ANTIMICROBIAL REACTIVE OXYGEN AND NITROGEN SPECIES: CONCEPTS AND CONTROVERSIES

Ferric C. Fang

Abstract | Phagocyte-derived reactive oxygen and nitrogen species are of crucial importance for host resistance to microbial pathogens. Decades of research have provided a detailed understanding of the regulation, generation and actions of these molecular mediators, as well as their roles in resisting infection. However, differences of opinion remain with regard to their host specificity, cell biology, sources and interactions with one another or with myeloperoxidase and granule proteases. More than a century after Metchnikoff first described phagocytosis, and more than four decades after the discovery of the burst of oxygen consumption that is associated with microbial killing, the seemingly elementary question of how phagocytes inhibit, kill and degrade microorganisms remains controversial. This review updates the reader on these concepts and the topical questions in the field.

PATHOPHYSIOLOGICAL
Functional changes that are associated with, or result from, disease or injury.

Departments of Laboratory Medicine and Microbiology, University of Washington School of Medicine, 1959 North East Pacific Street, Box 357242, Seattle, Washington 98195-7242, USA.
e-mail: fcfang@washington.edu
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> "When a thing ceases to be a subject of controversy, it ceases to be a subject of interest."
>
> *William Hazlitt*

Phagocytic cells are among the most important components of the innate immune response, which is the first line of host defence. Two of the most important antimicrobial systems of phagocytic cells are the NADPH phagocyte oxidase (also known as phox) and inducible nitric oxide synthase (iNOS) pathways, which are responsible for the generation of superoxide ($O_2^{-\bullet}$) and nitric oxide ($NO^{\bullet}$) radicals, respectively. Although these systems both depend on NADPH and molecular oxygen, and sometimes function together, the NADPH phagocyte oxidase and iNOS are separate enzyme complexes with independent regulation (FIG. 1). $O_2^{-\bullet}$ and other oxygen-derived intermediates that can modify organic molecules are referred to as 'reactive oxygen species' (ROS), whereas $NO^{\bullet}$ and its derivatives are collectively known as 'reactive nitrogen species' (RNS).

It is important to consider the biological relevance of these systems. Both ROS and RNS have essential roles in a broad range of physiological and PATHOPHYSIOLOGICAL processes that are relevant to infection (BOX 1). However, this review focuses on their antimicrobial actions. The importance of ROS and RNS for innate immunity can be best appreciated by examining the consequences of deficient production.

ROS and RNS — clinical importance

ROS and chronic granulomatous disease. The clinical significance of the NADPH phagocyte oxidase in host defence is easily shown, as defects in the genes that encode the gp91-phox, p47-phox, p22-phox or p67-phox subunits of the NADPH phagocyte oxidase result in chronic granulomatous disease (CGD), which is characterized by a reduced life expectancy and recurrent infections with microorganisms including *Staphylococcus aureus, Aspergillus fumigatus, Salmonella* (non-typhoidal serovars), *Serratia marcescens* and *Burkholderia cepacia*. More than one-half of all CGD patients have a mutation in the *gp91-phox* gene on the X-chromosome. Mutations that cause Rac2 (REF. 1) or glucose-6-phosphate dehydrogenase[2] deficiency can also result in CGD. Murine models of CGD that lack functional *gp91-phox* or *p47-phox* alleles seem to replicate the immune defects

POLYMORPHONUCLEAR PHAGOCYTES
White blood cells with multi-lobed nuclei and cytoplasmic granules that are involved in inflammatory responses.

that are observed in human CGD[3,4]. Treatment with interferon-γ (IFN-γ) can reduce the incidence of infection in both human and murine CGD[5,6], but the mechanism of action is not understood[7].

RNS and iNOS promoter polymorphisms. In humans, iNOS deficiency has not yet been demonstrated. However, several iNOS promoter polymorphisms have been linked to increased iNOS expression and resistance to malaria[8–10]. Increased iNOS expression has been associated with reductions in both the severity of malarial symptoms[8] and the likelihood of reinfection[10]. Conversely, low plasma concentrations of arginine, which is the substrate for iNOS, have been associated with reduced $NO^{\bullet}$ synthesis and the development of cerebral malaria[11].

Knockout mice that lack iNOS[12] have increased susceptibility to various infections, including *Mycobacterium tuberculosis, Listeria monocytogenes, Leishmania* spp. and *Salmonella enterica* (reviewed in REF. 13). In experimental *Salmonella* infections, ROS have an important role in the early host response to infection, after which RNS has a sustained role in limiting residual bacterial replication[14]. The sequential functions of ROS and RNS are also reflected in assays of *Salmonella* killing by macrophages[15]. This might be one method by which a rapid initial reduction in the microbial burden through ROS-dependent killing can be achieved, while allowing the subsequent production of the less cytotoxic RNS to control residual infection until the microorganisms are cleared by adaptive immune mechanisms. Intriguingly, mouse models that lack both iNOS and the NADPH phagocyte oxidase are more immunocompromised than animals that lack just one of these systems[16] and frequently become infected with commensal bacteria that are usually non-pathogenic. This might indicate that ROS and RNS can compensate for one another to control less virulent microorganisms. RNS might have a particularly important role in the maintenance of latent infections[17–19]. With this in mind, the detection of iNOS activity in the lungs of patients with tuberculosis[20] and the skin of patients with leishmaniasis[21] is extremely interesting. It has also recently been proposed that ROS might be involved in the maintenance of *Leishmania major* latency[22].

Deficiencies in cytokine production or response. Interleukin-12 (IL-12) and IFN-γ signalling is important for resistance to mycobacterial and *Salmonella* infections in mice, partly owing to the effects on iNOS expression[23,24]. In humans, mutations in the IL-12 receptor or other elements of the IL-12–IFN-γ signalling pair have been associated with increased susceptibility to mycobacterial and *Salmonella* infections[25,26]. More recently, polymorphisms in the IL-12 promoter have been correlated with $NO^{\bullet}$ production and resistance to malaria[26].

Generation of ROS and RNS

The NADPH phagocyte oxidase and iNOS are expressed in both POLYMORPHONUCLEAR PHAGOCYTES and mononuclear phagocytes, although the amount of ROS that is produced is greater in neutrophils than in macrophages, and macrophages generally produce considerably more RNS than neutrophils[13].

NADPH phagocyte oxidase. The essential components of the NADPH phagocyte oxidase complex are two membrane proteins, gp91-phox and p22-phox, and three cytosolic proteins, p47-phox, p67-phox and Rac (reviewed in REFS 27,28). A further non-essential cytosolic component, p40-phox, might have a regulatory role.

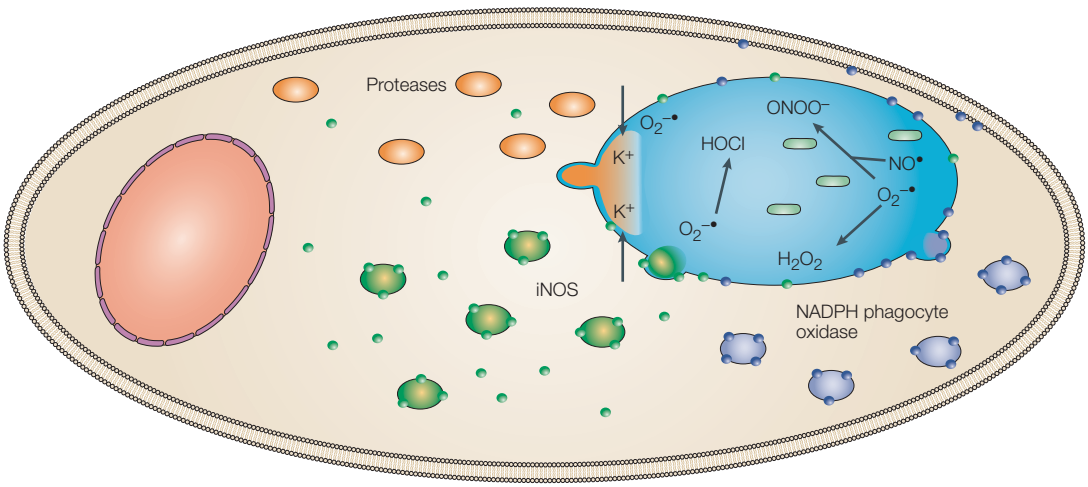

Figure 1 | **Antimicrobial mechanisms of phagocytes.** A simplified idealized phagocytic cell is shown. Nitric oxide ($NO^{\bullet}$) is generated by inducible nitric oxide synthase (iNOS). Superoxide ($O_2^{-\bullet}$) is generated by the reduced NADPH phagocyte oxidase and can subsequently be converted to hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl; by myeloperoxidase) or peroxynitrite ($ONOO^-$; by interaction with $NO^{\bullet}$). $O_2^{-\bullet}$ might also drive the influx of potassium (K^+) into the phagosome, thereby promoting the release of granule-associated proteases from a sulphated proteoglycan matrix. Some evidence indicates that active NADPH phagocyte oxidase is transported to the phagosome or plasma membrane in vesicles, whereas iNOS seems to be present in both cytosolic and vesicle-associated forms.

