addition to MPO, these granules contain mainly hydrolases (acid hydrolases, lysozyme, neutral proteases, deoxyribonucleases, etc.), which are probably responsible for the decomposition of killed organisms (31). Secondary (or specific) cytoplasmic granules fuse with the external plasma membrane before the primary granules do, thereby secreting their contents (lactoferrin, lysozyme, and vitamin B_{12}-binding protein) extracellularly (283). The membranes of these secondary granules also contain a number of functionally significant proteins, including CD11b/CD18, the formyl-methionyl-leucyl-phenylalanine receptor, and cytochrome b_{558} (48). The fusion of these granules with the plasma membrane serves to reinforce or sustain various cellular responses (29).

The importance of the NADPH-oxidase system for host microbicidal activity is exemplified in individuals with chronic granulomatous disease (CGD), a group of inherited disorders which are each characterized by defects in the NADPH-oxidase complex resulting in a lack of phagocyte $^{\cdot}O_2^-$ production (299). The NADPH-oxidase requires the assembly of its membrane and cytosolic components for generation of the respiratory burst. Likewise, the genetic defects in this enzyme observed among CGD patients are characterized by their localization to the membrane or cytosol. Approximately 60% of CGD patients have an X-linked defect in the membrane b cytochrome component as a result of mutations in the gp91*phox* (55%) or p22*phox* (5%) gene encoding the large and small subunits, respectively (236, 267, 300). Patients with autosomal defects most commonly lack the cytosolic component, p47*phox*, and account for approximately 35% of cases (55, 68). Less than 5% of CGD patients lack the p67*phox* cytosolic component (68). Regardless of the location of the defect, the clinical manifestations of the different genetic forms of CGD are quite similar. These persons suffer from recurrent pyogenic infections with organisms that are normally rapidly killed by oxidants: *Staphylococcus aureus*, enteric gram-negative rods, *Aspergillus* spp., and *Candida* spp. Infectious complications, which can involve virtually any organ system, typically begin in infancy and recur throughout childhood and adolescence.

Although associated with states of neutropenia, infections with other pathogenic organisms such as *P. aeruginosa* are infrequently encountered in CGD patients (299). In vitro data have demonstrated the ability of neutrophils to destroy *P. aeruginosa* (127, 145, 196, 221, 328). This process is markedly enhanced in the presence of serum which opsonizes the organism with complement and immunoglobulin to promote more efficient phagocytosis by the neutrophil (328). However, additional in vitro observations suggest that oxidants are not critical for neutrophil-mediated killing of *P. aeruginosa*. Neutrophils from CGD patients have the same capacity as normal neutrophils to kill *P. aeruginosa* (145). Without the presence of ambient O_2, neutrophils are unable to generate $^{\cdot}O_2^-$ and H_2O_2. However, their abilities to kill *P. aeruginosa* under aerobic and anaerobic conditions appear to be similar (196). This is in contrast to findings with *S. aureus*, in which neutrophils are unable to kill the organism under anaerobic conditions in vitro (197). It may be that $^{\cdot}O_2^-$-independent mechanisms of neutrophil killing, such as those involving granule-derived proteins and proteases, are more important in *P. aeruginosa* elimination (311). However, these findings do not eliminate the possibility that neutrophil-derived oxidants increase the effectiveness of the O_2-independent killing mechanisms.

Phagocyte-derived H_2O_2 may also be converted intra- or extracellularly to HOCl and the longer-lived chloramines in the presence of chloride and myeloperoxidase. In addition, MPO can catalyze the reaction of $^{\cdot}O_2^-$ and HOCl to form $^{\cdot}OH$ (232). All of these compounds are known to have a number of cytotoxic effects in vitro (313). However, their overall significance in in vivo microbicidal activity is unclear, as patients with MPO deficiency demonstrate delayed killing of fungi and bacteria but are normally resistant to most infections (237). Of all patients recognized with this disorder (a prevalence of approximately 1 in 2,000 of the general population) (225), only a few have had serious infections (58, 166). The majority of these patients had visceral or disseminated candidiasis (58, 179). Three of these patients had concomitant diabetes mellitus (58, 179), perhaps indicating that the clinical morbidity associated with MPO deficiency requires an additional defect in host defense.

Phagocytes may also participate in mode 1 and 2 bacterial killing by generating H_2O_2 as described by Imlay et al. (149, 153). As discussed above, the interaction of exogenous H_2O_2 at low concentrations with intracellular Fe^{2+} in *E. coli* results in DNA damage mediated by the ferryl radical. Bacterial exposure to higher H_2O_2 concentrations resulted in killing by a separate oxidative mechanism.

Perhaps a more physiologically significant mechanism involved in phagocyte-mediated oxidant generation and microbial toxicity involves the iron-catalyzed intra- or extracellular reaction of $^{\cdot}O_2^-$ and H_2O_2 to form $^{\cdot}OH$. Although there is a limited amount of free iron available for this reaction to take place in vivo, multiple potential host and microbial catalytic iron complexes exist, as discussed previously. In vitro studies have demonstrated that increased bacterial iron concentrations enhance $^{\cdot}OH$-mediated killing of *S. aureus* by H_2O_2, human monocytes, and neutrophil-derived cytoplasts (142, 256). However, the role of $^{\cdot}OH$-mediated killing of *S. aureus* by intact human neutrophils remains unresolved (70, 257). In addition, killing of *Leishmania donovani chagasi* promastigotes by H_2O_2 appears to involve iron-dependent $^{\cdot}OH$ formation (329), but these studies have not yet been extended to phagocyte systems. The role of iron in microbe-phagocyte interactions is clearly complicated, since Byrd and Horwitz have shown that conditions that modulate phagocyte iron concentration appear to affect intracellular microbicidal activity against *Legionella pneumophila* and *M. tuberculosis* in opposite ways (46, 47).

Recently, $NO^{\cdot}$ has been increasingly recognized as another phagocyte-derived oxidant involved in microbicidal activity. Its synthesis requires a $NO^{\cdot}$ synthase, of which there exist constitutive and inducible isoforms (see above) (216). The inducible enzyme has been definitively demonstrated in murine phagocytic cells and can be induced by a number of cytokines and lipopolysaccharides (216, 295). Despite efforts by many investigators, however, the ability to detect $NO^{\cdot}$ production by human mononuclear phagocytes has been modest at best under conditions where $NO^{\cdot}$ production by murine phagocytes is readily apparent (49, 117, 159, 160, 223). Recent data have demonstrated that human mononuclear phagocytes can produce constitutive $NO^{\cdot}$ synthase (255, 312). The inducible $NO^{\cdot}$ synthase mRNA and protein are generated in response to lipopolysaccharide and/or gamma interferon stimulation, demonstrating that human phagocytes appear to possess the necessary "machinery" to synthesize $NO^{\cdot}$. More direct evidence for $NO^{\cdot}$ production by human macrophages has been demonstrated by the recent findings of Nicholson et al. (227). An average of 65% of alveolar macrophages in bronchoalveolar lavage specimens from 11 patients with untreated, culture-positive pulmonary tuberculosis contained $NO^{\cdot}$ synthase mRNA and functional $NO^{\cdot}$ synthase expression. Of note, only 10% of alveolar macrophages from normal subjects demon-

strated similar findings. However, despite these reports, the quantity of NO˙ generated under a number of conditions was very small (312). In addition, studies where NO˙ production is equated with nitrite production may falsely overestimate the true quantity of NO˙ synthesis, as shown by Klebanoff and Nathan, who demonstrated that human neutrophils can synthesize nitrites via the catalase-catalyzed conversion of azide to nitrite in the presence of phagocyte-generated H_2O_2 in vitro (168).

The primary microbicidal effect of phagocyte-derived NO˙ appears to involve intracellular pathogens. A clear role in pyogenic bacterial infections has not been demonstrated. By treating murine-activated macrophages in vitro with N^G-monomethyl-L-arginine, a competitive inhibitor of nitrate and nitrite synthesis from L-arginine, a number of investigators have implicated NO˙ as having microbiostatic and/or microbicidal activity against pathogens such as *Cryptococcus neoformans* (117), *Toxoplasma gondii* (1), *Mycobacterium bovis* (100), *Leishmania major* (180, 182), *Schistosoma mansoni* (160), and others (223, 261). Further studies involving an in vivo model of murine leishmaniasis have demonstrated that NO˙ plays an important role in containing the extent of infection and decreasing the overall organism load (92, 181). A similar result was observed by Boockvar et al. in an in vivo model of murine listeriosis (28).

Although the importance of NO˙ production to murine macrophage function is now well established, these data are not directly applicable to human phagocytes, because the existence of a role for NO˙ in human phagocyte microbicidal activity is less clear. Recent data by Vouldoukis et al. suggest that the killing of *L. major* by human macrophages is mediated by NO˙, whose production is induced after cell activation via ligation of the low-affinity receptor for immunoglobulin E (FcERII/CD23 surface antigen) (307). This receptor is upregulated in cutaneous leishmaniasis. Additional in vitro data imply that tumor necrosis factor alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor stimulate human macrophages to restrict the growth of virulent *Mycobacterium avium* by a mechanism involving NO˙ (80). The previously discussed findings by Nicholson et al. (227) also suggest that NO˙ may be an important component of host defense against pulmonary tuberculosis. Likewise, Bukrinsky et al. reported that lipopolysaccharide or TNF-α-activated HIV-infected monocytes exhibit enhanced NO˙ production (44). In support of these findings, the authors detected RNA encoding the inducible NO˙ synthase in postmortem brain tissue from an AIDS patient with advanced HIV encephalitis. Nitric oxide may also contribute to the killing of staphylococci by neutrophil cytoplasts (anucleate, granule-poor, motile cells) which rapidly took up and killed the bacteria by a mechanism inhibited by N^G-monomethyl-L-arginine (194). In contrast to these various studies, a direct comparative study between murine and human macrophages revealed that activated murine but not human macrophages demonstrated enhanced NO˙ production and antimicrobial activity against *Toxoplasma gondii*, *Chlamydia psittaci*, and *Leishmania donovani* (223). Although not directly compared with murine macrophages, NO˙ production contributes minimally to human macrophage-mediated killing of *Cryptococcus neoformans* and *Schistosoma mansoni* (49, 158), organisms which are killed by a NO˙-mediated mechanism in murine macrophages (117, 160). This suggests that NO˙ makes a minimal contribution to the overall microbicidal activity against these pathogens in the human host.

Thus, there are increasing data supporting the concept that human phagocytes can produce NO˙, albeit in small quantities relative to their murine counterparts. However, these data are somewhat difficult to interpret, as the frequency with which negative findings are reported by laboratories is often quite low. This capability to synthesize NO˙ appears to be mediated via the classic NOS pathway. The microbicidal activity of human, like murine, phagocyte-derived NO˙, if and when it is generated, could contribute to host protection against intracellular organisms. However, the contribution of NO˙ relative to the other phagocyte antimicrobial mechanisms known to be effective against these and other pathogens has yet to be established.

Other oxidant sources and their contribution to microbicidal activity. Although phagocytes are the primary source of microorganism exposure to oxidants in mammalian hosts, other mechanisms of oxidant production exist and probably contribute to microbial oxidant stress. As discussed in a previous section, microorganisms such as *Nocardia* and *Lactobacillus* spp. produce NO˙ and H_2O_2, respectively, which may in turn have toxic effects on other microorganisms in close proximity (64, 89, 167, 332). In addition, endothelial cells produce NO˙, $˙O_2^-$, and H_2O_2 in response to a number of stimuli, including inflammation (216). Feng et al. have recently suggested that endothelial cell-derived NO˙ could protect these cells from infection with *Rickettsia conorii* (96). In an experimental model, pulmonary (tracheal and alveolar) epithelial cells also demonstrate luminal H_2O_2 production, which is enhanced after stimulation by phorbol myristate acetate and platelet-activating factor (165). Epithelial cells from cystic fibrosis patients have been shown to consume two- to threefold more oxygen than do normal cells, providing indirect evidence of a highly oxidative environment in a population known to have a chronically high organism load (296). These endothelial cell- or epithelial cell-derived compounds may exert microbial oxidant stress either alone or via their reaction by-products such as $ONOO^-$ and/or ˙OH in the extracellular space. Additionally, these oxidants may interact with phagocyte-derived oxidants, cytokines, and other compounds to potentiate the microbial insult.

Several antimicrobial agents used in the treatment of clinical infections, in addition to blocking key enzymes and other metabolic functions of microorganisms, produce reactive oxygen intermediates that are capable of damaging other biomolecules. For example, β-lactam antibiotics (penicillins and cephalosporins) have been shown to oxidatively damage DNA and deoxyribose in the presence of iron and copper salts, consistent with an ˙OH-mediated mechanism (246). In addition, the polyunsaturated structure of the polyene antifungal antibiotics (amphotericin, natamycin, and nystatin) gives them the propensity to oxidize to form peroxy radicals and thiobarbituric acid-reactive aldehyde fragments (281). These interactions can then lead to the generation of other oxygen-centered radicals capable of inciting further microbial injury. These newly recognized antibiotic effects may prove to be an important component of their biologic activities.

Likewise, a number of compounds undergo rapid redox cycling under aerobic conditions, potentially resulting in an additional source of extracellular oxidants for microbial encounter (135, 191). These compounds are univalently reduced to free radicals by cellular systems. In the presence of O_2, these reduced molecules are then reoxidized, with the resulting transfer of that electron to O_2, hence forming $˙O_2^-$ and H_2O_2, the latter via $˙O_2^-$ dismutation. Examples of such compounds include pharmacologic agents such as adriamycin, bleomycin, and nitrofurantoin (191).

The *P. aeruginosa* secretory product pyocyanin works by a similar mechanism. This compound is a phenazine-derived pigment that can undergo redox cycling to induce both intra- and

extracellular $\dot{}O_2^-$ and H_2O_2 production from O_2 in both eukaryotic and prokaryotic cells (134, 135, 218). This process contributes to cell death through the diversion of electron flow from normal biologic pathways into those leading to toxic oxidant generation. This pyocyanin-induced production of $\dot{}O_2^-$ and H_2O_2 also can lead to the formation of $\dot{}OH$ in the presence of a catalytic iron source (38, 208). Pyocyanin production increases under conditions of nutritional deprivation and oxidative stress (136). Interestingly, however, *P. aeruginosa* itself is relatively insensitive to pyocyanin and seemingly escapes oxidant-mediated injury during production of or exposure to this compound (136). This may be explained in part by its low endogenous levels of NADH/NADPH, its lack of NADPH:pyocyanin oxidoreductase, and/or its high levels of SOD and catalase.

In addition to its redox capabilities, pyocyanin has numerous in vivo and in vitro effects which could play a role in the pathogenesis of clinical infections. For example, the addition of pyocyanin, at concentrations detectable in pulmonary secretions of individuals with *P. aeruginosa* infection, to human ciliated nasal epithelial cells (4, 317, 318) and sheep tracheal epithelial cells (157) results in a loss of ciliary function as well as a decrease in in vivo tracheal mucus velocity in the sheep model (222). The effect on sheep cilia could be negated by the simultaneous presence of catalase, suggesting an oxidant-mediated mechanism (222). This process may contribute to the difficulty that cystic fibrosis patients experience in mobilizing their secretions (222). Other effects of pyocyanin potentially relevant to microbial killing and inflammatory tissue injury include those on stimulated neutrophils to alter $\dot{}O_2^-$ production and degranulation, host cell $NO^{\cdot}$ production, and lymphocyte proliferation and differentiation (207, 219, 220, 230, 282, 305, 310).