Box 1 | **Further roles of ROS and RNS in infection**

Signalling

The most fundamental role of ROS and RNS in biology might be signal transduction, and the production of these molecules might have eventually evolved into high-output systems with antimicrobial ability. Moderate production of ROS and RNS, through reversible interaction with thiols or metals, is involved in the regulation of diverse processes, including neurotransmission, phagocyte activation, iron metabolism, cell proliferation and apoptosis[38,40,211–213], and they can function in these settings with exquisite spatial and temporal precision. It is therefore not surprising that the absence of either iNOS or the NADPH phagocyte oxidase has important effects on the phagocyte transcriptome, as analysed using microarrays[214,215]. Experimental evidence increasingly supports an important role of ROS/RNS-dependent signalling during infection[216–218]. For example, observations in *Chlamydia*-infected mice indicate that either insufficient or excessive $NO^{\bullet}$ production can be immunosuppressive[219], and that $NO^{\bullet}$-mediated suppression of lymphocyte proliferation might actually be counter-regulated by ROS[220].

Regulation of vascular tone

The 1998 Nobel Prize in Medicine and Physiology was awarded to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad for discoveries that related to $NO^{\bullet}$ signalling in the cardiovascular system, and the importance of RNS in vascular homeostasis is now widely appreciated[221]. During sepsis, the vasodilatory functions of $NO^{\bullet}$ can result in hypotension, which indicates that NOS inhibition during sepsis might be beneficial. However, attempts to inhibit $NO^{\bullet}$ production must be tempered by an awareness of the beneficial actions of RNS in infection, including the preservation of tissue perfusion, immunomodulation, cytoprotection and antimicrobial actions[222], as well as by concerns about the deleterious effects of an increased afterload on cardiac output after administration of an NOS inhibitor[223]. Although a Phase III trial of a nonselective NOS inhibitor did not show clinical benefit[224], hope remains that the selective inhibition of iNOS might still be useful in patients with septic shock.

Tissue injury

The molecular moieties that are targeted by ROS and RNS are not unique to microorganisms, and the potential for these molecules to cause host-tissue injury is an important concern. Oxidative and nitrative tissue modifications have been detected in a range of infectious settings, including viral pneumonitis[225–227], encephalitis[228], *Pseudomonas aeruginosa* pneumonia[229], pneumococcal meningitis[230] and *Helicobacter pylori* gastritis[130]. Treatment with NOS inhibitors or antioxidants in some of these experimental models has reduced both tissue injury and mortality[225–227,230], which indicates that such interventions should be evaluated in a clinical setting. Some of the benefits of adjunctive immunomodulatory therapy in infection[231] might, in fact, be attributable to the effects on ROS and RNS[232,233].

Control of inflammation

Paradoxically, ROS and RNS can ameliorate, as well as mediate, tissue damage. Owing to their signalling and other non-antimicrobial functions, these molecules are important in limiting neutrophil sequestration and vascular injury during Gram-negative sepsis[234], and in controlling the inflammatory response to fungal hyphae[235]. The NADPH phagocyte oxidase is also required for the efficient degradation of ingested microorganisms by neutrophils[236]. These activities are likely to be clinically important, as patients with chronic granulomatous disease (CGD) often have problems with persistent chronic inflammatory lesions in the lung, liver, skin, lymphatic tissue and mucosal surfaces[237], which are not always associated with persistent infection. Therefore, ROS might both mediate and limit inflammatory tissue damage; oxidative tissue injury is observed in *H. pylori* gastritis[238], yet CGD mice show more intense mucosal inflammation after experimental *H. pylori* infection[239]. RNS also seem to control, as well as participate in, inflammation[240]. For example, a beneficial role of $NO^{\bullet}$ in the downregulation of adhesion-molecule expression in cerebral malaria has been proposed[241], and $NO^{\bullet}$ has recently been shown to control vascular inflammation and thrombosis by the regulation of *N*-ethylmaleimide-sensitive factor-dependent exocytosis[242].

The gp91-phox and p22-phox proteins together comprise the heterodimeric flavocytochrome b_{558}. PROINFLAMMATORY CYTOKINES, such as tumour-necrosis factor (TNF)-α and granulocyte–macrophage colony-stimulating factor (GM-CSF), can prime the NADPH phagocyte oxidase, perhaps through increased phosphoinositol 3-kinase activity[29], so that subsequent induction by phagocytosis or soluble AGONIST PEPTIDES, such as bacterial *N*-formyl peptides, elicits an increased response. Phophatidylinositol 3-phosphate might promote oxidase assembly by direct binding to p40-phox (REF. 30). Cytokines (for example, IFN-γ) and microbial products (for example, lipopolysaccharide) can also modulate the transcription of some of the genes that encode oxidase components[31].

Phosphorylation of cytosolic p47, for example, by protein kinase C or Akt, has an important role in oxidase activation by inducing a conformational change that allows p47-phox to interact with flavocytochrome b_{558} and p67-phox. The Rac GTP-binding protein is also required for oxidase assembly and activation, and might have an additional regulatory role in the induction of signalling pathways that lead to the translocation of cytosolic components to the membrane-bound flavocytochrome[32]. The flavocytochrome contains binding sites for flavin and NADPH, and two haem groups. In the presence of p67-phox and Rac, electrons are transferred from NADPH to FAD, then to the haem centres of gp91 and finally to oxygen, which results in the generation of $O_2^{-\bullet}$. This radical can be converted to hydrogen peroxide

PROINFLAMMATORY CYTOKINES
Secreted proteins with autocrine or paracrine action that regulate the inflammatory response. There are many types of cytokine, which elicit different cellular responses, including the control of cell proliferation and differentiation, the regulation of immune responses and haematopoiesis.

AGONIST PEPTIDES
Peptides that mimic cognate antigen, which results in cellular activation.

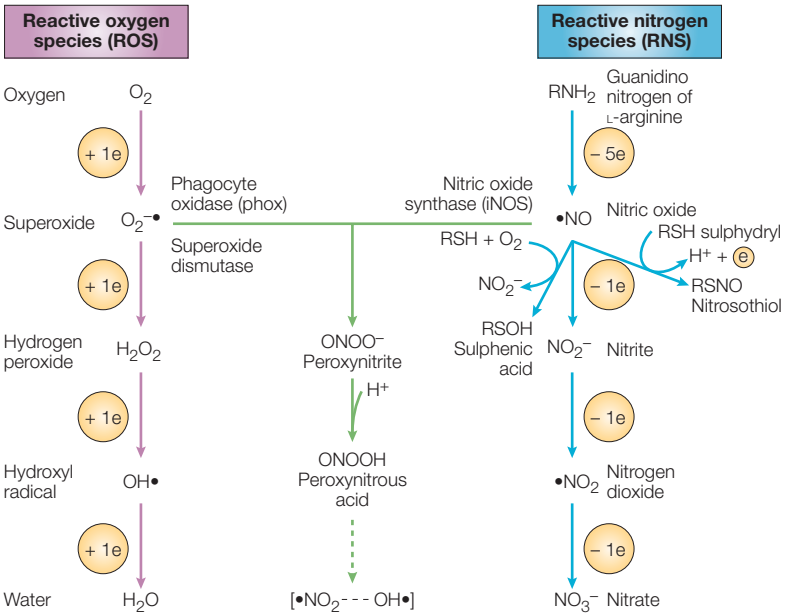

Figure 2 | **Reactive oxygen and nitrogen intermediate production in mammalian cells.** Nitroxyl anion (NO), a one-electron reduction product of nitric oxide (NO•), is unlikely to arise from NO• under physiological conditions. The reaction of reactive nitrogen species with cysteine sulphydryls can result in either *S*-nitrosylation or oxidation to the sulphenic acid, as well as disulphide-bond formation, all of which are potentially reversible. The peroxynitrite anion ($ONOO^-$) and peroxynitrous acid (ONOOH) have distinct patterns of reactivity. ONOOH spontaneously decomposes through a series of species that resemble the reactive radicals hydroxyl (•OH) and/or nitrogen dioxide ($NO_2^\bullet$). When the concentration of L-arginine is limiting, nitric oxide synthase (NOS) can produce superoxide ($O_2^{-\bullet}$) along with NO•, which favours the formation of peroxynitrite. Reproduced with permission from REF. 13 © (2000) National Academy of Sciences, USA.

(H_2O_2), the hydroxyl radical (•OH) or other ROS. There are two forms of the Rac GTPase — Rac1 seems to have an important role in the assembly of the phagocyte oxidase complex of human mononuclear cells[33], whereas Rac2 is more important in the assembly of the phagocyte oxidase of neutrophils[1]. Another GTP-binding protein, Rap1A, associates with cytochrome b_{558} and might also modulate oxidase activation, but its role is poorly defined at present[28,34]. The phagocyte oxidase is ELECTROGENIC and tends to depolarize the membrane potential; however, this is thought to be balanced by proton channels that open to prevent depolarization to levels that would be inhibitory to the oxidase[35].

Inducible nitric oxide synthase. Although the respiratory burst that is generated by the NADPH phagocyte oxidase has been recognized for almost 50 years[36], the importance of NO• in host defence has only recently been appreciated. Research in the diverse fields of vascular biology, toxicology and tumour immunology serendipitously converged in the 1980s (reviewed in REF. 37) with the realization that enzymatically generated NO• has diverse roles in biology, most notably as a mediator of signal transduction[38]. One important biological role of NO• is as an antimicrobial effector molecule that is produced by phagocytic cells. Although NO• production has been studied most extensively in macrophages, non-phagocytic cells and a subset of DENDRITIC CELLS[39,40] might also use NO• to inhibit microorganisms. Incidentally, not all of the host-derived NO• is synthesized by enzymes; dietary nitrate can be reduced by oral bacteria to nitrite and then converted to RNS by gastric acid[41], which creates a formidable antimicrobial barrier to ingested enteric pathogens.

NOS is present in cells as three ISOFORMS. The NOS2 isoform (iNOS), which can produce large quantities of NO•, is most relevant to phagocyte–microorganism interactions. NOS has an amino-terminal oxidase domain with a haem centre and binding sites for L-arginine and tetrahydrobiopterin, and is linked by a short calmodulin-binding domain to a carboxy-terminal reductase domain with binding sites for NADPH, FAD and flavin mononucleotide (FMN). Functional NOS is a dimer, and dimerization is promoted by haem incorporation, tetrahydrobiopterin and L-arginine. NOS transfers electrons from NADPH to FAD, then to FMN and finally to the haem iron of the adjacent NOS subunit to catalyse the formation of NO• and citrulline from L-arginine and oxygen through the intermediate N^{ω}-hydroxy-L-arginine (reviewed in REF. 42).

In contrast to the NADPH phagocyte oxidase or the constitutive NOS isoforms, iNOS activity is mainly regulated at the transcriptional level. Although phagocytes are typically induced to produce ROS immediately after a microbial stimulus, RNS production requires *de novo* protein synthesis. Stimulation of microbial pattern-recognition receptors[43] together with signalling from proinflammatory cytokines (such as IFNs, IL-1β and TNF-α) triggers signalling cascades that lead to iNOS transcription, including the p38 mitogen-activated protein kinase (MAPK), NF-κB and Janus-activated kinase–signal transducer and activator of transcription–interferon regulatory factor 1 (JAK–STAT–IRF1) pathways[44–47]. Post-transcriptional and post-translational regulation also occur[48], and substrate or cofactor availability can limit NO• production under certain circumstances[49,50]. However, unlike neuronal NOS (nNOS) and endothelial NOS (eNOS), iNOS activity is not controlled by intracellular calcium concentrations[51].

Molecular targets of ROS and RNS

ROS and RNS endow phagocytic cells with a broad array of interacting mediator molecules, which make it difficult to assign specific antimicrobial actions to individual molecular species. Although the product of the reaction catalysed by the phagocyte oxidase complex is $O_2^{-\bullet}$ and the product of that catalysed by iNOS is NO•, subsequent spontaneous, or catalysed, reactions involving $O_2^{-\bullet}$ or NO• can result in the formation of additional intermediates, such as H_2O_2, •OH, singlet oxygen ($^1O_2$), HYPOHALOUS acids (for example, hypochlorous acid; HOCl), nitrogen dioxide ($NO_2^\bullet$), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), dinitrosyl iron complexes, nitrosothiols or nitroxyl (HNO) (FIG. 2). Each of these species has different reactivity, stability, compartmentalization and biological activity[52], and several species can be present simultaneously in biologically relevant conditions. ROS and RNS can interact with numerous targets in a microbial cell, including thiols, metal centres, protein tyrosines,

ELECTROGENIC
Generating an electrical potential across a membrane.

DENDRITIC CELLS
'Professional' antigen-presenting cells that are found in the T-cell areas of lymphoid tissues and as minor cellular components in most tissues. They have a branched or dendritic morphology and are the most potent stimulators of T-cell responses.

ISOFORMS
Forms of a protein with slightly different amino-acid sequences that often have diverse activities, functions and/or distributions.

HYPOHALOUS
A compound in which a hydroxyl group is combined with a halogen atom.

nucleotide bases and lipids[13,53]. This accounts, in part, for the versatility of these molecules as antimicrobial effectors and for the challenges that are faced by microorganisms that attempt to resist phagocyte killing. Many of the chemical modifications that result from interactions with ROS and RNS are reversible, which might reflect the evolutionary transformation of these molecules from signalling mediators to cytotoxic species[38]. Some of the most important antimicrobial actions of ROS and RNS are shown in FIG. 3.

ROS targets. Studies in *Escherichia coli* have shown that at low concentrations of H_2O_2 the main mechanism of ROS-dependent antibacterial activity is DNA damage[54,55], whereas lethality at higher concentrations of H_2O_2 results from the ROS-mediated damage of several cellular targets. DNA damage is dependent on the presence of iron, which indicates that hydroxyl or ferryl radicals are toxic intermediates that are produced by the FENTON REACTION[56]. Oxidative attack of DNA bases can produce 8-hydroxyguanine, urea, hydroxymethyl urea and thymine glycol, among other products, whereas sugar modification can result in strand breaks. Proteins can undergo a range of oxidative modifications, in particular at cysteine, methionine, tyrosine, phenylalanine and tryptophan residues. Protein carbonyls that are formed by the oxidation of arginine, proline or lysine can be readily identified after oxidative injury, and proteins such as alcohol dehydrogenase E, elongation factor G, DnaK, OppA, enolase, OmpA and the F_0F_1-ATPase have been oxidized following the exposure of *E. coli* to H_2O_2 (REF. 57). The PEROXIDATION of bacterial lipids has been observed after ingestion by neutrophils[58]. However, it is not known whether this is a correlate, or a cause, of bacterial killing. The presence of saturated and monounsaturated fatty acids in bacterial membranes probably limits the potential for chain-peroxidation reactions[59], but the membrane lipids might be more important sites of oxidative damage in eukaryotic pathogens[60].

RNS targets. The antimicrobial actions of RNS are perhaps more complex than those of ROS and are dependent on the local redox environment[61]. Recent evidence indicates that NO$^\bullet$ or *S*-nitrosothiols can reversibly inhibit bacterial DNA replication through a mechanism that involves zinc mobilization from metalloproteins[62]. Nitric oxide by itself can also inhibit bacterial respiration[63,64], which might be one factor that induces a dormant or persistent state in certain microorganisms, including *M. tuberculosis*[17,65]. Interactions between NO$^\bullet$ and tyrosyl radicals seem to account for the inhibition of ribonucleotide reductase by RNS[66], which limits the availability of precursors for the synthesis and repair of DNA. When H_2O_2 is also present, the inhibition of respiration by NO$^\bullet$ can potentiate oxidative injury by accelerating flavin reduction and promoting Fenton chemistry[67]. The destabilization of iron–sulphur clusters by ROS and RNS can release free iron and further exacerbate this process[68,69]. In the presence of oxygen, the conversion of NO$^\bullet$ to $NO_2^\bullet$, N_2O_3 or $ONOO^-$ can result in oxidative modifications, which resemble those that are mediated by ROS alone[70–72]. Moreover, $ONOO^-$ or nitrite ion, together with H_2O_2 and MYELOPEROXIDASE (MPO), can nitrate tyrosine residues, but the contribution of this protein modification to microbial killing is unclear[73].