MECHANISMS OF MICROBIAL DEFENSE AGAINST OXIDANTS

Avoidance of Encounters with Phagocyte-Derived Oxidants

As microbial killing by phagocytes is a multistep process, microorganisms have likewise developed a sequential series of defense strategies to counteract this process. Some microorganisms secrete toxins to kill the phagocyte before they can be killed by it. Examples include the production of streptolysin by *Streptococcus* spp. (22), leukocidin by *Staphylococcus aureus* (263), and the *Clostridium septicum* toxin (193). Other organisms resist phagocytic uptake by covering their surfaces with hydrophobic capsules (*Neisseria meningitidis*, pneumococci) (103, 141, 147, 158, 258–260, 324). Pathogenic mucoid strains of *P. aeruginosa* synthesize alginate, an exopolysaccharide. In addition to aiding in avoiding phagocytic uptake, alginate has the ability to scavenge reactive oxygen intermediates, suppress leukocyte function, and promote bacterial adhesion (11, 82, 87, 177, 239, 280). This may be of particular clinical relevance, as airway isolates from individuals with cystic fibrosis commonly demonstrate alginate production (82, 239). *P. aeruginosa* also requires a unique glucose-dependent pathway for phagocytosis by macrophages (13). This may enhance its pathogenicity in the bronchoalveolar space, where concentrations of glucose and other carbohydrate are low. Acidification within the phagocytic vacuole is an important process to maximize the spontaneous dismutation of $\dot{}O_2^-$, hydrolase activity, and phagosome-lysosome fusions. Inhibition of this acidification process has been described for *Legionella pneumophila* (146) and *Toxoplasma gondii* (279). Although phagocyte-derived oxidants are important mediators in microbial killing, some organisms can survive the encounter to then inhibit phagosome-lysosome fusion and avoid enzymatic attack by hydrolytic enzymes. This process is poorly understood but has been demonstrated among some mycobacteria (143), *T. gondii* (143), *Chlamydia* spp. (143), and others (41, 146). Other organisms, such as *Listeria monocytogenes* (60), *Shigella flexneri* (274), and *Trypanosoma cruzi* (5), are able to escape from the phagosome by the secretion of membrane-damaging cytolysins.

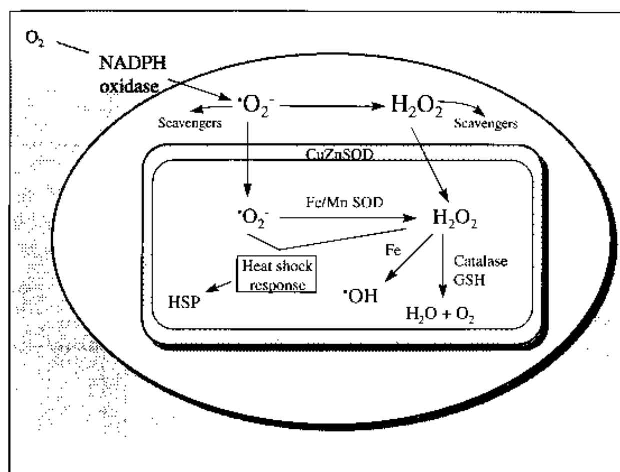

FIG. 2. Overview of bacterial defense mechanisms against oxidative killing inside the phagosome.

Another key virulence factor allowing for the avoidance of host defense mechanisms has been identified in a number of *Yersinia* spp. In *Yersinia enterocolitica*, a 51-kDa periplasmic protein encoded by the *yop-51* gene shares amino acid sequence identity with the catalytic domain of several protein tyrosine phosphatases (PTPases) (120). Activation of protein tyrosine kinases is an important signaling mechanism in many cells, including macrophages. By interfering with host signaling pathways, *Yersinia* spp. have the potential to modify the host immune response, which probably explains the importance of this process as a virulence factor. The *yop-51* gene resides on a naturally occurring 70-kb plasmid, and its mutation alters the virulence of the organism (26). An analogous gene, *yopH*, encodes a similar PTPase in *Y. pseudotuberculosis*. Further work has characterized the crystalline structure and active site of these proteins (294, 331). The specific gene and corresponding PTPase have not been determined for *Y. pestis*, but preliminary studies reveal that the *yop-51/yopH* gene is highly conserved in this organism (242).

Defense Strategies Specific for Oxidants

Nonenzymatic. Exposure to intraphagosomal oxidants is a fatal event for many microorganisms. However, some organisms have evolved an ability to inhibit the NADPH- oxidase-dependent oxidative burst and thus to inhibit reactive oxidant production within the phagosome (Fig. 2). This appears to be particularly important for intracellular pathogens as it aids in survival within the phagosome. For example, the lipophosphoglycan present on the membrane of *Leishmania major* and *L. donovani* (analogous to lipopolysaccharide in bacteria) inhibits protein kinase C activity in macrophages (30, 104), resulting in suppression of the respiratory burst and ultimately of $\dot{}O_2^-$ production. This inhibition of macrophage protein kinase C activity also impedes macrophage chemotactic locomotion and interleukin-1 (IL-1) production. *Legionella pneumophila* se-

cretes a compound shown to inhibit the neutrophil oxidative burst (272). *Leishmania donovani* (114) and *Legionella micdadei* (271) produce extracellular acidic phosphatases that block $^{\cdot}O_2^-$ formation in vitro. The mechanisms of these effects have not been further elucidated, however.

Antioxidant scavengers unique to specific pathogens have also evolved to protect microorganisms from phagocyte-derived oxidants. As noted above, *P. aeruginosa* produces alginate, an exopolysaccharide capable of scavenging oxidants (177, 280). In addition, the phenolic glycolipid of mycobacteria and the lipophosphoglycan of *L. donovani* are effective in scavenging $^{\cdot}OH$ and $^{\cdot}O_2^-$; these characteristics may enhance the intracellular survival of the organisms (61, 226). *Cryptococcus neoformans* is known to produce large amounts of mannitol both in vivo and in vitro (323). Mannitol in high concentrations has the ability to scavenge reactive oxygen species. Thus, its production by *C. neoformans* may be a protective mechanism by which the organism protects itself from oxidative killing by host phagocytes. Chaturvedi et al. have recently demonstrated, by using a low-mannitol-producing mutant of *C. neoformans*, that the ability of *C. neoformans* to produce and accumulate mannitol may influence its tolerance to heat and osmotic stresses and its pathogenicity in mice (62) through the scavenging of reactive oxygen intermediates (63).

The formation of heat shock proteins (HSP) by ingested bacteria may represent another adaptive mechanism. HSP production can be induced by increased temperature and/or oxidant exposure as a means of protection against both heat and oxidant damage. In *Mycobacterium tuberculosis* and *Mycobacterium leprae*, a strongly immunogenic antigen can be recognized by use of monoclonal antibodies (327). Production of this protein can be induced by stress, which may include phagocytosis. Severe stresses also increase the production of antioxidant enzymes such as SOD. There exists some evidence that HSP may also play a role in the regulation of antioxidant enzyme production in *E. coli* (293); this is discussed in more detail below.

Little is known about microbial defense against $NO^{\cdot}$. During the process of denitrification, microorganisms appear to limit toxicity by keeping endogenous $NO^{\cdot}$ levels very low (334). In an in vitro model, extracellularly generated $NO^{\cdot}$ was inactivated by the *P. aeruginosa*-derived phenazine pigment pyocyanin (310). Once phagocytized, microorganisms may have evolved a strategy to inhibit host nitric oxide synthase analogous to what has evolved for the NADPH-oxidase complex. However, at present, this has not been reported.

Enzymatic. Microorganisms have developed highly specific and effective enzymatic pathways of oxidant inactivation, including those catalyzed by SOD, catalase/peroxidase, and glutathione (GSH) in combination with glutathione peroxidase and glutathione reductase (122, 137). (See Fig. 2 and Table 1 for chemical reactions.)

Glutathione serves as a substrate for the H_2O_2-removing enzyme glutathione peroxidase. It can then be redox cycled via glutathione reductase for further H_2O_2 removal. GSH is also an $^{\cdot}OH$ scavenger. Eukaryotic cells depleted of GSH exhibit increased susceptibility to oxidant-mediated killing (205). There are also data suggesting that GSH depletion is involved in HIV replication (162, 286). The importance of this antioxidant system in prokaryotes, however, has not been clearly established. GSH reductase-negative *E. coli* mutants do not demonstrate an increased susceptibility to H_2O_2-mediated stress compared with the isogenic parental strain (118). However, there are data suggesting that GSH may facilitate the deactivation of *E. coli* aconitase and other [4Fe-4S]-containing dehydratases that have been oxidatively inactivated by $^{\cdot}O_2^-$ (111). Proteins immunologically related to GSH have been demonstrated in other bacterial species and in other strains of *E. coli* (240). Recently, Moore and Sparling have identified a GSH peroxidase homolog gene, *gpxA*, in *Neisseria meningitidis*. The amino acid sequence of this gene is highly homologous to GSH peroxidases found in other bacterial species (217). Thus, there may be several types of GSH-metabolizing proteins in bacteria which serve a similar purpose, and their distribution may even vary within a single species. Protozoa such as trypanosomes and leishmaniae produce trypanothione (93). It may have an analogous function to GSH in that it functions to maintain thiol redox within the organism and as a defense mechanism against oxidants, xenobiotics, and heavy metals. The importance of trypanothione to parasite survival can be exemplified by organism exposure to D,L-α-difluoromethylornithine, an antiparasitic agent used in the treatment of human African trypanosomiasis. D,L-α-Difluoromethylornithine inhibits parasite ornithine decarboxylase, which results in decreased cellular trypanothione levels among other effects (93). Why these organisms have evolved to produce trypanothione in addition to GSH is unclear.

Considerably more data are available on the distribution, structure, and regulation of microbial catalases and peroxidases (186). The antioxidant action of these enzymes is to catalytically convert H_2O_2 to H_2O and O_2. Nearly all aerobic and facultatively anaerobic microorganisms, with the exception of the *Streptococcus* spp., synthesize at least one form of catalase and/or peroxidase (201). The majority of obligate anaerobes lack this capability (201). These proteins are characterized by structural diversity between different organisms and even within the same organism. The most common form consists of a homotetramer with one protoheme IX per subunit. Most bacteria produce two catalases, whereas others such as *Klebsiella pneumoniae* and *P. aeruginosa* have the ability to produce multiple catalases under specific growth conditions (115, 136).

The two structurally distinct catalases of *E. coli*, termed hydroperoxidase I (HPI) and hydroperoxidase II (HPII), have been the most extensively studied (65, 66). HPI, a bifunctional catalase-peroxidase encoded by *katG*, contains two protoheme IX groups associated with a tetramer of identical 80-kDa subunits and is localized in the periplasmic space. HPII, a monofunctional catalase encoded by *katE*, consists of six heme *d* isomers associated with a hexameric structure of 84.2-kDa subunits and is found solely in the cytoplasm. The relative levels of HPI and HPII are controlled by two different regulons that respond to different environmental stimuli (186). HPI is synthesized preferentially in response to oxidative stress (H_2O_2), whereas HPII is produced in response to nutrient depletion as occurs in the stationary growth phase (188). Thus, not only are HPI and HPII different structurally and genetically, but also the processes controlling their synthesis respond to different stimuli and involve different mechanisms. The two catalases of *Bacillus subtilis* have been studied in comparison and appear to show some resemblance to *E. coli* HPI and HPII with regard to their structure and mechanism of control (185, 187). Among other bacterial species, the catalases of several other members of the *Enterobacteriaceae* family exhibit homology to *E. coli* HPI and HPII (298).

The complexity of bacterial catalase expression and regulation can be demonstrated by the reported correlation between the loss of catalase production and isoniazid resistance among *Mycobacterium tuberculosis* isolates (98, 330). Different amounts of catalase production have been found in a number of organisms in response to nutrient depletion and in association with their susceptibility to phagocyte killing (136, 188,

197). For example, the growth of *P. aeruginosa* in limited-succinate media resulted in increased catalase activity and the appearance of additional catalase isoforms compared to the catalase activity in the same organisms grown under nutritionally replete conditions (136, 210). Mandell demonstrated that neutrophils easily killed low- but not high-catalase-producing *Staphylococcus aureus* strains (197). This difference correlated with *in vivo* lethality in a mouse model. Likewise, catalase-deficient *E. coli* mutants exhibit an increased susceptibility to phagocyte-mediated killing (121).

Another mechanism of oxidant inactivation used by microorganisms involves SOD. The production of this group of enzymes is a key defense strategy aimed at the elimination of $^{\cdot}O_2^-$. Not only does this decrease the possibility of direct $^{\cdot}O_2^-$-mediated toxicity, but also it prevents $^{\cdot}O_2^-$-mediated reduction of iron and subsequent $^{\cdot}OH$ generation via the Haber-Weiss reaction. There are three common forms of SOD found in nature (12). Eukaryotes and some higher fungi predominantly produce CuZnSODs, homodimers (molecular weight 32,000) with two noncovalently linked identical subunits containing one atom each of copper and zinc. A few species of bacteria have also been found to contain CuZnSOD; some of these include *Stenotrophomonas* (*Pseudomonas*) *maltophila* (289), *Brucella abortus* (18), several *Haemophilus* spp. (171, 172, 176), *E. coli* (21), *N. meningitidis* (173), *L. pneumophila* (291), and *Salmonella* spp. (52). All bacteria, including obligate anaerobes, produce either FeSOD, MnSOD, or both. Like CuZnSOD, these enzymes exist as subunits (molecular weight 23,000) linked as dimers in FeSOD and dimers and tetramers in MnSOD. The metal content of both isozymes varies between 1 and 2 atoms per dimer. Most SODs are cytoplasmically located, although a few are located on or secreted through the cytoplasmic membrane (16, 21, 270). In general, FeSOD predominates in anaerobic organisms whereas MnSOD is more commonly found among aerobic organisms. Although variations in the FeSOD content have been observed in bacteria producing both isoenzymes, it is the control of MnSOD that is usually responsible for modulating the total level of SOD in bacteria.

Like catalase, microbial SOD regulation and genetics have been most extensively studied in *E. coli* (302), where SOD expression is dependent on a number of environmental stimuli. FeSOD, encoded by the *sodB* gene, is produced constitutively in *E. coli* grown aerobically or anaerobically but is upregulated when grown anaerobically in the presence of nitrate. MnSOD becomes the predominant form when the cell is exposed to oxidative stress. In addition, a hybrid form containing Fe and Mn has also been isolated in vitro (85). It appears that, functionally, FeSOD provides *E. coli* with the first line of defense against $^{\cdot}O_2^-$ and MnSOD is subsequently recruited in circumstances of increased oxidative stress. MnSOD gene (*sodA*) expression is governed by a number of regulon proteins, such as the Fur proteins and those under the control of the *sox* gene locus, including the Arc protein (119, 228, 304). These proteins are made in response to such stimuli as iron availability and oxygen/oxidant exposure, respectively. Interestingly, the Arc regulatory protein is also involved in the control of aconitase synthesis, suggesting that increased MnSOD is necessary to protect increased cellular concentrations of aconitase (109). Evidence suggests that MnSOD regulation also occurs at the posttranscriptional and posttranslational levels (244).

In recent work, Hassett et al. have begun to characterize a similar system in *P. aeruginosa*, which, like *E. coli*, possesses both an iron- and manganese-cofactored SOD (136, 138). Notably, *P. aeruginosa* has approximately four to five times the SOD activity reported for *E. coli* (136). When cloned, the genes encoding the MnSOD (*sodA*) and FeSOD (*sodB*) revealed a 50 and 67% sequence homology with the respective *E. coli* SODs. The relative quantities of the FeSOD or MnSOD isoenzyme produced appear, as in *E. coli*, to be dependent on nutritional availability and the degree of oxidative stress (136).