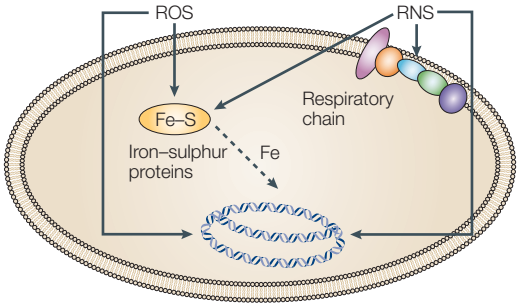

Figure 3 | **Microbial targets of reactive oxygen and nitrogen species.** A simplified, idealized microbial cell is shown. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) mainly interact with thiols, metal centres and DNA. Direct DNA damage is of central importance to the antimicrobial actions of ROS, whereas RNS inhibit respiration and interfere with DNA replication through the inactivation of zinc metalloproteins. Both ROS and RNS can mobilize iron from iron–sulphur-containing dehydratases, which further potentiates ROS toxicity. The diversity of targets for ROS and RNS acting individually, synergistically or with other systems results in a broad spectrum of antimicrobial activity that encompasses bacteria, fungi, parasites and viruses.

Microbial defences against ROS and RNS

Pathogenic microorganisms might subvert, or resist, the actions of ROS and RNS through a range of strategies, which can be grouped into evasion, suppression, enzymatic inactivation, scavenging, iron sequestration, stress responses and repair mechanisms[13,53,74]. As the targets of ROS and RNS overlap, it is not surprising that several microbial-resistance strategies function against both molecules.

Evasion of ROS and RNS. Microorganisms can avoid uptake by phagocytic cells through numerous methods, including the avoidance of recognition by phagocytic receptors and the disruption of phagocyte signalling pathways[75]. *Yersinia* spp. are known to translocate the tyrosine phosphatase YopH and the GTPase-activation protein YopE into macrophages to disrupt integrin-mediated signalling pathways and actin rearrangements, respectively[76,77]. By contrast, *S. enterica* is phagocytosed but subsequently translocates microbial proteins to the macrophage cytosol, which interfere with the delivery of ROS and RNS into the phagocytic vacuole[78,79]. *Francisella tularensis* has a more stealthy approach and undergoes phase variation, which produces a phenotype that is less stimulatory for the production of RNS[80].

Suppression of ROS and RNS. In addition to its antiphagocytic functions, the *Yersinia* YopH protein seems to suppress the production of ROS[81] . *Bordetella pertussis* inhibits the respiratory burst by producing an adenylate cylase toxin and increasing cAMP levels[82]. The haemozoin pigment of *Plasmodium falciparum*

FENTON REACTION
The reduction of hydrogen peroxide by ferrous iron.

PEROXIDATION
A type of reaction in which oxygen atoms are formed, which leads to the production of peroxides.

MYELOPEROXIDASE
Peroxidase from neutrophils that takes part in the bactericidal activity of these cells. The name originates from the first isolation from the blood of patients with myeloid leukaemia.

suppresses the production of both ROS and RNS by mononuclear cells[83]. *Anaplasma phagocytophilum*, which is a remarkable organism that multiplies in neutrophils, is thought to block late ROS production after initially inducing ROS release[84]. Some investigators have described a reduction in p22-phox concentrations after *A. phagocytophilum* uptake by neutrophils[85], whereas others have reported reduced expression of the genes encoding Rac2 and gp91-phox[86]. The antimicrobial actions of RNS also extend to some viral pathogens[53,87], and seem to result from both host and viral proteins[88]. Recently, the adenovirus E1A protein was shown to interfere with NF-κB activation of iNOS transcription[89], which might represent one mechanism by which the adenovirus evades innate immunity.

Enzymic detoxification of ROS and RNS. Detoxification by enzymes that convert ROS to less toxic species has been extensively documented for microbial catalases, superoxide dismutases and peroxidases. In some instances, these antioxidant enzymes are required for virulence in infection models[90–93]. Several enzymes that are involved in the detoxification of RNS have recently been identified, including microbial haemoglobins[94,95], NO• reductase[96], *S*-nitrosoglutathione reductase[97] and peroxynitrite reductase[98]. Evidence that implicates these enzymes in the pathogenesis of infection is still awaited in most cases; however, the flavohaemoglobin Hmp promotes the survival of *Salmonella* in NO•-producing macrophages[64], and a recent report implicated flavohaemoglobin and *S*-nitrosoglutathione reductase in the virulence of *Cryptococcus neoformans*[99].

Scavenger molecules. Low-molecular-weight thiols have an important role in either promoting the degradation of ROS and RNS, or in reversing oxidative and nitrosative protein modifications. Glutathione is the predominant low-molecular-weight thiol in enteric bacteria, whereas mycothiol is found in actinomycetes[100] and trypanothione is produced by some protozoa[101]. Glutathione peroxidase is required for the virulence of group A streptococci in mice[102]. Exposed methionine residues can also scavenge ROS and RNS as they can be repaired by methionine sulphoxide reductases[103]. Homocysteine is an interesting low-molecular-weight thiol that can antagonize the antimicrobial actions of RNS in *Salmonella* spp.[104] Increased homocysteine concentrations can antagonize endothelium-dependent vascular relaxation and are associated with an increased risk of atherothrombosis in humans[105], which indicates that similar molecular interactions might underlie these seemingly disparate biological functions. Melanin and mannitol, which are produced by fungi, might scavenge ROS and RNS[106,107], and the extracellular alginate that is produced by *Pseudomonas aeruginosa* might have a similar function[108].

Sequestration of iron. As iron(II) can function as a Fenton catalyst for the conversion of H_2O_2 into more toxic ROS, microorganisms can avoid toxic ROS by compartmentalizing their iron stores and controlling iron uptake. Compartmentalization is achieved by the production of the multimeric storage proteins ferritin and bacterioferritin[109]. Dps, which is a ferritin-like protein, might have a particularly important role in the sequestration of iron in the vicinity of DNA[110]. The iron-responsive proteins Fur and DtxR regulate bacterial iron-uptake systems at the transcriptional level[111]. There is increasing evidence that bacteria that are deficient in iron storage are more susceptible to oxidative stress[112–114]. Interestingly, a *fur* mutant of *E. coli* has increased susceptibility to NO• (REF. 115), which indicates that iron homeostasis is an important determinant of bacterial susceptibility to RNS and ROS. Free iron can interact with NO• to form dinitrosyl iron complexes, which might potentiate nitrosative stress by the stabilization of nitrosothiols[116]; further investigation of iron–RNS interactions is warranted.

Stress responses. Bacteria mainly respond to oxidative or nitrosative stress at the transcriptional level. The OxyR and SoxRS proteins of enteric bacteria are the prototype members of families of regulators that respond to H_2O_2 or $O_2^{-\bullet}$, respectively[117,118]. OxyR undergoes reversible cysteine oxidation in response to oxidative stress[119], whereas reversible oxidation of a [2Fe–2S] cluster results in activation of the SoxR protein. Other regulators, such as σ^B, σ^R, PerR, OhrR and BosR, have been shown to sense redox change in other bacteria[120–122]. Stimulation of these regulatory proteins results in the coordinated expression of genes that are involved in resistance to oxidative stress or the repair of oxidative damage. Some regulators, such as the stationary-phase sigma factor σ^S, can also control the expression of antioxidant proteins without being induced by oxidative stress *per se*[123].

Under specific conditions, OxyR and SoxRS can respond to RNS. This is not surprising, because both ROS and RNS can target thiols or iron–sulphur clusters, and there is considerable overlap between antioxidant and antinitrosative defences. However, a recent microarray analysis of *E. coli* indicated that the NorR and Fur regulatory proteins might have a more dominant role in response to nitrosative stress[124]. In the case of Fur, the formation of Fur–iron–NO complexes seems to account for the de-repression of Fur-regulated genes in the presence of NO•[115]. Fur de-repression in response to NO• — even though a *fur* mutation was shown to enhance NO• susceptibility — is a paradoxical finding that remains to be satisfactorily explained. Members of the FNR [4Fe–4S]-containing redox-sensor protein family also respond to NO• (REF. 125), which probably reflects an evolutionary link between the control of denitrification pathways and defence against nitrosative stress[126].

Repair processes. The ability to repair oxidative and nitrosative damage is an important component of resistance to ROS- and RNS-induced damage. Bacteria that are deficient in DNA repair mechanisms have increased susceptibility to both ROS and RNS[54,61,62,72,127,128], increased susceptibility to phagocytes[127,129,130] and reduced virulence[130,131]. The inhibition of DNA replication by ROS or RNS results in induction of the SOS response[62,116,132], which is a coordinated stress response that includes several repair functions and does not

Box 2 | **Questions and controversies**

- **Is NO• produced by human macrophages?**
- **What is the role of myeloperoxidase in ROS-dependent antimicrobial actions?**
- **What is the importance of xanthine oxidase in ROS production and host defence?**
- **Is vesicular transport important for ROS and RNS delivery to the phagosome?**
- **Do ROS and RNS kill microorganisms synergistically?**
- **Is the principal antimicrobial role of ROS to activate granule-associated proteases?**
- **How important is the generation of ROS by antibodies?**

involve polymerases. ROS also induce transcription of the *suf* operon[133], which includes genes that are involved in the formation and repair of iron–sulphur clusters and is an important molecular target of oxidative and nitrosative stress. An intriguing role of the PROTEASOME in the RNS resistance and virulence of *M. tuberculosis* has recently been described[128]. Proteasomes are required by eukaryotes for the degradation of irreversibly damaged proteins, and are also found in mycobacteria and other actinomycetes. These new observations indicate that the repair or turnover of proteins with nitrosative or oxidative modifications might be crucial for RNS resistance.

Controversies in ROS and RNS research

The modern era of oxidative-stress research is usually thought to have begun 50 years ago with the proposal by Gerschman and co-workers[134] that free radicals are responsible for the toxic effects of oxygen. Soon afterwards it was observed that neutrophils consume oxygen and generate ROS in response to various stimuli[36]; this 'respiratory burst' and its associated antimicrobial actions are now known to be dependent on the NADPH phagocyte oxidase. The production of iNOS and RNS by activated mononuclear phagocytes was only discovered several decades later[37], but a plethora of studies providing evidence for the distinct antimicrobial contribution of RNS rapidly followed[53]. It is therefore surprising that several active controversies remain regarding fundamental aspects of the contribution of ROS and RNS to the antimicrobial actions of phagocytic cells (BOX 2), which are discussed below.

NO• production by human macrophages. Initial reports of cytokine-inducible NO• production in murine macrophages[43] could not be reproduced in human monocyte-derived macrophages[135], which led to speculation that iNOS might not be expressed in human phagocytes. However, ~250 reports have subsequently described iNOS mRNA, protein activity or biological functions in human macrophages[136]. iNOS has also been detected in human neutrophils[137]. Although some scepticism remains[138], most of the evidence now indicates that human phagocytes express iNOS and produce NO• in response to inflammatory stimuli such as infection[139]. The reason for the earlier controversy seems to have been an insufficient understanding of the signals that are required for iNOS activation in human cells. Macrophages that are obtained from patients with infection or other inflammatory conditions almost invariably express iNOS[8,20,140–142], whereas peripheral blood-monocyte-derived macrophages that are obtained from normal donors and stimulated *in vitro* generally do not. The iNOS promoters of genes from rodent and human macrophages are divergent[46], and further analysis of human iNOS transcriptional regulation might eventually facilitate the study of NO• production by human peripheral blood-mononuclear cells.

Role of myeloperoxidase. MPO is a tetrameric granule-associated haem protein that comprises 5% of the dry weight of neutrophils[143] and a slightly lower proportion of monocytes. MPO is not produced by differentiated macrophages *in vitro*, but seems to be present in some macrophages *in vivo*[144]. MPO can generate oxidants from H_2O_2 and a range of co-substrates, most notably chlorine[145] and nitrite[146]. HOCl, which can be produced by MPO, is strongly bactericidal[147] and markedly increases the antibacterial potency of ROS[148,149], which might indicate that MPO is important in host defence. *In vitro*, an MPO–H_2O_2–halide system oxidizes bacterial thiols, releases sulphide from iron–sulphur centres and inhibits respiration[150,151]. MPO-dependent chlorination of bacteria has been observed in neutrophils[152], but the amount of bacterial chlorination is relatively minor compared with the chlorination of neutrophil proteins[153]. H_2O_2 that is produced by streptococci is believed to interact with MPO and halide ions to produce antistreptococcal activity in patients with CGD, and strains of *Streptococcus pyogenes* or *Haemophilus* spp. that do not produce H_2O_2 seem to regain virulence in mice or humans with CGD[154,155]. However, in contrast to CGD, MPO deficiency is associated with mild compromise of the immune system[156], which is characterized by an enhanced susceptibility to *Candida albicans* infection if the patient also has diabetes mellitus. An MPO-knockout mouse seems to have an innate immune defect that is similar to that observed in cases of human MPO deficiency[157], although the effects on vascular disease seem to differ from those observed in humans[158]. It can therefore be inferred that the NADPH phagocyte oxidase does not require MPO for its antimicrobial actions, although MPO might augment host defence, particularly against *Candida* spp. Increasing attention is being focused on the possible roles of MPO in atherosclerosis and other inflammatory diseases[159,160].

Role of xanthine oxidase. Xanthine oxidase (XO) is a molybdenum-containing enzyme that can generate ROS by the process of degrading hypoxanthine or xanthine to uric acid. The production of XO in response to proinflammatory cytokines[161] and enhanced microbial replication in phagocytes and infected mice after the administration of XO inhibitors[162–164] have led to the suggestion that XO has an important role in innate immunity. However, there are several reasons to be cautious about this hypothesis. Differences in purine metabolism between humans and mice might limit substrate availability for XO in human phagocytes[165]. The profound immunodeficiency of patients or mice with CGD clearly shows that XO cannot compensate for the loss of the NADPH phagocyte oxidase, and activated peritoneal

PROTEASOME
In eukaryotes the 26S proteasome is a large multisubunit protease complex that selectively degrades multi-ubiquitylated proteins. It contains a 20S particle that carries the catalytic activity and two regulatory 19S particles.

macrophages from *gp91-phox*$^{-/-}$ mice in fact produce no detectable ROS *in vitro*[15]. The importance of MPO in enhancing host resistance to *C. albicans*[157] is not observed in CGD mice[166], which indicates that the NADPH oxidase is the only source of H_2O_2 that can be converted to HOCl *in vivo*. XO can modulate the cytokine response to inflammatory stimuli[167], which might indirectly affect microbial proliferation in experimental infection models. Allopurinol, which is the most widely used XO inhibitor, can also function as a scavenger of $^{\bullet}OH$[168], which raises concerns about its specificity. Perhaps most troubling of all is the lack of immunocompromise that is observed in humans with hereditary XO deficiency or xanthinuria[169] — some patients present with renal-stone formation, whereas others are completely asymptomatic. Mice that lack XO have not yet been evaluated with regard to phagocyte function, partly because *XO*$^{-/-}$ offspring have a shortened lifespan owing to a failure of XO heterozygous mothers to lactate normally[170]. The abundance of XO, and the availability of uric acid at mucosal surfaces and in the liver, might indicate that XO has a role in host defence at these specific locations. Moreover, some studies using allopurinol have shown increased microbial proliferation in the liver[163,164]. Probably the best evidence so far for a role of XO in innate immunity comes from the unusual relationship between African Cape buffaloes and trypanosomes; these animals are resistant to trypanosome infection owing to a combination of circulating XO and an infection-associated decline in serum catalase activity[171].

Importance of vesicular trafficking. In neutrophils, most cytochrome b_{558} is found in the membranes of intracellular granules[172], with the remainder in the plasma membrane. After activation or phagocytosis, the assembled NADPH oxidase can be detected in plasma or phagosome membranes together with the concomitant release of ROS into the extracellular environment or vacuolar space[173–175]. Therefore, NADPH phagocyte oxidase is thought to assemble at the plasma or phagosomal membranes[28]; one study using immunofluorescence microscopy detected the p47-phox and p67-phox components only at these locations[176]. However, other investigators studying neutrophils that have been loaded with cytochalasin b and stimulated with phorbol myristate acetate (PMA) or formyl-methionyl-leucyl-phenylalanine (fMLP) have indicated that assembly of the NADPH oxidase might be more complex[177,178]. In these studies, p47-phox and cerium perhydrate, which is a marker of ROS production, were visualized at the plasma and phagosomal membranes; however, they were also observed in association with intracellular compartments that are not derived from the plasma membrane[177,179]. Dichlorodihydrofluorescein-dependent fluorescence, which is another indicator of ROS, can also be detected in apparent vesicular compartments of normal neutrophils, but not those from patients with CGD[180]. These observations indicate that the active oxidase might assemble in secretory granules that subsequently target and fuse with either the plasma or phagosomal membranes to release ROS.