Some pathogens have evolved the ability to localize SOD activity to their extracellular environment as a means of resisting oxidant attack. For example, *Mycobacterium tuberculosis* secretes an FeSOD, whereas *Legionella pneumophila* and *E. coli* possess a periplasmic CuZnSOD (21, 270). *Nocardia asteroides* has a unique SOD associated with the outer cell wall, which can be selectively secreted extracellularly. This SOD differs significantly from those isolated from other bacteria in that it contains equimolar amounts of Fe, Mn, and Zn (16). Although *N. asteroides* induces an oxidative burst in human phagocytes, it is not readily killed by this mechanism. Subsequent in vitro and in vivo studies have demonstrated that this resistance to phagocyte-mediated killing is dependent on the production and secretion of SOD by the organism (15, 17). Initial killing and/or enhanced clearance of *N. asteroides* was observed in organs obtained from infected mice given a monoclonal anti-SOD antibody-treated *N. asteroides*. This effect was not observed in mice given a nonspecific nocardial antibody. Thus, the extracellular localization of bacterial SOD may be an important determinant in the pathogenesis of infection for *N. asteroides* and other pathogens.

The importance of microbial SOD production can be appreciated when studying SOD-deficient organisms. MnSOD- and FeSOD-negative mutants have been obtained from *E. coli* (303). However, with the recent discovery of the periplasmic *E. coli* CuZnSOD (21), studies with these mutants warrant qualification. Nonetheless, these organisms exhibit extreme sensitivity to oxidizing agents such as paraquat and methylene blue and are more susceptible to phagocyte-mediated killing (53, 121). Fe- and Mn-SOD-deficient double mutants demonstrate a marked increase in oxygen-dependent mutagenesis (94). Amino acid biosynthesis and membrane integrity also appear to be affected (151, 152). However, in vitro data obtained with *E. coli* suggest that overexpression of SOD may also be deleterious by accelerating H_2O_2 production in the organism upon its exposure to oxidative stress (276).

ROLE OF OXIDANTS IN VIRAL INFECTIONS

There is recent evidence that oxidants, whether derived from phagocytes or other sources, play a role in the pathogenesis of viral infections. The majority of work has centered around HIV. HIV infection is associated with a proinflammatory state in the host, resulting in high levels of circulating cytokines, including TNF-α, IL-1α, IL-1β, IL-2, IL-6, alpha interferon (IFN-α), and IFN-γ (262). Although it has been shown that some of these cytokines can activate HIV replication in the infected host cell directly (241), cytokine activation of phagocytes and other cells can also stimulate oxidant production. Oxidants also have direct effects on HIV replication. Legrand-Poels et al. demonstrated that the addition of exogenous H_2O_2 to a latently HIV-infected T-cell line (U1) resulted in increased replication of the HIV-1 provirus (178). Schreck et al. confirmed these findings in Jurkat T cells and provided further insight into the mechanism of activation (275). This process, like direct cytokine activation of HIV, is mediated by the induction of NF-κB, a ubiquitous transcription factor that is recognized by the HIV promoter (275). Likewise, Sandstrom et al. showed that HIV gene expression enhanced T-cell susceptibility to H_2O_2-induced apoptosis (273).

HIV-infected cells may be uniquely sensitive to oxidant

stress, as a number of studies have shown them to exhibit low levels of GSH, the main intracellular defense against oxidants. HIV-infected patients demonstrate decreased GSH levels in blood and peripheral blood mononuclear cells relative to those in normal patients, and this decrease becomes more pronounced with advanced disease (81). More specifically, Staal et al. found that in patients with symptomatic AIDS, GSH concentrations in CD8 and CD4 T cells are 62 and 63%, respectively, of those found in seronegative controls (286). The greatest decreases in GSH levels were seen in those patients with advanced infection. Not only does this decrease in intracellular GSH levels leave the infected cell susceptible to the direct effects of oxidants, but also it leads to increased NF-κB expression, resulting in further activation of HIV replication (285). Using a HeLa cell line transfected with the *tat* gene from HIV-1, Flores et al. found that the expression of the regulatory Tat protein, essential for virus replication, suppresses the expression of cellular MnSOD (102). These cells also exhibited other evidence of increased oxidative stress manifested by elevated levels of carbonyl proteins and decreased cellular sulfhydryl content (102). Thus, HIV-mediated modification of host antioxidant enzymes may be an important component in mediating ongoing HIV infection and the ultimate progression to severe immunodeficiency. This process may be further altered in the presence of opportunistic pathogens.

These advances in the understanding of the pathogenesis of HIV infection have prompted investigations into the use of antioxidants as therapy for HIV-infected individuals. In vitro studies with an HIV-infected human promonocytic cell line have demonstrated that HIV expression can be decreased by treatment of the cells with GSH, glutathione ester, or *N*-acetylcysteine (162, 195). Each of these compounds increases intracellular thiol concentrations and, as a result, inhibits NF-κB and ultimately HIV expression. These observations have led to studies of HIV-infected patients to determine whether the administration of *N*-acetylcysteine or L-2-oxothiazolidine-4-carboxylic acid (Procysteine) may alter disease progression (81, 161). Although both of these compounds were found to increase intracellular GSH levels in treated patients, there were no significant differences in CD4 cell counts, viral load, or proviral DNA frequency. Additional in vitro data suggest that the oxidant scavenger ascorbate also suppresses HIV replication in chronically and acutely infected T cells (129). This interaction appears to be synergistic when cells are exposed to ascorbate and *N*-acetylcysteine concurrently (128). In vivo studies with this combination have not been reported to date. Although oxidants may play a role in the pathogenesis of HIV infection, applying these findings for the development of potential therapeutic strategies in HIV-infected patients has been of limited benefit thus far.

Oxidants may also be involved in the pathogenesis of other viral infections. As in HIV-infected cells, H_2O_2 effectively induces synthesis of viral antigens in several lymphoid cell lines that harbor the Epstein-Barr virus genome (234). In contrast, H_2O_2 markedly decreases the release of progeny hepatitis B virus (HBV) particles in cultured hepatoma cells without causing any significant difference in the overall pattern of host protein synthesis (333). These findings may be important in the pathophysiology of chronic HBV infection. In one circumstance, turning off viral gene expression may be a way for the host to eradicate HBV infection. However, this mechanism may allow the virus to evade complete destruction by shutting off viral expression in infected hepatocytes adjacent to an area of active inflammation. This would allow a few cells to escape antigen-specific killing and resume viral replication once the inflammation subsides. Levels of vitamin E in plasma are notably low in patients with chronic liver disease (306). It is widely established that vitamin E is an important cell membrane antioxidant which acts as a free radical scavenger. One might speculate that its deficiency in this setting may further perpetuate tissue damage caused by oxidant release from injured hepatocytes in patients with chronic viral hepatitis and ultimately with cirrhosis. However, there have been no controlled clinical trials assessing the therapeutic role of vitamin E in these patients.

Human papillomavirus infection has been linked to an increased risk of acquiring human cervical carcinoma, and a recent study by Fernandez et al. suggests a potential oxidant-dependent mechanism which could be involved (97). They demonstrate that approximately 50% of healthy women possess polyamine oxidase and/or diamine oxidase in their cervical mucus. These enzymes were shown to act on spermine and spermidine (polyamines present in seminal fluid) to generate H_2O_2 and reactive aldehydes, which are likely to exert local mutagenic effects in vivo. These transformed cervical cells may exhibit prolonged survival in the presence of HPV infection through HPV suppression of apoptosis in the keratinocytes. Thus, the authors suggest that the effects of HPV infection of cervical cells may be synergistic with the effects of polyamine oxidation occurring in the cervical environment of sexually active women. The regulation of HPV replication may also be modified by oxidants, as the intracellular redox environment has been shown to affect the posttranslational DNA-binding activity of three E2 proteins (199).

Virus-host cell interactions in relation to oxidant production also appear to be important in the pathogenesis of influenza A virus infection. Although neutrophils predominate in the early inflammatory response to influenza A virus (106), the ability of this virus to adversely affect neutrophil and monocyte function in infected patients is well established (133) and may contribute to secondary bacterial infections. The influenza A virus hemagglutinin molecule appears to be an important mediator in this process of abnormal leukocyte function (56, 131). Although exposure to the virus leads to neutrophil activation and generation of a respiratory burst, the neutrophil response is atypical with regard to calcium fluxes, phospholipase C activation, and release of H_2O_2 but not $\dot{O}_2^-$ (130, 132). Daigneault et al. have further characterized this unique virus-phagocyte interplay, specifically through studies of the hemagglutinin-neutrophil receptor interaction (78). Clearly, further understanding of the role of oxidants in viral replication and virus-host cell interactions for these and other viruses could potentially lead to new therapeutic interventions.

UNTOWARD CONSEQUENCES OF OXIDANT PRODUCTION FOR THE HOST

At sites of infection, host-derived oxidants not only place the offending organisms under oxidative stress but also cause stress to neighboring host tissues. As discussed above, these oxidants are derived primarily from phagocytes; however, they can be produced by other cell types inherently or via induction by redox-active agents. Tissue injury at sites of infection may be the result of the host inflammatory response to the pathogen rather than cytotoxic components of the microorganism. The role of oxidants in such processes will briefly be reviewed, given their intimate relationship with the pathophysiology of many infectious diseases. Readers are referred to the myriad of excellent recent reviews on oxidant-mediated tissue injury (76, 124–126, 209, 216, 309).

Many aspects of acute and chronic inflammatory tissue injury appear to be mediated by oxidants released by neutrophils

and other phagocytes (216, 313). This process is enhanced by adherence of the phagocyte to the target cell surface (309). This adherence and subsequent movement of phagocytes from the blood to sites of inflammation require a complex signaling system involving a family of glycoproteins termed selectins. Selectins are synthesized by endothelial cells and stored in their secretory granules. When endothelial cells are activated by compounds such as thrombin or histamine (released in response to inflammation), the granules fuse with the outer membrane to expose the selectins on the cell surface. Phagocytes recognize these proteins, and this promotes their adherence to the endothelium and primes them for degranulation (190, 203). Following leukocyte activation, phagocyte-derived proteins termed integrins bind to their respective receptors on the endothelial cell. This interaction further strengthens adhesion and directs the migration of the phagocyte beneath the endothelium. Thus, this process targets phagocytes to areas of inflammation, where, through the further recruitment of phagocytes, oxidant-mediated tissue injury may result. Phagocyte-derived H_2O_2 can also indirectly lead to inflammatory tissue injury by upregulating selectin expression on endothelial cells and promoting further neutrophil localization (238).

Inflammatory tissue injury may also result via oxidant-induced cellular production of proinflammatory cytokines (72, 164, 206). Likewise, the production of these cytokines may potentiate further cellular oxidant release. Many of these interactions are mediated through the transcription-regulating factor, NF-κB (266). For example, in a rat model of neutrophilic alveolitis, endotoxin-induced NF-κB activation is thought to mediate the production of cytokine-induced neutrophil chemoattractant (analogous to human IL-8) by alveolar macrophages (25). This process is believed to be important for the recruitment of neutrophils and ultimately for the inflammatory tissue injury seen in this model. Oxidants can also activate NF-κB, promoting the production and release of cytokines such as IL-1 and TNF-α (8). Joint inflammation can also be induced by bacterial products, immune complexes, and crystals which recruit and activate phagocytes to form reactive oxygen species (125, 126) primarily. This process can result in tissue destruction via oxidant interactions with host proteoglycans, collagen, and elastin (124).

Injury to pulmonary epithelial and pulmonary vascular endothelial cells can also occur as a consequence of microbial infection in the case of acute necrotizing pneumonia and chronic lung infection seen in cystic fibrosis patients (278, 309). A similar injury pattern can be observed with infection-related pulmonary complications such as acute respiratory distress syndrome and hyperoxic lung injury. The principal mechanism by which this lung injury occurs remains to be determined, but it appears to involve alterations in a number of parameters of epithelial and endothelial cell function inducible by phagocyte-derived $^{\cdot}O_2^-$ and/or H_2O_2 (278). Iron-dependent formation of $^{\cdot}OH$ appears to be involved in the ability of phagocytes to damage endothelial cells in vitro, with the endothelial cells serving as the source of catalytic iron (107, 175). Peroxynitrite also has been shown to inhibit pulmonary epithelial cell ion channels, suggesting that this species could contribute to diffusion barrier disruption under conditions in which both $^{\cdot}O_2^-$ and $NO^{\cdot}$ are present concurrently (14).

MPO-derived oxidants released in response to a microbial stimulus may also contribute to inflammatory tissue injury directly via their toxic effects (148) and indirectly by their ability to inactivate serine protease inhibitors such as α_1-antitrypsin (54, 83). These antiproteases play a critical role in limiting the activity of proteases such as human neutrophil elastase released at local sites of inflammation (202). Thus, protease inhibitor inactivation by MPO-derived oxidants may lead to emphysematous changes analogous to those seen in individuals congenitally deficient in α_1-antitrypsin. Such processes have been hypothesized to contribute to lung injury associated with chronic bronchitis and other forms of chronic obstructive pulmonary disease (198, 233, 315). Others have suggested this process may also be involved in the lung disease observed in cystic fibrosis patients (214, 297).

Data supporting a role for $NO^{\cdot}$ and its derivatives in mediating inflammatory tissue injury in humans have been limited mainly to studies of autoimmune diseases (95, 216, 288). Evidence supporting $NO^{\cdot}$ production at sites of infection is lacking, however, as there are no definitive data demonstrating its formation by human phagocytes in vivo. In fact, recent literature suggests that $NO^{\cdot}$ may also have antioxidant properties (163, 288, 319). However, bacterially derived lipopolysaccharide induces $NO^{\cdot}$ production in endothelial cells. This process may contribute to the vasodilation and hypotension observed in septic shock (216).

Like microorganisms, host cells have evolved a complex system to defend themselves against oxidant injury. As discussed above, eukaryotic cells synthesize CuZnSOD as a means of $^{\cdot}O_2^-$ elimination. This enzyme is located in the cytosol and is usually constitutively expressed (12). Synthesis of a manganese-containing enzyme (MnSOD) can also be induced in the mitochondrial matrix under conditions of increased oxidative stress, specific cytokine stimulation, or heat shock (12). Since the H_2O_2 formed by the dismutation of $^{\cdot}O_2^-$ is also cytotoxic, eukaryotic cells have developed various mechanisms for its removal analogous to those found in prokaryotic microorganisms. This is accomplished by regulation of intracellular levels of catalase, the two GSH-dependent enzymes, GSH, and/or NADPH. Intra- and extracellular oxidant scavengers, such as ascorbic acid, vitamin E, β-carotene, and α-tocopherol, also probably play an important role in limiting cellular susceptibility to oxidant-mediated injury (313). Preventing the formation of $^{\cdot}O_2^-$ and H_2O_2 is the primary mechanism by which cells can limit the formation of other potent oxidants such as $^{\cdot}OH$ and the MPO-derived oxidants. Hydroxyl radical generation via the Haber-Weiss reaction can also be controlled by limiting the availability of redox-active iron catalysts through the formation of less active iron complexes such as extracellular lactoferrin and transferrin (7, 9, 42, 45, 320) and intracellular ferritin (10, 59). Heme oxygenase mRNA expression in mammalian cells is also known to be increased following cell exposure to oxidant stress. Although disputed by some investigators (231), the proposed mechanisms of protection afforded by heme oxygenase induction are twofold (6, 292). Heme oxygenase decreases the availability of intracellular iron capable of participating in the Haber-Weiss reaction by catalyzing the conversion of free heme to bile pigments. These bile pigments in turn exert antioxidant effects. Little is known about how host cells protect themselves from injury by $NO^{\cdot}$. It is likely that regulation of its production by the cell-specific $NO^{\cdot}$ synthase will prove important.

The extent of oxidant-mediated cytotoxicity observed at sites of inflammation is dependent on the balance between host- and microorganism-derived prooxidant and antioxidant forces. When this balance is swayed in favor of the prooxidants, not only microbial but also host cell cytotoxicity results, leading to clinical manifestations such as the sepsis syndrome, acute respiratory disease syndrome, lung destruction in diseases such as cystic fibrosis and α_1-antitrypsin deficiency, and joint destruction in inflammatory arthritides. Further understanding of the mechanisms that regulate the prooxidant-antioxidant balance will probably have significant therapeutic implications in

the management of these and other diseases characterized by inflammatory tissue injury.

CONCLUSIONS

Defining the many roles of reactive oxygen species in host-microbial interactions has proved complex. Although these oxidants are consistent by-products of normal cellular metabolism, the concentration and potential biotoxicity can be markedly enhanced under conditions of exogenous oxidative stress, by exposure to pharmacologic agents, and, particularly, by phagocytes as a means of host defense against invading microorganisms. These oxidants can have beneficial and detrimental functions in both the host and the microorganism. Therefore, both have evolved complex adaptive mechanisms for protection against these compounds, including enzymatic and nonenzymatic oxidant-scavenging systems. These systems act as virulence factors for the microorganism which enable it to survive in a hostile environment. Despite the marked progress in this field recently, there are still many unanswered questions regarding the role of oxidants in microbial pathophysiology that will probably prove to be a promising research area in the future.

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REFERENCES

1. **Adams, L. B., J. B. Hibbs, Jr., R. R. Taintor, and J. L. Krahenbuhl.** 1990. Microbiostatic effect of murine-activated macrophages for *Toxoplasma gondii*. Role for synthesis of inorganic nitrogen oxides from L-arginine. J. Immunol. **144:**2725–2729.
2. **Albrich, J. M., J. H. Gilbaugh III, K. B. Callahan, and J. K. Hurst.** 1986. Effects of the putative neutrophil-generated toxin, hypochlorous acid, on membrane permeability and transport systems of *Escherichia coli*. J. Clin. Invest. **78:**177–184.
3. **Almagor, M., I. Kahane, and S. Yatziv.** 1984. Role of superoxide anion in host cell injury induced by *Mycoplasma pneumoniae* infection. J. Clin. Invest. **73:**842–847.
4. **Amitani, R., R. Wilson, A. Rutman, R. Read, C. Ward, D. Burnett, R. A. Stockley, and P. J. Cole.** 1991. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. Am. J. Respir. Cell Mol. Biol. **4:**26–32.
5. **Andrews, N. W., C. K. Abrams, S. L. Slatin, and G. Griffiths.** 1990. A *T. cruzi*-secreted protein immunologically related to the complement component C9: evidence for membrane pore-forming activity at low pH. Cell **61:**1277–1287.
6. **Applegate, L. A., P. Luscher, and R. M. Tyrrell.** 1991. Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Res. **51:**974–978.
7. **Aruoma, O. I., and B. Halliwell.** 1987. Superoxide-dependent and ascorbate-dependent formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Are lactoferrin and transferrin promoters of hydroxyl-radical generation? Biochem. J. **241:**273–278.
8. **Baeuerle, P. A., and T. Henkel.** 1994. Function and activation of NF-kappaB in the immune system. Annu. Rev. Immunol. **12:**141–179.
9. **Baldwin, D. A., E. R. Jenny, and P. Aisen.** 1984. The effect of human serum transferrin and milk lactoferrin on hydroxyl radical formation from superoxide and hydrogen peroxide. J. Biol. Chem. **259:**13391–13394.
10. **Balla, G., H. S. Jacob, J. Balla, M. Rosenberg, K. Nath, F. Apple, J. W. Eaton, and G. M. Vercellotti.** 1992. Ferritin: a cytoprotective antioxidant stratagem of endothelium. J. Biol. Chem. **267:**18148–18153.
11. **Baltimore, R. S., and M. Mitchell.** 1980. Immunologic investigations of mucoid strains of *Pseudomonas aeruginosa*: comparison of susceptibility to opsonic antibody in mucoid and non-mucoid strains. J. Infect. Dis. **141:**238–246.
12. **Bannister, J. V., W. H. Bannister, and G. Rotilio.** 1987. Aspects of the structure, function, and applications of superoxide dismutase. Crit. Rev. Biochem. **22:**111–180.
13. **Barghouthi, S., K. D. E. Everett, and D. P. Speert.** 1995. Nonopsonic phagocytosis of *Pseudomonas aeruginosa* requires facilitated transport of D-glucose by macrophages. J. Immunol. **154:**3420–3428.
14. **Bauer, M. L., J. S. Beckman, R. J. Bridges, C. M. Fuller, and S. Matalon.** 1992. Peroxynitrite inhibits sodium uptake in rat colonic membrane vesicles. Biochim. Biophys. Acta Biomembr. **1104:**87–94.
15. **Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman.** 1985. Role of superoxide dismutase and catalase as determinants of pathogenicity of *Nocardia asteroides*: importance in resistance to microbicidal activities of human polymorphonuclear neutrophils. Infect. Immun. **47:**135–141.
16. **Beaman, B. L., S. M. Scates, S. E. Moring, R. Deem, and H. P. Misra.** 1983. Purification and properties of a unique superoxide dismutase from *Nocardia asteroides*. J. Biol. Chem. **258:**91–96.
17. **Beaman, L., and B. L. Beaman.** 1990. Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of *Nocardia asteroides* within the intact host. Infect. Immun. **58:**3122–3128.
18. **Beck, B. L., L. B. Tabatabai, and J. E. Mayfield.** 1990. A protein isolated from *Brucella abortus* is a Cu-Zn superoxide dismutase. Biochemistry **29:**372–376.
19. **Beckman, J. S., T. W. Beckman, J. Chen, P. A. Marshall, and B. A. Freeman.** 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial cell injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. USA **87:**1620–1624.
20. **Bennett, R. M., J. Davis, S. Campbell, and S. Portnoff.** 1983. Lactoferrin binds to cell membrane DNA: association of surface DNA with an enriched population of B cells and monocytes. J. Clin. Invest. **71:**611–618.
21. **Benov, L. T., and I. Fridovich.** 1994. *Escherichia coli* expresses a copper- and zinc-containing superoxide dismutase. J. Biol. Chem. **269:**25310–25314.
22. **Bernheimer, A. W., and L. L. Schwartz.** 1960. Leucocidal agents of haemolytic streptococci. J. Pathol. Bacteriol. **79:**37–46.
23. **Biemond, P., H. G. Van Eijk, A. J. G. Swaak, and J. F. Koster.** 1984. Iron mobilized from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes: possible mechanism in inflammation disease. J. Clin. Invest. **74:**1576–1579.
24. **Birgens, H. S., N. E. Hansen, H. Karle, and L. O. Kristensen.** 1983. Receptor binding of lactoferrin to human monocytes. Br. J. Haematol. **54:**383–391.
25. **Blackwell, T. S., E. P. Holden, T. R. Blackwell, J. E. DeLarco, and J. W. Christman.** 1994. Cytokine-induced neutrophil chemoattractant mediates neutrophilic alveolitis in rats: association with nuclear factor kappaB activation. Am. J. Respir. Cell Mol. Biol. **11:**464–472.
26. **Bliska, J. B., K. Guan, J. E. Dixon, and S. Falkow.** 1991. Tyrosine phosphatase hydrolysis of host proteins by an essential *Yersinia* virulence determinant. Proc. Natl. Acad. Sci. USA **88:**1187–1191.
27. **Bluard-Deconinck, J., J. Williams, R. W. Evans, J. van Snick, P. A. Osinski, and P. L. Masson.** 1978. Iron-binding fragments from the N-terminal and C-terminal regions of human lactoferrin. Biochem. J. **171:**321–327.
28. **Boockvar, K. S., D. L. Granger, R. M. Poston, M. Maybodi, M. K. Washington, J. B. Hibbs, Jr., and R. L. Kurlander.** 1994. Nitric oxide produced during murine listeriosis is protective. Infect. Immun. **62:**1089–1100.
29. **Borregaard, N., J. M. Heiple, E. R. Simons, and R. A. Clark.** 1983. Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase. Translocation during activation. J. Cell Biol. **97:**52–61.
30. **Brandonisio, O., M. A. Panaro, R. Marzio, A. Marangi, S. M. Faliero, and E. Jirillo.** 1994. Impairment of the human phagocyte oxidative responses caused by *Leishmania* lipophosphoglycan (LPG): in vitro studies. FEMS Immunol. Med. Microbiol. **8:**57–62.
31. **Bretz, U., and M. Baggiolini.** 1974. Biochemical and morphological characterization of azurophil and specific granules of human neutrophilic polymorphonuclear leukocytes. J. Cell Biol. **63:**251.
32. **Brines, R. D., and J. H. Brock.** 1983. The effect of trypsin and chymotrypsin on the in vitro antimicrobial and iron-binding properties of lactoferrin in human milk and bovine colostrum: unusual resistance of human apolactoferrin to proteolytic digestion. Biochim. Biophys. Acta **759:**229–235.
33. **Britigan, B. E., and B. L. Edeker.** 1991. *Pseudomonas* and neutrophil products modify transferrin and lactoferrin to create conditions that favor hydroxyl radical formation. J. Clin. Invest. **88:**1092–1102.
34. **Britigan, B. E., D. J. Hassett, G. M. Rosen, D. R. Hamill, and M. S. Cohen.** 1989. Neutrophil degranulation inhibits potential hydroxyl radical formation: differential impact of myeloperoxidase and lactoferrin release on hydroxyl radical production by iron supplemented neutrophils assessed by spin trapping. Biochem. J. **264:**447–455.
35. **Britigan, B. E., M. B. Hayek, B. N. Doebbeling, and R. B. Fick, Jr.** 1993. Transferrin and lactoferrin undergo proteolytic cleavage in the *Pseudomonas aeruginosa*-infected lungs of patients with cystic fibrosis. Infect. Immun. **61:**5049–5055.
36. **Britigan, B. E., and G. Rasmussen.** 1996. Unpublished data.
37. **Britigan, B. E., G. T. Rasmussen, and C. D. Cox.** 1994. *Pseudomonas* siderophore pyochelin enhances neutrophil-mediated endothelial cell injury. Am. J. Physiol. Lung Cell. Mol. Physiol. **266:**L192–L198.
38. **Britigan, B. E., T. L. Roeder, G. T. Rasmussen, D. M. Shasby, M. L.**