In contrast to neutrophils, macrophages are generally not thought to have a granular pool of cytochrome b_{558} (REF. 181). However, there is some evidence that vesicular trafficking might have a role in assembly of the NADPH oxidase of macrophages. Earlier studies detected a small amount of ROS production in cytoplasmic vesicles[182]. Investigators using specific antibodies found evidence of cytochrome b_{558}-containing intracellular vesicles in macrophages as well as in neutrophils[183]. Oxidase activity (as detected by the conversion of cerium chloride to cerium perhydrate) has also been observed in non-phagosomal membrane-bound compartments in macrophages after the phagocytosis of bacteria[184]. Recent studies of phagocyte interactions with *Salmonella* have provided further evidence for functional oxidase in intracellular membrane-bound compartments in macrophages. An antibody to the p22-phox component detected cytochrome b_{558} in the plasma membrane of peritoneal macrophages, with increasing membrane localization after stimulation with PMA. However, following the phagocytosis of virulent *S. enterica* serovar Typhimurium, cytochrome b_{558} was instead observed in what seemed to be intracellular vesicles, where it was accompanied by cytosolic NADPH oxidase components[78]. The oxidase-carrying vesicles localized to the phagosome when mutant bacteria that lacked *Salmonella*-pathogenicity island 2 (SPI2) were used to infect the macrophages. The SPI2-virulence genes encode a secretory system that translocates bacterial effector proteins to the macrophage cytoplasm. These effectors inhibit ROS production by the phagocyte specifically in the phagosomal compartment, even though the overall amount of ROS that is released (as detected by chemiluminescent substrates) is preserved[78]. The authors suggested that trafficking of oxidase-containing vesicles to the phagosome, but not the assembly of the oxidase in vesicles, was disrupted by SPI2. Subsequent work further demonstrated that the putative trafficking process requires an intact TNF-α–TNFRp55 signalling pathway[185]. Collectively, these observations indicate that vesicular transport has an important role in NADPH phagocyte oxidase function, both in neutrophils and macrophages. This might be crucial for the control of ROS production in established phagosomal vacuoles.

The iNOS enzyme might also be transported to the phagosomes in vesicles. Approximately one-half of the iNOS in primary macrophages is found in a particulate fraction that contains 50–80-nm vesicles[186]. The *Salmonella* SPI2 locus might also interfere with the transport of iNOS-containing vesicles to the phagosome[79], although the ability of $NO^{\bullet}$ to pass freely across membranes makes the importance of iNOS-associated vesicular trafficking less clear[187]. In fact, macrophages that are unable to mobilize iNOS to the phagosomal membrane seem to retain antibacterial activity[188].

Synergy between ROS and RNS. Under certain conditions, the combined antimicrobial activities of ROS and RNS are greater than that of either molecule alone[63]. However, the importance of this synergy in host defence

is a matter of some debate. Several mechanisms can account for ROS–RNS synergy, including the production of cytotoxic species, such as $ONOO^-$ (which is formed by the combination of $NO^\bullet$ and $O_2^{-\bullet}$)[189] or the flavin reductase-dependent enhancement of Fenton chemistry that results from the inhibition of respiration during nitrosative stress[67]. Production of both ROS and RNS seems to be required for the killing of *Mycoplasma pulmonis* or *C. albicans* by macrophages[190,191]. ROS and RNS synergy also seems to be important in the killing of *Rhodococcus equi* by macrophages; mice that are deficient in either the NADPH oxidase or iNOS are impaired in their ability to clear a systemic *R. equi* infection[192]. However, during experimental *Salmonella* infection, the contributions of ROS- and RNS-related antimicrobial actions seem to be temporally separated — with ROS-mediated bacterial killing occurring early and RNS-dependent bacteriostatic actions observed subsequently[14,15]. This absence of synergy might partly reflect antagonism or disruption of normal host defences by the pathogen[15,79,193]. Alternatively, the sequential production of ROS and RNS might optimize early microbial killing, while reducing the amount of collateral tissue injury that is caused by the inflammatory response.

Synergy between ROS and proteases. A new synergistic interaction between ROS and neutrophil granule-associated proteases has recently been proposed[194], which represents a radical departure from the usual view of ROS-related antimicrobial activity. Mice that lack cathepsin G and elastase were found to be susceptible to *S. aureus, C. albicans* and *Aspergillus* infection, similar to CGD mice[194], despite the preservation of the respiratory burst[195]. There is also evidence to indicate that $O_2^{-\bullet}$ that is generated by the NADPH phagocyte oxidase promotes a massive influx of potassium ions through large conductance calcium-activated channels into the phagosome, thereby releasing cationic granule proteins from their anionic sulphated proteoglycan matrix[196]. It has therefore been proposed that ROS have a central role in activating non-oxidative antimicrobial systems, rather than acting as antimicrobial effectors *per se*. This ingenious model deserves further attention and provides a rationale for the synergy between ROS and neutrophil proteases.

However, several observations argue for caution before discarding the conventional theory that ROS exert antimicrobial actions through oxidative modifications of microbial targets[197]. One recent study has shown that bacteria that are phagocytosed by neutrophils sense and respond to oxidative stress, and that failure to do so compromises their resistance to killing[198]. Many other reports, which are detailed above and elsewhere[74], have shown virulence defects in bacteria that lack specific well-defined antioxidant functions. Other investigators have not found a role for either elastase[199] or cathepsin G[200] in resistance to *S. aureus* infection, whereas the NADPH oxidase is clearly important[3]. The antimicrobial activity of macrophages from both humans[201] and mice[15] with CGD is markedly impaired, even though they lack neutrophil elastase and cathepsin G. These findings provide compelling evidence that ROS have a direct antimicrobial role in addition to promoting the activation of proteases.

CGD neutrophils can kill catalase-deficient bacteria that produce H_2O_2 (REF. 202), despite the fact that this molecule would not be expected to promote potassium influx and protease activation. Furthermore, erythrocytes can interfere with the antimicrobial actions of phagocytes in the peritoneal cavity by scavenging ROS and RNS[203]. This indicates that ROS can exert antibacterial actions in the extracellular environment where potassium concentrations cannot conceivably achieve the levels that are required for protease release from the granule matrix[194]. The non-oxidative killing of *P. aeruginosa* by neutrophils does not require the NADPH phagocyte oxidase[204], in contrast to the killing of *B. cepacia*[204,205], which indicates that oxidative and non-oxidative systems do not necessarily operate together. Finally, the specific role of potassium ions in the newly proposed model has been called into question on the basis of conflicting physiological data[206]. It has been proposed that valinomycin, which is a conductive IONOPHORE, should have enhanced, rather than counteracted, potassium accumulation in the phagosome and there is also concern that the microprobe measurements of Reeves and colleagues failed to support the proposed large increase in ionic strength[206]. At present, although the underappreciated synergy between ROS and neutrophil granule proteases[194] is intriguing, this work should not be interpreted to exclude the direct antimicrobial effects of phagocyte-derived ROS.

Generation of ROS by antibodies. Phagocytes and, to a lesser extent, non-phagocytic cells have long been regarded as the sources of antimicrobial ROS. However, a series of recent publications has provided evidence that antibodies can generate sufficient quantities of ROS to kill bacteria and that one of the most crucial antimicrobial species produced might actually be ozone[207]. This hypothesis hinges on the specificity of indigo carmine bleaching to detect ozone[208], but this assumption has been questioned[209]. Although intriguing, the speculation regarding the biological significance of antibody-catalysed ROS formation must be regarded as preliminary[210].

Conclusions

The versatility of ROS and RNS as signalling and cytotoxic molecules allows these molecules to have several roles in infection, through a range of mechanisms. Their ability to target many essential processes of microbial pathogens and to synergize with one another, or with oxygen-independent antimicrobial systems, results in broad antimicrobial activity that is difficult for microorganisms to completely resist or circumvent. Analyses of genetically determined variability in ROS and RNS production by humans or laboratory mice have helped to clarify the role of these molecules in host defence. Nevertheless, the remaining uncertainties and controversies ensure that biologists who are interested in the interactions between hosts and microorganisms still have many fascinating questions to answer.

IONOPHORE
Small hydrophobic molecules that dissolve in lipid bilayers and increase the permeability of membranes to ions.