McCormick, and C. D. Cox. 1992. Interaction of the *Pseudomonas aeruginosa* secretory products pyocyanin and pyochelin generates hydroxyl radical and causes synergistic damage to endothelial cells: implications for pseudomonas-associated tissue injury. J. Clin. Invest. **90:**2187–2196.
39. **Britigan, B. E., G. M. Rosen, B. Y. Thompson, Y. Chai, and M. S. Cohen.** 1986. Stimulated neutrophils limit iron-catalyzed hydroxyl radical formation as detected by spin trapping techniques. J. Biol. Chem. **261:**17026–17032.
40. **Britigan, B. E., J. S. Serody, M. B. Hayek, L. M. Charniga, and M. S. Cohen.** 1991. Uptake of lactoferrin by mononuclear phagocytes inhibits their ability to form hydroxyl radical and protects them from membrane autoperoxidation. J. Immunol. **147:**4271–4277.
41. **Buchmeier, N. A., and F. Heffron.** 1991. Inhibition of macrophage phagosome-lysosome fusion by *Salmonella typhimurium*. Infect. Immun. **59:**2232–2238.
42. **Buettner, G. R.** 1987. The reaction of superoxide, formate radical, and hydrated electron with transferrin and its model compound, Fe(III)-ethylenediamine-N,N′-bis [2-(2-hydroxyphenyl)acetic acid] as studied by pulse radiolysis. J. Biol. Chem. **262:**11995–11998.
43. **Buettner, G. R.** 1993. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. Arch. Biochem. Biophys. **300:**535–543.
44. **Bukrinsky, M. I., H. S. L. M. Nottet, H. Schmidtmayerova, L. Dubrovsky, C. R. Flanagan, M. E. Mullins, S. A. Lipton, and H. E. Gendelman.** 1995. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. J. Exp. Med. **181:**735–745.
45. **Bullen, J. J., H. J. Rogers, and E. Griffiths.** 1978. Role of iron in bacterial infection. Curr. Top. Microbiol. Immunol. **80:**1–35.
46. **Byrd, T. F.** 1993. Iron is required for cytokine-mediated restriction of *Mycobacterium tuberculosis* multiplication in human monocytes. Clin. Res. **41:**324A. (Abstract.)
47. **Byrd, T. F., and M. A. Horwitz.** 1991. Lactoferrin inhibits or promotes *Legionella pneumophila* intracellular multiplication in nonactivated and interferon gamma-activated human monocytes depending upon its degree of iron saturation. Iron-lactoferrin and nonphysiologic iron chelates reverse monocyte activation against *Legionella pneumophila*. J. Clin. Invest. **88:**1103–1112.
48. **Calafat, J., T. W. Juijpers, and H. Janssen.** 1993. Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b_{558} and the adhesion molecule CD11b/CD18. Blood **81:**3122–3129.
49. **Cameron, M. L., D. L. Granger, J. B. Weinberg, W. J. Kozumbo, and H. S. Koren.** 1990. Human alveolar and peritoneal macrophages mediate fungistasis independently of L-arginine oxidation to nitrite or nitrate. Am. Rev. Respir. Dis. **142:**1313–1319.
50. **Campbell, E. J.** 1982. Human leukocyte elastase, cathepsin G, and lactoferrin: family of neutrophil granule glycoproteins that bind to an alveolar macrophage receptor. Proc. Natl. Acad. Sci. USA **79:**6941–6945.
51. **Candeias, L. P., K. B. Patel, M. R. L. Stratford, and P. Wardman.** 1993. Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. FEBS Lett. **333:**151–153.
52. **Canvin, J., P. R. Langford, K. E. Wilks, and J. S. Kroll.** 1996. Identification of *sodC* encoding periplasmic [Cu,Zn]-superoxide dismutase in *Salmonella*. FEMS Microbiol. Lett. **136:**215–220.
53. **Carlioz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5:**623–630.
54. **Carp, H., and A. Janoff.** 1979. In vitro suppression of serum-elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes. J. Clin. Invest. **63:**793–797.
55. **Casimir, C., M. Chetty, M.-C. Bohler, R. Garcia, A. Fischer, C. Griscelli, B. Johnson, and A. W. Segal.** 1992. Identification of the defective NADPH-oxidase component in chronic granulomatous disease: a study of 57 European families. Eur. J. Clin. Invest. **22:**403–406.
56. **Cassidy, L. F., D. S. Lyles, and J. S. Abramson.** 1989. Depression of polymorphonuclear leukocyte functions by purified influenza virus hemagglutinin and sialic acid-binding lectins. J. Immunol. **142:**4401–4406.
57. **Castro, L., M. Rodriguez, and R. Radi.** 1994. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. J. Biol. Chem. **269:**29409–29415.
58. **Cech, P., A. Papathanassiou, G. Boreux, P. Roth, and P. A. Miescher.** 1979. Hereditary myeloperoxidase deficiency. Blood **53**(3):403–411.
59. **Cermak, J., J. Balla, H. S. Jacob, G. Balla, H. Enright, K. Nath, and G. M. Vercellotti.** 1993. Tumor cell heme uptake induces ferritin synthesis resulting in altered oxidant sensitivity: possible role in chemotherapy efficacy. Cancer Res. **53:**5308–5313.
60. **Chakraborty, T., and W. Goebel.** 1988. Recent developments in the study of virulence in *Listeria monocytogenes*. Curr. Top. Microbiol. Immunol. **138:**41–58.
61. **Chan, J., T. Fujiwara, P. Brennan, M. McNeil, S. J. Turco, J.-C. Sibille, M. Snapper, P. Aisen, and B. R. Bloom.** 1989. Microbial glycolipids. Possible virulence factors that scavenge oxygen radicals. Proc. Natl. Acad. Sci. USA **86:**2453–2457.
62. **Chaturvedi, V., T. Flynn, W. G. Niehaus, and B. Wong.** 1996. Stress tolerance and pathogenic potential of a mannitol mutant of *Cryptococcus neoformans*. Microbiology **142:**937–943.
63. **Chaturvedi, V., B. Wong, and S. L. Newman.** 1996. Oxidative killing of *Cryptococcus neoformans* by human neutrophils: evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. J. Immunol. **156:**3836–3840.
64. **Chen, Y., and J. P. N. Rosazza.** 1994. A bacterial nitric oxide synthase from a *Nocardia* species. Biochem. Biophys. Res. Commun. **203:**1251–1258.
65. **Clairborne, A., and I. Fricovich.** 1979. Purification of the *o*-Dianisidine peroxidase from *Escherichia coli* B. Physicochemical characterization and analysis of its dual catalatic and peroxidatic activities. J. Biol. Chem. **254:**4245–4252.
66. **Clairborne, A., D. P. Malinowski, and I. Fridovich.** 1979. Purification and characterization of hydroperoxidase II of *Escherichia coli* B. J. Biol. Chem. **254:**11664–11668.
67. **Clark, R. A.** 1990. The human neutrophil respiratory burst oxidase. J. Infect. Dis. **161:**1140–1147.
68. **Clark, R. A., H. L. Malech, J. I. Gallin, H. Nunoi, B. D. Volpp, D. W. Pearson, W. M. Nauseef, and J. T. Curnutte.** 1989. Genetic variants of chronic granulomatous disease: prevalence of deficiencies of two cytosolic components of the NADPH oxidase system. N. Engl. J. Med. **321:**647–652.
69. **Coffman, T. J., C. D. Cox, B. L. Edeker, and B. E. Britigan.** 1990. Possible role of bacterial siderophores in inflammation. Iron bound to the *Pseudomonas* siderophore pyochelin can function as a hydroxyl radical catalyst. J. Clin. Invest. **86:**1030–1037.
70. **Cohen, M. S., B. E. Britigan, Y. S. Chai, S. Pou, T. L. Roeder, and G. M. Rosen.** 1991. Phagocyte-derived free radicals stimulated by ingestion of iron-rich *Staphylococcus aureus*: a spin-trapping study. J. Infect. Dis. **163:**819–824.
71. **Cohen, M. S., J. Mao, G. T. Rasmussen, J. S. Serody, and B. E. Britigan.** 1992. Interaction of lactoferrin and lipopolysaccharide (LPS): effects on the antioxidant property of lactoferrin and the ability of LPS to prime human neutrophils for enhanced superoxide formation. J. Infect. Dis. **166:**1375–1378.
72. **Colletti, L. M., D. G. Remick, G. D. Burtch, S. L. Kunkel, R. M. Strieter, and D. A. Campbell, Jr.** 1990. Role of tumor necrosis factor-α in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J. Clin. Invest. **85:**1936–1943.
73. **Coulthard, C. E., W. F. Short, R. Michaelis, G. Sykes, G. E. Skrimshire, A. F. Standfast, J. H. Birkenshaw, and H. Raistrick.** 1966. Notatin: an antibacterial glucose-aerodehydrogenase from *Penicillium notatum* Westling. J. Immunol. **150:**634–635.
74. **Cox, C. D., and P. Adams.** 1985. Siderophore activity of pyoverdin for *Pseudomonas aeruginosa*. Infect. Immun. **48:**130–138.
75. **Cox, C. D., K. L. Rinehart, Jr., M. L. Moore, and C. J. Cook, Jr.** 1981. Pyochelin: novel structure of an iron-chelating growth promoter for *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **78:**4256–4260.
76. **Cross, C. E., B. Halliwell, E. T. Borish, W. A. Pryor, R. L. Saul, J. M. McCord, and D. Harman.** 1987. Oxygen radicals and human disease. Ann. Intern. Med. **107:**526–545.
77. **Czapski, G.** 1984. On the use of ·OH scavengers in biological systems. Isr. J. Chem. **24:**29–32.
78. **Daigneault, D. E., K. L. Hartshorn, L. S. Liou, G. M. Abbruzzi, M. R. White, S.-K. Oh, and A. I. Tauber.** 1992. Influenza A virus binding to human neutrophils and cross-linking requirements for activation. Blood **80:**3227–3234.
79. **Daugherty, A., J. L. Dunn, D. L. Rateri, and J. W. Heinecke.** 1994. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. J. Clin. Invest. **94:**437–444.
80. **Denis, M.** 1991. Tumor necrosis factor and granulocyte macrophage-colony stimulating factor stimulate human macrophages to restrict growth of virulent *Mycobacterium avium* and to kill avirulent *M. avium*: killing effector mechanism depends on the generation of reactive nitrogen intermediates. J. Leukocyte Biol. **49:**380–387.
81. **De Quay, B., R. Malinverni, and B. H. Lauterburg.** 1992. Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine. AIDS **6:**815–819.
82. **Deretic, V., M. J. Schurr, J. C. Boucher, and D. W. Martin.** 1994. Conversion of *Pseudomonas aeruginosa* to mucoidy in cystic fibrosis: environmental stress and regulation of bacterial virulence by alternative sigma factors. J. Bacteriol. **176:**2773–2780.
83. **Desrochers, P. E., K. Mookhtiar, H. E. Van Wart, K. A. Hasty, and S. J. Weiss.** 1992. Proteolytic inactivation of α_{11}-proteinase inhibitor and α_1-antichymotrypsin by oxidatively activated human neutrophil metalloproteinases. J. Biol. Chem. **267:**5005–5012.
84. **Doring, G., M. Pfestorf, K. Botzenhart, and M. A. Abdallah.** 1988. Impact of proteases on iron uptake of *Pseudomonas aeruginosa* pyoverdin from transferrin and lactoferrin. Infect. Immun. **56:**291–293.
85. **Dougherty, H. W., S. J. Sadowski, and E. E. Baker.** 1978. A new iron-

containing superoxide dismutase from *Escherichia coli*. J. Biol. Chem. **253:** 5220–5223.
86. **Duane, P. G., J. B. Rubins, H. R. Weisel, and E. N. Janoff.** 1993. Identification of hydrogen peroxide as a *Streptococcus pneumoniae* toxin for rat alveolar epithelial cells. Infect. Immun. **61:**4392–4397.
87. **Eftekhar, F., and D. P. Speert.** 1988. Alginase treatment of mucoid *Pseudomonas aeruginosa* enhances phagocytosis by human monocyte-derived macrophages. Infect. Immun. **56:**2788–2793.
88. **Ellison, R. T., III, and T. J. Giehl.** 1991. Killing of gram-negative bacteria by lactoferrin and lysozyme. J. Clin. Invest. **88:**1080–1091.
89. **Eschenbach, D. A., P. R. Davick, B. L. Williams, S. J. Klebanoff, K. Young-Smith, C. M. Critchlow, and K. K. Holmes.** 1989. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. J. Clin. Microbiol. **27:**251–256.
90. **Esparza, I., and J. H. Brock.** 1980. The effect of trypsin digestion on the structure and iron-donating properties of transferrins from several species. Biochim. Biophys. Acta **622:**297–307.
91. **Evans, R. W., and J. Williams.** 1978. Studies of the binding of different iron donors to human serum transferrin and isolation of iron-binding fragments from the N- and C-terminal regions of the protein. Biochem. J. **173:**543–552.
92. **Evans, T. G., L. Thai, D. L. Granger, and J. B. Hibbs, Jr.** 1993. Effect of in vivo inhibition of nitric oxide production in murine leishmaniasis. J. Immunol. **151:**907–915.
93. **Fairlamb, A. H., and A. Cerami.** 1992. Metabolism and functions of trypanothione in the kinetoplastida. Annu. Rev. Microbiol. **46:**695–729.
94. **Farr, S. B., R. D'Ari, and D. Touati.** 1986. Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. Proc. Natl. Acad. Sci. USA **83:**8268–8272.
95. **Farrell, A. J., D. R. Blake, R. M. J. Palmer, and S. Moncada.** 1992. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann. Rheum. Dis. **51:**1219–1222.
96. **Feng, H.-M., V. L. Popov, and D. H. Walker.** 1994. Depletion of gamma interferon and tumor necrosis factor alpha in mice with *Rickettsia conorii*-infected endothelium: impairment of rickettsicidal nitric oxide production resulting in fatal, overwhelming rickettsial disease. Infect. Immun. **62:**1952–1960.
97. **Fernandez, C., R. M. Sharrard, M. Talbot, B. D. Reed, and N. Monks.** 1995. Evaluation of the significance of polyamines and their oxidases in the aetiology of human cervical carcinoma. Br. J. Cancer **72:**1194–1199.
98. **Ferrazoli, L., M. Palaci, M. A. Da Silva Telles, S. Y. Ueki, A. Kritski, L. R. M. Marques, O. C. Ferreira, and L. W. Riley.** 1995. Catalase expression, *katG*, and MIC of isoniazid for *Mycobacterium tuberculosis* isolates from Sao Paulo, Brazil. J. Infect. Dis. **171:**237–240.
99. **Finkelstein, R. A., C. V. Sciortino, and M. A. McIntosh.** 1983. Role of iron in microbe-host interactions. Rev. Infect. Dis. **5:**5759–5777.
100. **Flesch, I. E. A., and S. H. E. Kaufmann.** 1991. Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates. Infect. Immun. **59:**3213–3218.
101. **Flint, D. H., E. Smyk-Randall, J. F. Tuminello, B. Draczynska-Lusiak, and O. R. Brown.** 1993. The inactivation of dihydroxy-acid dehydratase in *Escherichia coli* treated with hyperbaric oxygen occurs because of the destruction of its Fe-S cluster, but the enzyme remains in the cell in a form that can be reactivated. J. Biol. Chem. **268:**25547–25552.
102. **Flores, S. C., J. C. Marecki, K. P. Harper, S. K. Bose, S. K. Nelson, and J. M. McCord.** 1993. Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in HeLa cells. Proc. Natl. Acad. Sci. USA **90:**7632–7636.
103. **Foley, M. J., and W. B. Wood, Jr.** 1959. Studies on the pathogenicity of group A streptococci. II. The antiphagocytic effects of the M protein and the capsular gel. J. Exp. Med. **110:**617–628.
104. **Frankenburg, S., V. Leibovici, N. Mansbach, S. J. Turco, and G. Rosen.** 1990. Effect of glycolipids of *Leishmania* parasites on human monocyte activity: inhibition by lipophosphoglycan. J. Immunol. **145:**4284–4289.
105. **Freeman, B.** 1994. Free radical chemistry of nitric oxide: looking at the dark side. Chest **105**(Suppl.):79S–84S.
106. **Fujisawa, H., S. Tsuru, M. Taniguchi, Y. Zinnaka, and K. Nomoto.** 1987. Protective mechanisms against pulmonary infection with influenza virus. I. Relative contribution of polymorphonuclear leukocytes and of alveolar macrophages to protection during the early phase of intranasal infection. J. Gen. Virol. **68:**425–432.
107. **Gannon, D. E., J. Varani, S. H. Phan, J. H. Ward, J. Kaplan, G. O. Till, R. H. Simon, U. S. Ryan, and P. A. Ward.** 1987. Source of iron in neutrophil-mediated killing of endothelial cells. Lab. Invest. **57:**37–44.
108. **Gardner, P. R., and I. Fridovich.** 1991. Superoxide sensitivity of the *Escherichia coli* 6-phosphogluconate dehydratase. J. Biol. Chem. **266:**1478–1483.
109. **Gardner, P. R., and I. Fridovich.** 1991. Superoxide sensitivity of the *Escherichia coli* aconitase. J. Biol. Chem. **266:**19328–19333.
110. **Gardner, P. R., and I. Fridovich.** 1992. Inactivation-reactivation of aconitase in *Escherichia coli*. A sensitive measure of superoxide radical. J. Biol. Chem. **267:**8757–8763.
111. **Gardner, P. R., and I. Fridovich.** 1993. Effect of glutathione on aconitase in *Escherichia coli*. Arch. Biochem. Biophys. **301:**98–102.
112. **Gaudu, P., D. Touati, V. Nivière, and M. Fontecave.** 1994. The NAD(P)H: flavin oxidoreductase from *Escherichia coli* as a source of superoxide radicals. J. Biol. Chem. **269:**8182–8188.
113. **Ghigo, D., R. Todde, H. Ginsburg, C. Costamagna, P. Gautret, F. Bussolino, D. Ulliers, G. Giribaldi, E. Deharo, G. Gabrielli, G. Pescarmona, and A. Bosia.** 1995. Erythrocyte stages of *Plasmodium falciparum* exhibit a high nitric oxide synthase (NOS) activity and release an NOS-inducing soluble factor. J. Exp. Med. **182:**677–688.
114. **Glew, R. H., A. K. Saha, S. Das, and A. T. Remaley.** 1988. Biochemistry of the *Leishmania* species. Microbiol. Rev. **52:**412–432.
115. **Goldberg, I., and A. Hochman.** 1989. Purification and characterization of a novel type of catalase from the bacterium *Kleibsiella pneumoniae*. Biochim. Biophys. Acta **991:**330–336.
116. **Goldstein, S., and G. Czapski.** 1986. The role and mechanism of metal ions and their complexes in enhancing damage in biological systems or in protecting these systems from the toxicity of O_2. J. Free Radicals Biol. Med. **2:**3–11.
117. **Granger, D. L., J. B. Hibbs, Jr., J. R. Perfect, and D. T. Durack.** 1988. Specific amino acid (L-arginine) requirement for microbiostatic activity of murine macrophages. J. Clin. Invest. **81:**1129–1136.
118. **Greenberg, J. T., and B. Demple.** 1986. Glutathione in *Escherichia coli* is dispensable for resistance to H_2O_2 and gamma radiation. J. Bacteriol. **168:**1026–1029.
119. **Greenberg, J. T., P. Monach, J. H. Chou, P. D. Josephy, and B. Demple.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**6181–6185.
120. **Guan, K.-L., and J. E. Dixon.** 1993. Bacterial and viral protein tyrosine phosphatases. Semin. Cell Biol. **4:**389–396.
121. **Gunther, M. R., M. Jinghe, G. A. Shetty, et al.** 1994. Use of isogenic mutants of *Escherichia coli* to demonstrate formation of hydroxyl radical during bacterial killing by neutrophils. Clin. Res. **42:**149A. (Abstract.)
122. **Haas, A., and W. Goebel.** 1992. Microbial strategies to prevent oxygen-dependent killing by phagocytes. Free Radical Res. Commun. **16:**137–157.
123. **Haber, F., and J. Weiss.** 1934. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. R. Soc. London Ser. A **147:**332–351.
124. **Halliwell, B., and J. M. C. Gutteridge.** 1985. The importance of free radicals and catalytic metal ions in human diseases. Mol. Aspects Med. **8:**89–193.
125. **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.
126. **Halliwell, B., J. M. C. Gutteridge, and D. Blake.** 1985. Metal ions and oxygen radical reactions in human inflammatory joint disease. Philos. Trans. R. Soc. London Ser. B **311:**659–671.
127. **Hammer, M. C., A. L. Balthch, N. T. Sutphen, R. P. Smith, and J. V. Conroy.** 1981. *Pseudomonas aeruginosa*: quantitation of maximum phagocytic and bactericidal capabilities of normal and human granulocytes. J. Lab. Clin. Med. **98:**938–948.
128. **Harakeh, S., and R. J. Jariwalla.** 1991. Comparative study of the anti-HIV activities of ascorbate and thiol-containing reducing agents in chronically HIV-infected cells. Am. J. Clin. Nutr. **54:**1231S–1235S.
129. **Harakeh, S., R. J. Jariwalla, and L. Pauling.** 1990. Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. Proc. Natl. Acad. Sci. USA **87:**7245–7249.
130. **Hartshorn, K. L., M. Collamer, M. R. White, J. H. Schwartz, and A. I. Tauber.** 1990. Characterization of influenza A virus activation of the human neutrophil. Blood **75:**218–226.
131. **Hartshorn, K. L., D. E. Daigneault, M. R. White, and A. I. Tauber.** 1992. Anomalous features of human neutrophil activation by influenza A virus are shared by related viruses and sialic acid-binding lectins. J. Leukocyte Biol. **51:**230–236.
132. **Hartshorn, K. L., D. E. Daigneault, M. R. White, M. Tuvin, J. L. Tauber, and A. I. Tauber.** 1992. Comparison of influenza A virus and formyl-methionyl-leucyl-phenylalanine activation of the human neutrophil. Blood **79:**1049–1057.
133. **Hartshorn, K. L., and A. I. Tauber.** 1988. The influenza virus-infected phagocyte. A model of deactivation. Hematol. Oncol. Clin. North Am. **2:**301–315.
134. **Hassan, H. M., and I. Fridovich.** 1979. Intracellular production of superoxide radical and hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. **196:**385–395.
135. **Hassan, H. M., and I. Fridovich.** 1980. Mechanism of the antibiotic action of pyocyanine. J. Bacteriol. **141:**156–163.
136. **Hassett, D. J., L. Charniga, K. Bean, D. E. Ohman, and M. S. Cohen.** 1992. Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. Infect. Immun. **60:**328–336.
137. **Hassett, D. J., and M. S. Cohen.** 1989. Bacterial adaptation to oxidative

stress: implications for pathogenesis and interaction with phagocytic cells. FASEB J. **3:**2574–2582.

138. **Hassett, D. J., W. A. Woodruff, D. J. Wozniak, M. L. Vasil, M. S. Cohen, and D. E. Ohman.** 1993. Cloning and characterization of the *Pseudomonas aeruginosa sodA* and *sodB* genes encoding manganese- and iron-cofactored superoxide dismutase: demonstration of increased manganese superoxide dismutase activity in alginate-producing bacteria. J. Bacteriol. **175:**7658–7665.
139. **Hausladen, A., and I. Fridovich.** 1994. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. J. Biol. Chem. **269:**29405–29408.
140. **Heinecke, J. W., W. Li, G. A. Francis, and J. A. Goldstein.** 1993. Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. J. Clin. Invest. **91:**2866–2872.
141. **Hendley, J. O., K. R. Powell, R. Rodewald, H. H. Holzgrefe, and R. Lyles.** 1975. Demonstration of a capsule on *Neisseria gonorrhoeae*. N. Engl. J. Med. **296:**608–611.
142. **Hoepelman, I. M., W. A. Bezemer, C. M. J. E. Vandenbroucke-Grauls, J. J. M. Marx, and J. Verhoef.** 1990. Bacterial iron enhances oxygen radical-mediated killing of *Staphylococcus aureus* by phagocytes. Infect. Immun. **58:**26–31.
143. **Hof, H.** 1991. Microbial strategies for intracellular survival. Infection **19**(Suppl. 4)**:**S202–S205.
144. **Hogg, N., V. M. Darley-Usmar, M. T. Wilson, and S. Moncada.** 1992. Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. Biochem. J. **281:**419–424.
145. **Holmes-Gray, B.** 1977. Metabolic stimulation and bactericidal function of polymorphonuclear leukocytes. J. Reticuloendothel. Soc. **22:**87–88.
146. **Horwitz, M. A., and F. R. Maxfield.** 1984. *Legionella pneumophila* inhibits acidification of its phagosome in human monocytes. J. Cell Biol. **99:**1936–1943.
147. **Houston, T., and J. C. Rankin.** 1907. The opsonic and agglutinative power of blood serum in cerebro-spinal fever. Br. Med. J. **2:**1414–1419.
148. **Hurst, J. K., and W. C. Barrette, Jr.** 1989. Leukocyte oxygen activations and microbicidal oxidative toxins. Crit. Rev. Biochem. Mol. Biol. **24:**271–328.
149. **Imlay, J. A., S. M. Chin, and S. Linn.** 1988. Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro*. Science **240:**640–642.
150. **Imlay, J. A., and I. Fridovich.** 1991. Superoxide production by respiring membranes of *Escherichia coli*. Free Radical Res. Commun. **12–13:**59–66.
151. **Imlay, J. A., and I. Fridovich.** 1991. Assay of metabolic superoxide production in *Escherichia coli*. J. Biol. Chem. **266:**6957–6965.
152. **Imlay, J. A., and I. Fridovich.** 1992. Suppression of oxidative envelope damage by pseudoreversion of a superoxide dismutase-deficient mutant of *Escherichia coli*. J. Bacteriol. **174:**953–961.
153. **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.
154. **Imlay, J. A., and S. Linn.** 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **169:**2967–2976.
155. **Ischiropoulos, H., L. Zhu, J. Chen, M. Tsai, J. C. Martin, C. D. Smith, and J. S. Beckman.** 1992. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch. Biochem. Biophys. **298:**431–437.
156. **Iyengar, R., D. J. Stuehr, and M. A. Marletta.** 1987. Macrophage synthesis of nitrite, nitrate, and N-nitrosamines: precursors and role of the respiratory burst. Proc. Natl. Acad. Sci. USA **84:**6369–6373.
157. **Jackowski, J. T., Z. Szepfalusi, D. A. Wanner, Z. Seybold, M. W. Sielczak, I. T. Lauredo, T. Adams, W. M. Abraham, and A. Wanner.** 1991. Effects of *P. aeruginosa*-derived bacterial products on tracheal ciliary function: role of O_2 radicals. Am. J. Physiol. Lung Cell. Mol. Physiol. **260:**L61–L67.
158. **James, J. F., and J. Swanson.** 1977. The capsule of the gonococcus. J. Exp. Med. **145:**1082–1086.
159. **James, S. L., K. W. Cook, and J. K. Lazdins.** 1990. Activation of human monocyte-derived macrophages to kill schistosomula of *Schistosoma mansoni in vitro*. J. Immunol. **145:**2686–2690.
160. **James, S. L., and J. Glaven.** 1989. Macrophage cytotoxicity against schistosomula of *Schistosoma mansoni* involves arginine-dependent production of reactive nitrogen intermediates. J. Immunol. **143:**4208–4212.
161. **Kalayjian, R. C., G. Skowron, R. Emgushov, M. Chance, S. A. Spell, P. R. Borum, L. S. Webb, K. H. Mayer, J. B. Jackson, B. Yen-Lieberman, et al.** 1994. A phase I/II trial of intravenous L-1-oxothiazolidine-4-carboxylic acid (procysteine) in asymptomatic HIV-infected subjects. J. Acquired Immune Defic. Syndr. **7:**369–374.
162. **Kalebic, T., A. Kinter, G. Poli, M. E. Anderson, A. Meister, and A. S. Fauci.** 1991. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and N-acetylcysteine. Proc. Natl. Acad. Sci. USA **88:**986–990.
163. **Kanner, J., S. Harel, and R. Granit.** 1991. Nitric oxide as an antioxidant. Arch. Biochem. Biophys. **289:**130–136.
164. **Kasama, T., K. Kobayashi, T. Fukushima, M. Tabata, I. Ohno, M. Negishi, H. Ide, T. Takahashi, and Y. Niwa.** 1989. Production of interleukin 1-like factor from human peripheral blood monocytes and polymorphonuclear leukocytes by superoxide anion: the role of interleukin 1 and reactive oxygen species in inflamed sites. Clin. Immunol. Immunopathol. **53:**439–448.
165. **Kinnula, V. L., K. B. Adler, N. J. Ackley, and J. D. Crapo.** 1992. Release of reactive oxygen species by guinea pig tracheal epithelial cells in vitro. Am. J. Physiol. Lung Cell. Mol. Physiol. **262:**L708–L712.
166. **Kitahara, M., H. J. Eyre, Y. Simonian, C. L. Atkin, and S. J. Hasstedt.** 1981. Hereditary myeloperoxidase deficiency. Blood **57:**888–893.
167. **Klebanoff, S. J., and R. W. Coombs.** 1991. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. J. Exp. Med. **174:**289–292.
168. **Klebanoff, S. J., and C. F. Nathan.** 1993. Nitrite production by stimulated human polymorphonuclear leukocytes supplemented with azide and catalase. Biochem. Biophys. Res. Commun. **197:**192–196.
169. **Koppenol, W. H.** 1986. The reaction of ferrous EDTA with hydrogen peroxide. Evidence against hydroxyl radical formation. J. Free Radical Biol. Med. **1:**281–285.
170. **Koppenol, W. H., J. J. Moreno, W. A. Pryor, H. Ischiropoulos, and J. S. Beckman.** 1992. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem. Res. Toxicol. **5:**834–842.
171. **Kroll, J. S., P. R. Langford, and B. M. Loynds.** 1991. Copper-zinc superoxide dismutase of *Haemophilus influenzae* and *H. parainfluenzae*. J. Bacteriol. **173:**7449–7457.
172. **Kroll, J. S., P. R. Langford, J. R. Saah, and B. M. Loynds.** 1993. Molecular and genetic characterization of superoxide dismutase in *Haemophilus influenzae* type b. Mol. Microbiol. **10:**839–848.
173. **Kroll, J. S., P. R. Langford, K. E. Wilks, and A. D. Keil.** 1995. Bacterial [Cu,Zn]-superoxide dismutase: phylogenetically distinct from the eukaryotic enzyme, and not so rare after all! Microbiology **141:**2271–2279.
174. **Kuo, C. F., T. Mashino, and I. Fridovich.** 1987. α,β-Dihydroxyisovalerate dehydratase. J. Biol. Chem. **262:**4724–4727.
175. **Kvietys, P. R., W. Inaven, B. R. Bacon, and M. B. Grisham.** 1989. Xanthine oxidase-induced injury to endothelium: role of intracellular iron and hydroxyl radical. Am. J. Physiol. Heart Circ. Physiol. **257:**H1640–H1646.
176. **Langford, P. R., B. M. Loynds, and J. S. Kroll.** 1992. Copper-zinc superoxide dismutase in *Haemophilus* species. J. Gen. Microbiol. **138:**517–522.
177. **Learn, D. B., E. P. Brestel, and S. Seetharama.** 1987. Hypochlorite scavenging by *Pseudomonas aeruginosa* alginate. Infect. Immun. **55:**1813–1818.
178. **Legrand-Poels, S., D. Vaira, J. Pincemail, A. Van de Vorst, and J. Piette.** 1990. Activation of human immunodeficiency virus type 1 by oxidative stress. AIDS Res. Hum. Retroviruses **6:**1389–1397.
179. **Lehrer, R. I., and M. J. Cline.** 1971. Leukocyte candidacidal activity and resistance to systemic candidiasis in patients with cancer. Cancer **27:**1211–1217.
180. **Liew, F. Y., Y. Li, and S. Millott.** 1990. Tumor necrosis factor-α synergizes with IFN-gamma in mediating killing of *Leishmania major* through the induction of nitric oxide. J. Immunol. **145:**4306–4310.
181. **Liew, F. Y., S. Millott, C. Parkinson, R. M. J. Palmer, and S. Moncada.** 1990. Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J. Immunol. **144:**4794–4797.
182. **Liew, F. Y., L. Yun, and S. Milott.** 1990. Tumor necrosis factor-alpha with IFN-gamma in mediating killing of *Leishmania major* through the induction of nitric oxide. J. Immunol. **145:**4306–4310.
183. **Liochev, S. I., and I. Fridovich.** 1993. Modulation of the fumarases of *Escherichia coli* in response to oxidative stress. Arch. Biochem. Biophys. **301:**379–384.
184. **Locksley, R. M., C. B. Wilson, and S. J. Klebanoff.** 1982. Role for endogenous and acquired peroxidase in the toxoplasmacidal activity of murine and human mononuclear phagocytes. J. Clin. Invest. **69:**1099–1111.
185. **Loewen, P. C.** 1989. Genetic mapping of *katB*, a locus that affects catalase 2 levels in *Bacillus subtilis*. Can. J. Microbiol. **35:**807–810.
186. **Loewen, P. C.** 1992. Regulation of bacterial catalase synthesis, p. 97–115. *In* J. G. Scandalios (ed.), Molecular biology of free radical scavenging systems. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
187. **Loewen, P. C., and J. Switala.** 1987. Multiple catalases in *Bacillus subtilis*. J. Bacteriol. **169:**3601–3607.
188. **Loewen, P. C., J. Switala, and B. L. Triggs-Raine.** 1985. Catalases HPI and HPII in *Escherichia coli* are induced independently. Arch. Biochem. Biophys. **243:**144–149.
189. **Long, C. A., and B. H. J. Bielski.** 1980. Rate of reaction of superoxide radical with chloride-containing species. J. Phys. Chem. **84:**555–557.
190. **Lorant, D. E., M. K. Topham, R. E. Whatley, R. P. McEver, T. M. McIntyre, S. M. Prescott, and G. A. Zimmerman.** 1993. Inflammatory roles of P-selectin. J. Clin. Invest. **92:**559–570.
191. **Lown, J. W.** 1985. Molecular mechanisms of action of anticancer agents involving free radical intermediates. Adv. Free Radical Biol. Med. **1:**225–264.
192. **Lynch, R. E., and B. C. Cole.** 1980. *Mycoplasma pneumoniae*: a pathogen which manufactures superoxide but lacks superoxide dismutase. Proc. Fed. Eur. Biochem. Soc. **62:**49–56.
193. **MacLennan, J. D.** 1962. The histotoxic clostridial infections of man. Bacteriol. Rev. **26:**177–276.
194. **Malawista, S. E., R. R. Montgomery, and G. Van Blaricom.** 1992. Evidence