1. Ambruso, D. R. *et al*. Human neutrophil immunodeficiency syndrome is associated with an inhibitory *Rac2* mutation. *Proc. Natl Acad. Sci. USA* **97**, 4654–4659 (2000).
2. Gray, G. R. *et al*. Neutrophil dysfunction, chronic granulomatous disease, and non-spherocytic haemolytic anaemia caused by complete deficiency of glucose-6-phosphate dehydrogenase. *Lancet* **2**, 530–534 (1973).
3. Pollock, J. D. *et al*. Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. *Nature Genet.* **9**, 202–209 (1995).
4. Jackson, S. H., Gallin, J. I. & Holland, S. M. The *p47phox* mouse knock-out model of chronic granulomatous disease. *J. Exp. Med.* **182**, 751–758 (1995).
Presents a model of X-linked deficiency of the NADPH phagocyte oxidase.
5. The International Chronic Granulomatous Disease Cooperative Study Group. A controlled trial of interferon-γ to prevent infection in chronic granulomatous disease. *N. Engl. J. Med.* **324**, 509–516 (1991).
6. Jackson, S. H. *et al.* IFN-γ is effective in reducing infections in the mouse model of chronic granulomatous disease (CGD). *J. Interferon Cytokine Res.* **21**, 567–573 (2001).
7. Muhlebach, T. J. *et al.* Treatment of patients with chronic granulomatous disease with recombinant human interferon-γ does not improve neutrophil oxidative metabolism, cytochrome b_{558} content or levels of four anti-microbial proteins. *Clin. Exp. Immunol.* **88**, 203–206 (1992).
8. Levesque, M. C. *et al.* Nitric oxide synthase type 2 promoter polymorphisms, nitric oxide production, and disease severity in Tanzanian children with malaria. *J. Infect. Dis.* **180**, 1994–2002 (1999).
Provides the first genetic evidence of the role of iNOS in human resistance to infection.
9. Hobbs, M. R. *et al.* A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. *Lancet* **360**, 1468–1475 (2002).
10. Kun, J. F. *et al.* Nitric oxide synthase 2 (Lambarene) (G-954C), increased nitric oxide production, and protection against malaria. *J. Infect. Dis.* **184**, 330–336 (2001).
11. Lopansri, B. K. *et al.* Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet* **361**, 676–678 (2003).
12. MacMicking, J. D. *et al.* Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* **81**, 641–650 (1995).
13. Nathan, C. & Shiloh, M. U. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc. Natl Acad. Sci. USA* **97**, 8841–8848 (2000).
14. Mastroeni, P. *et al.* Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival *in vivo*. *J. Exp. Med.* **192**, 237–248 (2000).
15. Vazquez-Torres, A., Jones-Carson, J., Mastroeni, P., Ischiropoulos, H. & Fang, F. C. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages *in vitro*. *J. Exp. Med.* **192**, 227–236 (2000).
References 14 and 15 describe the sequential contributions of NADPH phagocyte oxidase and iNOS in host resistance to *Salmonella* infection.
16. Shiloh, M. U. *et al.* Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. *Immunity* **10**, 29–38 (1999).
Shows that NADPH oxidase and NO˙ synthase might compensate for one another as mice that are deficient in both develop spontaneous infections with commensal flora.
17. Voskuil, M. I. *et al.* Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J. Exp. Med.* **198**, 705–713 (2003).
18. Stenger, S., Donhauser, N., Thuring, H., Rollinghoff, M. & Bogdan, C. Reactivation of latent leishmaniasis by inhibition of inducible nitric oxide synthase. *J. Exp. Med.* **183**, 1501–1514 (1996).
Shows a clear role for NO˙ in the maintenance of latent infection.
19. Scanga, C. A. *et al.* Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect. Immun.* **67**, 4531–4538 (1999).
20. Choi, H. S., Rai, P. R., Chu, H. W., Cool, C. & Chan, E. D. Analysis of nitric oxide synthase and nitrotyrosine expression in human pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* **166**, 178–186 (2002).
21. Qadoumi, M., Becker, I., Donhauser, N., Rollinghoff, M. & Bogdan, C. Expression of inducible nitric oxide synthase in skin lesions of patients with American cutaneous leishmaniasis. *Infect. Immun.* **70**, 4638–4642 (2002).
22. Blos, M. *et al.* Organ-specific and stage-dependent control of *Leishmania major* infection by inducible nitric oxide synthase and phagocyte NADPH oxidase. *Eur. J. Immunol.* **33**, 1224–1234 (2003).
23. Mastroeni, P. *et al.* Interleukin-12 is required for control of the growth of attenuated aromatic-compound-dependent salmonellae in BALB/c mice: role of γ-interferon and macrophage activation. *Infect. Immun.* **66**, 4767–4776 (1998).
24. Xing, Z., Zganiacz, A. & Santosuosso, M. Role of IL-12 in macrophage activation during intracellular infection: IL-12 and mycobacteria synergistically release TNF-α and nitric oxide from macrophages via IFN-γ induction. *J. Leukoc. Biol.* **68**, 897–902 (2000).
25. de Jong, R. *et al.* Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* **280**, 1435–1438 (1998).
26. Morahan, G. *et al.* A promoter polymorphism in the gene encoding interleukin-12 p40 (IL12B) is associated with mortality from cerebral malaria and with reduced nitric oxide production. *Genes Immun.* **3**, 414–418 (2002).
27. Babior, B. M., Lambeth, J. D. & Nauseef, W. The neutrophil NADPH oxidase. *Arch. Biochem. Biophys.* **397**, 342–344 (2002).
An up-to-date mini-review of NADPH oxidase.
28. Vignais, P. V. The superoxide-generating NADPH oxidase: structural aspects and activation mechanism. *Cell Mol. Life Sci.* **59**, 1428–1459 (2002).
29. Cadwallader, K. A. *et al.* Regulation of phosphatidylinositol 3-kinase activity and phosphatidylinositol 3,4,5-trisphosphate accumulation by neutrophil priming agents. *J. Immunol.* **169**, 3336–3344 (2002).
30. Ellson, C. D. *et al.* PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40(phox). *Nature Cell Biol.* **3**, 679–682 (2001).
31. Cassatella, M. A. *et al.* Molecular basis of interferon-γ and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *J. Biol. Chem.* **265**, 20241–20246 (1990).
32. Dinauer, M. C. Regulation of neutrophil function by Rac GTPases. *Curr. Opin. Hematol.* **10**, 8–15 (2003).
33. Zhao, X., Carnevale, K. A. & Cathcart, M. K. Human monocytes use Rac1, not Rac2, in the NADPH oxidase complex. *J. Biol. Chem.* **278**, 40788–40792 (2003).
34. Bokoch, G. M., Quilliam, L. A., Bohl, B. P., Jesaitis, A. J. & Quinn, M. T. Inhibition of Rap1A binding to cytochrome b_{558} of NADPH oxidase by phosphorylation of Rap1A. *Science* **254**, 1794–1796 (1991).
35. DeCoursey, T. E., Morgan, D. & Cherny, V. V. The voltage dependence of NADPH oxidase reveals why phagocytes need proton channels. *Nature* **422**, 531–534 (2003).
36. Sbarra, A. J. & Karnovsky, M. L. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J. Biol. Chem.* **234**, 1355–1362 (1959).
37. Hibbs, J. B. Jr. Infection and nitric oxide. *J. Infect. Dis.* **185** (Suppl. 1), S9–S17 (2002).
38. Stamler, J. S., Lamas, S. & Fang, F. C. Nitrosylation. The prototypic redox-based signaling mechanism. *Cell* **106**, 675–683 (2001).
The authors propose that thiol nitrosylation is a model for redox-based signal transduction.
39. Serbina, N. V., Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A. & Pamer, E. G. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* **19**, 59–70 (2003).
40. Bogdan, C., Rollinghoff, M. & Diefenbach, A. The role of nitric oxide in innate immunity. *Immunol. Rev.* **173**, 17–26 (2000).
41. Benjamin, N. & Dykhuizen, R. in *Nitric Oxide and Infection* (ed. Fang, F. C.) 215–230 (Kluwer Academic/Plenum Publishers, New York, 1999).
42. Stuehr, D. J. Mammalian nitric oxide synthases. *Biochim Biophys. Acta* **1411**, 217–230 (1999).
43. Stuehr, D. J. & Marletta, M. A. Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proc. Natl Acad. Sci. USA* **82**, 7738–7742 (1985).
44. Xie, Q. W., Kashiwabara, Y. & Nathan, C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J. Biol. Chem.* **269**, 4705–4708 (1994).
45. Kamijo, R. *et al.* Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. *Science* **263**, 1612–1615 (1994).
46. Taylor, B. S. & Geller, D. A. Molecular regulation of the human inducible nitric oxide synthase (iNOS) gene. *Shock* **13**, 413–424 (2000).
Discusses the fundamental differences between human and murine iNOS promoters.
47. Vazquez-Torres, A. *et al.* Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: importance of the Kupffer cell network. *J. Immunol.* **172**, 6202–6208 (2004).
48. Kolodziejski, P. J., Musial, A., Koo, J. S. & Eissa, N. T. Ubiquitination of inducible nitric oxide synthase is required for its degradation. *Proc. Natl Acad. Sci. USA* **99**, 12315–12320 (2002).
49. El-Gayar, S., Thuring-Nahler, H., Pfeilschifter, J., Rollinghoff, M. & Bogdan, C. Translational control of inducible nitric oxide synthase by IL-13 and arginine availability in inflammatory macrophages. *J. Immunol.* **171**, 4561–4568 (2003).
50. Schoedon, G. *et al.* Regulation of the L-arginine-dependent and tetrahydrobiopterin-dependent biosynthesis of nitric oxide in murine macrophages. *Eur. J. Biochem.* **213**, 833–839 (1993).
51. Nathan, C. & Xie, Q. W. Nitric oxide synthases: roles, tolls, and controls. *Cell* **78**, 915–918 (1994).
52. Gaston, B. & Stamler, J. S. in *Nitric Oxide and Infection* (ed. Fang, F. C.) 37–55 (Kluwer Academic/Plenum Publishers, New York, 1999).
53. De Groote, M. A. & Fang, F. C. *in Nitric Oxide and Infection* (ed. Fang, F. C.) 231–261 (Kluwer Academic/Plenum Publishers, New York, 1999).
54. Imlay, J. A. & Linn, S. Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* **166**, 519–527 (1986).
Shows that the mechanism of bacterial killing by H_2O_2 is concentration dependent.
55. Imlay, J. A. & Linn, S. DNA damage and oxygen radical toxicity. *Science* **240**, 1302–1309 (1988).
56. McCormick, M. L., Buettner, G. R. & Britigan, B. E. Endogenous superoxide dismutase levels regulate iron-dependent hydroxyl radical formation in *Escherichia coli* exposed to hydrogen peroxide. *J. Bacteriol.* **180**, 622–625 (1998).
57. Tamarit, J., Cabiscol, E. & Ros, J. Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress. *J. Biol. Chem.* **273**, 3027–3032 (1998).
58. Shohet, S. B., Pitt, J., Baehner, R. L. & Poplack, D. G. Lipid peroxidation in the killing of phagocytized pneumococci. *Infect. Immun.* **10**, 1321–1328 (1974).
59. Imlay, J. A. Pathways of oxidative damage. *Annu. Rev. Microbiol.* **57**, 395–418 (2003).
60. Stadler, N., Hofer, M. & Sigler, K. Mechanisms of *Saccharomyces cerevisiae* PMA1 H^+-ATPase inactivation by Fe^{2+}, H_2O_2 and Fenton reagents. *Free Radic. Res.* **35**, 643–653 (2001).
61. De Groote, M. A. *et al.* Genetic and redox determinants of nitric oxide cytotoxicity in a *Salmonella typhimurium* model. *Proc. Natl Acad. Sci. USA* **92**, 6399–6403 (1995).
62. Schapiro, J. M., Libby, S. J. & Fang, F. C. Inhibition of bacterial DNA replication by zinc mobilization during nitrosative stress. *Proc. Natl Acad. Sci. USA* **100**, 8496–8501 (2003).
Indicates that zinc metalloproteins that are involved in DNA replication are a crucial target of nitrogen oxides.
63. Pacelli, R. *et al.* Nitric oxide potentiates hydrogen peroxide-induced killing of *Escherichia coli*. *J. Exp. Med.* **182**, 1469–1479 (1995).
64. Stevanin, T. M. *et al.* Flavohemoglobin Hmp affords inducible protection for *Escherichia coli* respiration, catalyzed by cytochromes bo′ or bd, from nitric oxide. *J. Biol. Chem.* **275**, 35868–35875 (2000).
Describe how NO˙ inhibits bacterial respiration, which is antagonized by a bacterial flavohaemoglobin that converts NO˙ to nitrate.
65. Shi, L., Jung, Y. J., Tyagi, S., Gennaro, M. L. & North, R. J. Expression of T_H1-mediated immunity in mouse lungs induces a *Mycobacterium tuberculosis* transcription pattern characteristic of nonreplicating persistence. *Proc. Natl Acad. Sci. USA* **100**, 241–246 (2003).
66. Lepoivre, M., Fieschi, F., Coves, J., Thelander, L. & Fontecave, M. Inactivation of ribonucleotide reductase by nitric oxide. *Biochem. Biophys. Res. Commun.* **179**, 442–448 (1991).
67. Woodmansee, A. N. & Imlay, J. A. A mechanism by which nitric oxide accelerates the rate of oxidative DNA damage in *Escherichia coli*. *Mol. Microbiol.* **49**, 11–22 (2003).
68. Flint, D. H., Tuminello, J. F. & Emptage, M. H. The inactivation of Fe–S clusters containing hydrolyases by superoxide. *J. Biol. Chem.* **268**, 22369–22376 (1993).