for reactive nitrogen intermediates in killing of staphylococci by human neutrophil cytoplasts. A new microbicidal pathway for polymorphonuclear leukocytes. J. Clin. Invest. **90:**631–636.
195. **Malorni, W., R. Rivabene, M. T. Santini, and G. Donelli.** 1993. *N*-Acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. FEBS Lett. **327:**75–78.
196. **Mandell, G. L.** 1974. Bactericidal activity of aerobic and anaerobic polymorphonuclear neutrophils. Infect. Immun. **9:**337–341.
197. **Mandell, G. L.** 1975. Catalase, superoxide dismutase, and virulence of Staphylococcus aureus: in vitro and in vivo studies with emphasis on staphylococcal-leukocyte interaction. J. Clin. Invest. **55:**561–566.
198. **Matheson, N. R., P. S. Wong, and J. Travis.** 1979. Enzymatic inactivation of human alpha-1-proteinase inhibitor by neutrophil myeloperoxidase. Biochem. Biophys. Res. Commun. **88:**402–409.
199. **McBride, A. A., R. D. Klausner, and P. M. Howley.** 1992. Conserved cysteine residue in the DNA-binding domain of the bovine papillomavirus type 1 E2 protein confers redox regulation of the DNA-binding activity *in vitro*. Proc. Natl. Acad. Sci. USA **89:**7531–7535.
200. **McCord, J. M., and I. Fridovich.** 1968. The reduction of cytochrome c by milk xanthine oxidase. J. Biol. Chem. **243:**5753–5760.
201. **McCord, J. M., B. B. Keele, Jr., and I. Fridovich.** 1971. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. USA **68:**499–506.
202. **McElvaney, N. G., H. Nakamura, P. Birrer, C. A. Hebert, W. L. Wong, M. Aphonso, J. B. Baker, M. A. Catalano, and R. G. Crystal.** 1992. Modulation of airway inflammation in cystic fibrosis. In vivo suppression of interleukin-8 levels on the respiratory epithelial surface by aerosolization of recombinant secretory leukoprotease inhibitor. J. Clin. Invest. **90:**1296–1312.
203. **McEver, R. P.** 1992. Leukocyte-endothelial cell interactions. Curr. Opin. Cell Biol. **4:**840–849.
204. **Meier, B., and G. G. Habermehl.** 1990. Evidence for superoxide dismutase and catalase in mollicutes and release of reactive oxygen species. Arch. Biochem. Biophys. **277:**74–79.
205. **Meister, A.** 1992. A train of research: from glutamine synthetase to selective inhibition of glutathione synthesis. Chemtracts Biochem. Mol. Biol. **3:**75–106.
206. **Metinko, A. P., S. L. Kunkel, T. J. Standiford, and R. M. Strieter.** 1992. Anoxia-hyperoxia induces monocyte-derived interleukin-8. J. Clin. Invest. **90:**791–798.
207. **Miller, K. M., D. G. Dearborn, and R. U. Sorensen.** 1987. In vitro effect of synthetic pyocyanine on neutrophil superoxide production. Infect. Immun. **55:**559–563.
208. **Miller, R. A., and B. E. Britigan.** 1995. Protease cleaved iron-transferrin augments oxidant-mediated endothelial cell injury via hydroxyl radical formation. J. Clin. Invest. **95:**2491–2500.
209. **Miller, R. A., and B. E. Britigan.** 1995. The formation and biologic significance of phagocyte-derived oxidants. J. Invest. Med. **43:**39–49.
210. **Miller, R. A., M. A. Pfaller, and B. E. Britigan.** 1995. Characterization of antioxidant enzymes among *Pseudomonas aeruginosa* clinical isolates, abstr. 27. *In* IDSA 33rd Annual Meeting Program.
211. **Miller, R. A., G. T. Rasmussen, C. D. Cox, and B. E. Britigan.** 1996. Protease cleavage of iron-transferrin augments pyocyanin-mediated endothelial cell injury via promotion of hydroxyl radical formation. Infect. Immun. **64:**182–188.
212. **Miyasaki, K. T., J. J. Zambon, C. A. Jones, and M. E. Wilson.** 1987. Role of high-avidity binding of human neutrophil myeloperoxidase in the killing of *Actinobacillus actinomycetemcomitans*. Infect. Immun. **55:**1029–1036.
213. **Miyazawa, K., C. Mantel, L. Lu, D. C. Morrison, and H. E. Broxmeyer.** 1991. Lactoferrin-lipopolysaccharide interactions: effect on lactoferrin binding to monocyte/macrophage-differentiated HL-60 cells. J. Immunol. **146:**723–729.
214. **Mohammed, J. R., B. S. Mohammed, L. J. Pawluk, D. M. Bucci, N. R. Baker, and W. B. Davis.** 1988. Purification and cytotoxic potential of myeloperoxidase in cystic fibrosis sputum. J. Lab. Clin. Med. **112:**711–720.
215. **Molloy, A. L., and C. C. Winterbourn.** 1990. Release of iron from phagocytosed *Escherichia coli* and uptake by neutrophil lactoferrin. Blood **75:** 984–989.
216. **Moncada, S., and A. Higgs.** 1993. Mechanisms of disease: the L-arginine-nitric oxide pathway. N. Engl. J. Med. **329:**2002–2012.
217. **Moore, T. D. E., and P. F. Sparling.** 1995. Isolation and identification of a glutathione peroxidase homolog gene, *gpxA*, present in *Neisseria meningitidis* but absent in *Neisseria gonorrhoeae*. Infect. Immun. **63:**1603–1607.
218. **Morrison, M. M., E. T. Seo, J. K. Howie, and D. T. Sawyer.** 1978. Flavin model systems. 1. The electrochemistry of 1-hydroxyphenazine and pyocyanine in aprotic solvents. J. Am. Chem. Soc. **100:**207–211.
219. **Muhlradt, P. F., H. Tsai, and P. Conradt.** 1986. Effects of pyocyanine, a blue pigment from *Pseudomonas aeruginosa*, on separate steps of T cell activation: interleukin 2 (IL2) production, IL2 receptor formation, proliferation and induction of cytolytic activity. Eur. J. Immunol. **16:**434–440.
220. **Muller, M., and T. C. Sorrell.** 1991. Production of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid by human neutrophils is inhibited by *Pseudomonas aeruginosa* phenazine derivatives. Infect. Immun. **59:**3316–3318.
221. **Muller, P. K., K. Krohn, and P. F. Muhlradt.** 1989. Effects of pyocyanine, a phenazine dye from *Pseudomonas aeruginosa*, on oxidative burst and bacterial killing in human neutrophils. Infect. Immun. **57:**2591–2596.
222. **Munro, N. C., A. Barker, A. Rutman, G. Taylor, D. Watson, W. J. McDonald-Gibson, R. Towart, W. A. Taylor, R. Wilson, and P. J. Cole.** 1989. Effect of pyocyanin and 1-hydroxyphenazine on in vivo tracheal mucus velocity. J. Appl. Physiol. **67:**316–323.
223. **Murray, H. W., and R. F. Teitelbaum.** 1992. L-Arginine-dependent reactive nitrogen intermediates and the antimicrobial effect of activated human mononuclear phagocytes. J. Infect. Dis. **165:**513–517.
224. **Nathan, C., and Q. Xie.** 1994. Nitric oxide synthases: roles, tolls, and controls. Cell **78:**915–918.
225. **Nauseef, W. M.** 1990. Myeloperoxidase deficiency. Hematol. Pathol. **4:**165–178.
226. **Neill, M. A., and S. J. Klebanoff.** 1989. The effect of phenolic glycolipid-1 from *Mycobacterium leprae* on the antimicrobial activity of human macrophages. J. Exp. Med. **167:**30–42.
227. **Nicholson, S., M. Bonecini-Almeida, J. R. Lapa e Silva, C. Nathan, Q. Xie, R. Mumford, J. R. Weidner, J. Calaycay, J. Geng, N. Boechat, et al.** 1996. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J. Exp. Med. **183:**2293–2302.
228. **Niederhoffer, E. C., C. M. Naranjo, K. L. Bradley, and J. A. Fee.** 1990. Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus. J. Bacteriol. **172:**1930–1938.
229. **Nielands, J. B.** 1981. Microbial iron compounds. Annu. Rev. Biochem. **50:**715–731.
230. **Nutman, J., M. Berger, P. A. Chase, D. G. Dearborn, K. M. Miller, R. L. Waller, and R. U. Sorensen.** 1987. Studies on the mechanism of T cell inhibition by the *Pseudomonas aeruginosa* phenazine pigment pyocyanine. J. Immunol. **138:**3481–3487.
231. **Nutter, L. M., E. E. Sierra, and E. O. Ngo.** 1994. Heme oxygenase does not protect human cells against oxidant stress. J. Lab. Clin. Med. **123:**506–514.
232. **Okolow-Zubkowska, M. J., and H. A. O. Hill.** 1982. An alternative mechanism for the production of hydroxyl radicals by stimulated neutrophils, p. 423–427. *In* F. Rossi and P. Patriarca (ed.), Biochemistry and function of phagocytes. Plenum Press, New York, N.Y.
233. **Ossanna, P. J., S. T. Test, N. R. Matheson, S. Regiani, and S. J. Weiss.** 1986. Oxidative regulation of neutrophil elastase-alpha-a-proteinase inhibitor interactions. J. Clin. Invest. **77:**1939–1951.
234. **Oya, Y., A. Tonomura, and K. Yamamoto.** 1987. The biological activity of hydrogen peroxide. III. Induction of Epstein-Barr virus via indirect action, as compared with TPA and teleocidin. Int. J. Cancer **40:**69–73.
235. **Pacelli, R., D. A. Wink, J. A. Cook, M. C. Krishna, W. DeGraff, N. Friedman, M. Tsokos, A. Samuni, and J. B. Mitchell.** 1995. Nitric oxide potentiates hydrogen peroxide-induced killing of *Escherichia coli*. J. Exp. Med. **182:**1469–1479.
236. **Parkos, C. A., M. C. Dinauer, L. E. Walker, R. A. Allen, A. J. Jesaitis, and S. H. Orkin.** 1995. Primary structure and unique expression of the 22-kilodalton light chain of human neutrophil cytochrome b. Proc. Natl. Acad. Sci. USA **92:**3319–3323.
237. **Parry, M. F., R. K. Root, J. A. Metcalf, K. K. Delaney, L. S. Kaplow, and W. J. Richar.** 1981. Myeloperoxidase deficiency: prevalence and clinical significance. Ann. Intern. Med. **95:**293–301.
238. **Patel, K. D., G. A. Zimmerman, S. M. Prescott, R. P. McEver, and T. M. McIntyre.** 1991. Oxygen radicals induce human endothelial cells to express GMP-140 and bind neutrophils. J. Cell Biol. **112:**749–759.
239. **Pedersen, S. S., A. Kharazmi, F. Espersen, and N. Hoiby.** 1990. *Pseudomonas aeruginosa* alginate in cystic fibrosis sputum and the inflammatory response. Infect. Immun. **58:**3363–3368.
240. **Piccolomini, R., C. Di Ilio, A. Aceto, N. Allocati, A. Faraone, L. Cellini, G. Ravagnan, and G. Federici.** 1989. Glutathione transferase in bacteria: subunit composition and antigenic characterization. J. Gen. Microbiol. **135:** 3119–3125.
241. **Poli, G., P. Bressler, A. Kinter, E. Duh, W. C. Timmer, A. Rabson, S. Justement, S. Stanley, and A. S. Fauci.** 1990. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor α by transcriptional and post-transcriptional mechanisms. J. Exp. Med. **172:**151–158.
242. **Portnoy, D. A., H. Wolf-Watz, I. Bolin, A. B. Beeder, and S. Falkow.** 1984. Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. Infect. Immun. **43:**108–114.
243. **Pou, S., W. S. Pou, D. S. Bredt, S. H. Snyder, and G. M. Rosen.** 1992. Generation of superoxide by purified brain nitric oxide synthase. J. Biol. Chem. **267:**24173–24176.
244. **Privalle, C. T., and I. Fridovich.** 1990. Anaerobic biosynthesis of the manganese-containing superoxide dismutase in *Escherichia coli*. Effects of diazenedicarboxylic acid bis(*N,N′*-dimethylamide) (diamide). J. Biol. Chem. **265:**21966–21970.
245. **Pryor, W. A., and G. L. Squadrito.** 1995. The chemistry of peroxynitrite: a

product from the reaction of nitric oxide with superoxide. Am. J. Physiol. Lung Cell. Mol. Physiol. **268:**L699–L722.

246. **Quinlan, G. J., and J. M. Gutteridge.** 1988. Oxidative damage to DNA and deoxyribose by beta-lactam antibiotics in the presence of iron and copper salts. Free Radical Biol. Med. **5:**149–158.
247. **Radi, R., J. S. Beckman, K. M. Bush, and B. A. Freeman.** 1991. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J. Biol. Chem. **266:**4244–4250.
248. **Radi, R., J. S. Beckman, K. M. Bush, and B. A. Freeman.** 1991. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. **288:**481–487.
249. **Rakita, R. M., B. R. Michel, and H. Rosen.** 1989. Myeloperoxidase-mediated inhibition of microbial respiration: damage to *Escherichia coli* ubiquinol oxidase. Biochemistry **28:**3031–3036.
250. **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.
251. **Ramsey, P. G., T. Martin, E. Chi, and S. J. Klebanoff.** 1982. Arming of mononuclear phagocytes by eosinophil peroxidase bound to *Staphylococcus aureus*. J. Immunol. **128:**415–420.
252. **Rashba-Step, J., N. J. Turro, and A. I. Cederbaum.** 1993. ESR studies on the production of reactive oxygen intermediates by rat liver microsomes in the presence of NADPH or NADH. Arch. Biochem. Biophys. **300:**391–400.
253. **Rauckman, E. J., G. M. Rosen, and B. B. Kitchell.** 1979. Superoxide radical as an intermediate in the oxidation of hydroxylamines by mixed function amine oxidase. Mol. Pharmacol. **15:**131–137.
254. **Raymond, K. N., G. Muller, and B. F. Matzanke.** 1984. Complexation of iron by siderophores. A review of their solution and structural chemistry and biological function. Top. Curr. Chem. **123:**50–101.
255. **Reiling, N., A. J. Ulmer, M. Duchrow, M. Ernst, H.-D. Flad, and S. Hauschildt.** 1994. Nitric oxide synthase: mRNA expression of different isoforms in human monocytes/macrophages. Eur. J. Immunol. **24:**1941–1944.
256. **Repine, J. E., R. B. Fox, and E. M. Berger.** 1981. Hydrogen peroxide kills *Staphylococcus aureus* by reacting with staphylococcal iron to form hydroxyl radical. J. Biol. Chem. **256:**7094–7096.
257. **Repine, J. E., R. B. Fox, E. M. Berger, and R. N. Harada.** 1981. Effect of staphylococcal iron content on the killing of *Staphylococcus aureus* by polymorphonuclear leukocytes. Infect. Immun. **32:**407–410.
258. **Richardson, W. P., and J. C. Sadoff.** 1977. Production of a capsule by *Neisseria gonorrhoeae*. Infect. Immun. **15:**663–664.
259. **Roberts, R. B.** 1967. The interaction *in vitro* between group B meningococci and rabbit polymorphonuclear leukocytes. Demonstration of type specific opsonins and bacteriocidins. J. Exp. Med. **126:**795–818.
260. **Roberts, R. B.** 1970. The relationship between group A and group C meningococcal polysaccharide and serum opsonins in man. J. Exp. Med. **131:**499–513.
261. **Rockett, K. A., M. M. Awburn, W. B. Cowden, and I. A. Clark.** 1991. Killing of *Plasmodium falciparum* in vitro by nitric oxide derivatives. Infect. Immun. **59:**3280–3283.
262. **Roederer, M., S. W. Ela, F. J. T. Staal, and L. A. Herzenberg.** 1992. N-Acetylcysteine: a new approach to anti-HIV therapy. AIDS Res. Hum. Retroviruses **8:**209–217.
263. **Rogolsky, M.** 1979. Nonenteric toxins of *Staphylococcus aureus*. Microbiol. Rev. **43:**320–360.
264. **Roos, D., C. M. Eckmann, M. Yazdanbakhsh, M. N. Hamers, and M. DeBoer.** 1984. Excretion of superoxide by phagocytes measured with cytochrome c entrapped in resealed erythrocyte ghosts. J. Biol. Chem. **259:**1770–1775.
265. **Rosen, H., J. Orman, R. M. Rakita, B. R. Michel, and D. R. VanDevanter.** 1990. Loss of DNA-membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**10048–10052.
266. **Rosenberg, Z. F., and A. S. Fauci.** 1991. Immunopathogenesis of HIV infection. FASEB J. **5:**2382–2390.
267. **Royer-Pokora, B., L. M. Kunkel, A. P. Monaco, S. C. Goff, P. E. Newburger, R. L. Baehner, F. S. Cole, J. T. Curnutte, and S. H. Orkin.** 1986. Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. Nature **322:**32–38.
268. **Rush, J. D., and W. H. Koppenol.** 1986. Oxidizing intermediates in the reaction of ferrous EDTA with hydrogen peroxide. J. Biol. Chem. **261:**6730–6733.
269. **Ryan, C. S., and I. Kleinberg.** 1995. Bacteria in human mouths involved in the production and utilization of hydrogen peroxide. Arch. Oral Biol. **40:**753–763.
270. **Sadosky, A. B., J. W. Wilson, H. M. Steinman, and H. A. Shuman.** 1994. The iron superoxide dismutase of *Legionella pneumophila* is essential for viability. J. Bacteriol. **176:**3790–3799.
271. **Saha, A. K., J. N. Dowling, K. L. LaMarco, S. Das, A. T. Remaley, N. Olomu, M. T. Pope, and R. H. Glew.** 1985. Properties of an acid phosphatase from *Legionella micdadei* which blocks superoxide anion production by human neutrophils. Arch. Biochem. Biophys. **243:**150–160.
272. **Sahney, N. N., B. C. Lambe, J. T. Summersgill, and R. D. Miller.** 1990. Inhibition of polymorphonuclear leukocyte function by *Legionella pneumophila* exoproducts. Microb. Pathog. **9:**117–125.
273. **Sandstrom, P. A., B. Roberts, T. M. Folks, and T. M. Buttke.** 1993. HIV gene expression enhances T cell susceptibility to hydrogen peroxide-induced apoptosis. AIDS Res. Hum. Retroviruses **9:**1107–1113.
274. **Sansonetti, P. J., A. Ryter, P. Clerc, A. T. Maurelli, and J. Mounier.** 1986. Multiplication of *Shigella flexneri* within HeLa cells: lysis of the phagocytic vacuole and plasmid-mediated contact hemolysis. Infect. Immun. **51:**461–469.
275. **Schreck, R., P. Rieber, and P. A. Baeuerle.** 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. EMBO J. **10:**2247–2258.
276. **Scott, M. D., S. R. Meshnick, and J. W. Eaton.** 1987. Superoxide dismutase-rich bacteria: paradoxical increase in oxidant toxicity. J. Biol. Chem. **262:**3640–3645.
277. **Selvaraj, R. J., J. M. Zgliczynski, B. B. Paul, and A. J. Sbarra.** 1978. Enhanced killing of myeloperoxidase-coated bacteria in the myeloperoxidase-H_2O_2-Cl- system. J. Infect. Dis. **137:**481–485.
278. **Sibille, Y., and H. Y. Reynolds.** 1990. Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am. Rev. Respir. Dis. **141:**471–501.
279. **Sibley, L. D., R. Lawson, and E. Weidner.** 1986. Superoxide dismutase and catalase in *Toxoplasma gondii*. Mol. Biochem. Parasitol. **19:**83–87.
280. **Simpson, J. A., S. E. Smith, and R. T. Dean.** 1989. Scavenging by alginate of free radicals released by macrophages. Free Radical Biol. Med. **6:**347–353.
281. **Sokol-Anderson, M. L., J. Brajtburg, and G. Medoff.** 1986. Amphotericin B-induced oxidative damage and killing of *Candida albicans*. J. Infect. Dis. **154:**76–83.
282. **Sorensen, R. U., J. D. Klinger, H. A. Cash, P. A. Chase, and D. G. Dearborn.** 1983. In vitro inhibition of lymphocyte proliferation by *Pseudomonas aeruginosa* phenazine pigments. Infect. Immun. **41:**321–330.
283. **Spitznagel, J. K., F. G. Dalldorf, M. S. Leffell, J. D. Folds, I. R. H. Welsh, H. H. Cooney, and L. E. Martin.** 1974. Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab. Invest. **30:**774–785.
284. **Squadrito, G. L., and W. A. Pryor.** 1995. The formation of peroxynitrite in vivo from nitric oxide and superoxide. Chem. Biol. Interact. **96:**203–206.
285. **Staal, F. J. T., M. Roederer, and L. A. Herzenberg.** 1990. Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **87:**9943–9947.
286. **Staal, F. J. T., M. Roederer, D. M. Israelski, J. Bubp, L. A. Mole, D. McShane, S. C. Deresinski, W. Ross, H. Sussman, P. A. Raju, M. T. Anderson, W. Moore, S. W. Ela, and L. A. Herzenberg.** 1992. Intracellular glutathione levels in T cell subsets decrease in HIV-infected individuals. AIDS Res. Hum. Retroviruses **8:**305–311.
287. **Stark, J. M., and S. K. Jackson.** 1990. Sensitivity to endotoxin is induced by increased membrane fatty-acid unsaturation and oxidant stress. J. Med. Microbiol. **32:**217–221.
288. **Stefanovic-Racic, M., J. Stadler, and C. H. Evans.** 1993. Nitric oxide and arthritis. Arthritis Rheum. **36:**1036–1044.
289. **Steinman, H. M.** 1985. Bacteriocuprien superoxide dismutases in pseudomonads. J. Bacteriol. **162:**1255–1260.
290. **Stiefel, E. I., and G. D. Watt.** 1979. *Azobacter* cytochrome$_{b557.5}$ is a bacterioferritin. Nature **279:**81–83.
291. **St. John, G., and H. M. Steinman.** 1996. Periplasmic copper-zinc superoxide dismutase of *Legionella pneumophila*: role in stationary-phase survival. J. Bacteriol. **178:**1578–1584.
292. **Stocker, R.** 1990. Induction of haem oxygenase as a defence against oxidative stress. Free Radical Biol. Med. **9:**101–112.
293. **Storz, G., L. A. Tartaglia, S. B. Farr, and B. N. Ames.** 1990. Bacterial defenses against oxidative stress. Trends Genet. **6:**363–368.
294. **Stuckey, J. A., H. L. Schubert, E. B. Fauman, Z.-Y. Zhang, J. E. Dixon, and M. A. Saper.** 1994. Crystal structure of *Yersinia* protein tyrosine phosphatase at 2.5 Å and the complex with tungstate. Nature **370:**571–575.
295. **Stuehr, D. J., and M. A. Marletta.** 1985. Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. Proc. Natl. Acad. Sci. USA **82:**7738–7742.
296. **Stutts, M. J., M. R. Knowles, J. T. Gatzy, and R. C. Boucher.** 1986. Oxygen consumption and ouabain binding sites in cystic fibrosis nasal epithelium. Pediatr. Res. **20:**1316–1320.
297. **Suter, S.** 1994. The role of bacterial proteases in the pathogenesis of cystic fibrosis. Am. J. Respir. Crit. Care Med. **150**(Suppl.)**:**S118–S122.
298. **Switala, J., B. L. Triggs-Raine, and P. C. Loewen.** 1990. Homology among bacterial catalase genes. Can. J. Microbiol. **36:**728–731.
299. **Tauber, A. I., N. Borregaard, E. Simons, and J. Wright.** 1983. Chronic granulomatous disease: a syndrome of phagocyte oxidase deficiencies. Medicine (Baltimore) **62:**286–308.
300. **Teahan, C., P. Rowe, and P. Parker.** 1987. The X-linked chronic granulomatous disease gene codes for the β-chain of cytochrome b_{245}. Nature **327:**720–721.
301. **Theil, E. C.** 1987. Ferritin: structure, gene regulation, and cellular function

in animals, plants, and microorganisms. Annu. Rev. Biochem. **56:**289–315.

302. **Touati, D.** 1989. The molecular genetics of superoxide dismutase in *E. coli*. An approach to understanding the biological role and regulation of SODs in relation to other elements of the defence system against oxygen toxicity. Free Radical Res. Commun. **8:**1–9.
303. **Touati, D.** 1992. Regulation and protective role of the microbial superoxide dismutases, p. 231–261. *In* J. G. Scandalios (ed.), Molecular biology of free radical scavenging systems. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
304. **Tsaveva, I. R., and B. Weiss.** 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J. Bacteriol. **172:**4197–4205.
305. **Ulmer, A. J., J. Pryjma, Z. Tarnok, M. Ernst, and H.-D. Flad.** 1990. Inhibitory and stimulatory effects of *Pseudomonas aeruginosa* pyocyanine on human T and B lymphocytes and human monocytes. Infect. Immun. **58:** 808–815.
306. **von Herbay, A., H. De Groot, U. Hegi, W. Stremmel, G. Strohmeyer, and H. Sies.** 1994. Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. J. Hepatol. **20:**41–46.
307. **Vouldoukis, I., V. Riveros-Moreno, B. Dugas, F. Ouaaz, P. Bécherel, P. Debré, S. Moncada, and M. D. Mossalayi.** 1995. The killing of *Leishmania major* by human macrophages is mediated by nitric oxide induced after ligation of the Fc_RII/CD23 surface antigen. Proc. Natl. Acad. Sci. USA **92:**7804–7808.
308. **Wack, M. F., T. Tabatabaie, M. M. Huycke, and R. A. Floyd.** 1995. Membrane-associated extracellular superoxide production by *Enterococcus faecalis*. J. Invest. Med. **43**(Suppl.)**:**460A. (Abstract.)
309. **Ward, P. A.** 1991. Mechanisms of endothelial cell injury. J. Lab. Clin. Med. **118:**421–426.
310. **Warren, J. B., R. Loi, N. B. Rendell, and G. W. Taylor.** 1990. Nitric oxide is inactivated by the bacterial pigment pyocyanin. Biochem. J. **266:**921–923.
311. **Wasiluk, K. R., K. M. Skubitz, and B. H. Gray.** 1991. Comparison of granule proteins from human polymorphonuclear leukocytes which are bactericidal toward *Pseudomonas aeruginosa*. Infect. Immun. **59:**4193–4200.
312. **Weinberg, J. B., M. A. Misukonis, P. J. Shami, S. N. Mason, D. L. Sauls, W. A. Dittman, E. R. Wood, G. K. Smith, B. McDonald, K. E. Bachus, A. F. Haney, and D. L. Granger.** 1995. Human mononuclear phagocyte inducible nitric oxide synthase (iNOS): analysis of iNOS mRNA, iNOS protein, biopterin, and nitric oxide production by blood monocytes and peritoneal macrophages. Blood **86:**1184–1195.
313. **Weiss, S. J.** 1986. Oxygen, ischemia and inflammation. Acta Physiol. Scand. Suppl. **548:**9–37.
314. **Weiss, S. J., M. D. Lampert, and S. T. Test.** 1983. Long-lived oxidants generated by human neutrophils: characterization and bioactivity. Science **222:**625–628.
315. **Weiss, S. J., and S. Regiani.** 1984. Neutrophils degrade subendothelial matrices in the presence of alpha-1-proteinase inhibitor. Cooperative use of lysosomal proteinases and oxygen metabolites. J. Clin. Invest. **73:**1297–1303.
316. **Whittenbury, R.** 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. **35:**13–26.
317. **Wilson, R., T. Pitt, G. Taylor, D. Watson, J. MacDermot, D. Sykes, D. Roberts, and P. Cole.** 1987. Pyocyanin and 1-hydroxyphenazine produced by *Pseudomonas aeruginosa* inhibit the beating of human respiratory cilia in vitro. J. Clin. Invest. **79:**221–229.
318. **Wilson, R., D. A. Sykes, D. Watson, A. Rutman, G. W. Taylor, and P. J. Cole.** 1988. Measurement of *Pseudomonas aeruginosa* phenazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelium. Infect. Immun. **56:**2515–2517.
319. **Wink, D. A., I. Hanbauer, M. C. Krishna, W. DeGraff, J. Gamson, and J. B. Mitchell.** 1993. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc. Natl. Acad. Sci. USA **90:**9813–9817.
320. **Winterbourn, C. C.** 1983. Lactoferrin-catalyzed hydroxyl radical production: additional requirements for a chelating agent. Biochem. J. **210:**15–19.
321. **Winterbourn, C. C.** 1987. The ability of scavengers to distinguish ˙OH production in the iron catalyzed Haber-Weiss reaction. Comparison of four assays for ˙OH. Free Radical Biol. Med. **3:**33–39.
322. **Winterbourn, C. C., J. J. M. Van den Berg, E. Roitman, and F. A. Kuypers.** 1992. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. Arch. Biochem. Biophys. **296:**547–555.
323. **Wong, B., J. R. Perfect, S. Beggs, and K. A. Wright.** 1990. Production of the hexitol D-mannitol by *Cryptococcus neoformans* in vitro and in rabbits with experimental meningitis. Infect. Immun. **58:**1664–1670.
324. **Wood, W. B., Jr., M. R. Smith, and B. Watson.** 1946. Studies on the mechanism of recovery in pneumonococcal pneumonia. IV. The mechanism of phagocytosis in the absence of antibody. J. Exp. Med. **84:**387–402.
325. **Wright, C. D., and R. D. Nelson.** 1988. Candidacidal activity of myeloperoxidase: characterization of myeloperoxidase-yeast complex formation. Biochem. Biophys. Res. Commun. **154:**809–817.
326. **Yamada, Y., T. Amagasaki, D. W. Jacobsen, and R. Green.** 1987. Lactoferrin binding by leukemia cell lines. Blood **70:**264–270.
327. **Young, D. B., J. Ivanyi, J. H. Cox, and J. R. Lamb.** 1987. The 65kDa antigen of mycobacteria—a common bacterial protein? Immunol. Today **8:**215–219.
328. **Young, L. S., and D. Armstrong.** 1972. Human immunity to *Pseudomonas aeruginosa*. I. In-vitro interaction of bacteria, polymorphonuclear leukocytes, and serum factors. J. Infect. Dis. **126:**257–276.
329. **Zarley, J. H., B. E. Britigan, and M. E. Wilson.** 1991. Hydrogen peroxide-mediated toxicity for *Leishmania donovani chagasi* promastigotes. Role of hydroxyl radical and protection by heat shock. J. Clin. Invest. **88:**1511–1521.
330. **Zhang, Y., B. Heym, B. Allen, D. Young, and S. Cole.** 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature **358:**591–593.
331. **Zhang, Z.-Y., Y. Wang, L. Wu, E. B. Fauman, J. A. Stuckey, H. L. Schubert, M. A. Saper, and J. E. Dixon.** 1994. The Cys(X)$_5$Arg catalytic motif in phosphoester hydrolysis. Biochemistry **33:**15266–15270.
332. **Zheng, H., T. M. Alcorn, and M. S. Cohen.** 1994. Effects of H_2O_2-producing lactobacilli on *Neisseria gonorrhoeae* growth and catalase activity. J. Infect. Dis. **170:**1209–1215.
333. **Zheng, Y.-W., and T. S. B. Yen.** 1994. Negative regulation of hepatitis B virus gene expression and replication by oxidative stress. J. Biol. Chem. **269:**8857–8862.
334. **Zumft, W. G.** 1993. The biological role of nitric oxide in bacteria. Arch. Microbiol. **160:**253–264.