69. Keyer, K. & Imlay, J. A. Superoxide accelerates DNA damage by elevating free-iron levels. *Proc. Natl Acad. Sci. USA* **93**, 13635–13640 (1996).
70. Wink, D. A. *et al.* DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* **254**, 1001–1003 (1991).
71. Burney, S., Caulfield, J. L., Niles, J. C., Wishnok, J. S. & Tannenbaum, S. R. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat. Res.* **424**, 37–49 (1999).
72. Spek, E. J. *et al.* Recombinational repair is critical for survival of *Escherichia coli* exposed to nitric oxide. *J. Bacteriol.* **183**, 131–138 (2001).
73. Evans, T. J. *et al.* Cytokine-treated human neutrophils contain inducible nitric oxide synthase that produces nitration of ingested bacteria. *Proc. Natl Acad. Sci. USA* **93**, 9553–9558 (1996).
74. Miller, R. A. & Britigan, B. E. Role of oxidants in microbial pathophysiology. *Clin. Microbiol. Rev.* **10**, 1–18 (1997).
Reviews some of the many examples of bacterial oxidative-stress resistance mechanisms that promote virulence.
75. Celli, J. & Finlay, B. B. Bacterial avoidance of phagocytosis. *Trends Microbiol.* **10**, 232–237 (2002).
76. Black, D. S. & Bliska, J. B. Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J.* **16**, 2730–2744 (1997).
77. Rosqvist, R., Forsberg, A. & Wolf-Watz, H. Intracellular targeting of the *Yersinia* YopE cytotoxin in mammalian cells induces actin microfilament disruption. *Infect. Immun.* **59**, 4562–4569 (1991).
78. Vazquez-Torres, A. *et al.* *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. *Science* **287**, 1655–1658 (2000).
Shows that *Salmonella* translocates proteins into the macrophage cytosol, which seems to interfere with vesicular transport.
79. Chakravortty, D., Hansen-Wester, I. & Hensel, M. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. *J. Exp. Med.* **195**, 1155–1166 (2002).
80. Cowley, S. C., Myltseva, S. V. & Nano, F. E. Phase variation in *Francisella tularensis* affecting intracellular growth, lipopolysaccharide antigenicity and nitric oxide production. *Mol. Microbiol.* **20**, 867–874 (1996).
81. Bliska, J. B. & Black, D. S. Inhibition of the Fc receptor-mediated oxidative burst in macrophages by the *Yersinia pseudotuberculosis* tyrosine phosphatase. *Infect. Immun.* **63**, 681–685 (1995).
82. Pearson, R. D., Symes, P., Conboy, M., Weiss, A. A. & Hewlett, E. L. Inhibition of monocyte oxidative responses by *Bordetella pertussis* adenylate cyclase toxin. *J. Immunol.* **139**, 2749–2754 (1987).
83. Prada, J., Malinowski, J., Muller, S., Bienzle, U. & Kremsner, P. G. Effects of *Plasmodium vinckei* hemozoin on the production of oxygen radicals and nitrogen oxides in murine macrophages. *Am. J. Trop. Med. Hyg.* **54**, 620–624 (1996).
84. Choi, K. S. & Dumler, J. S. Early induction and late abrogation of respiratory burst in *A. phagocytophilum*-infected neutrophils. *Ann. NY Acad. Sci.* **990**, 488–493 (2003).
85. Mott, J., Rikihisa, Y. & Tsunawaki, S. Effects of *Anaplasma phagocytophila* on NADPH oxidase components in human neutrophils and HL-60 cells. *Infect. Immun.* **70**, 1359–1366 (2002).
86. Carlyon, J. A., Chan, W. T., Galan, J., Roos, D. & Fikrig, E. Repression of *rac2* mRNA expression by *Anaplasma phagocytophila* is essential to the inhibition of superoxide production and bacterial proliferation. *J. Immunol.* **169**, 7009–7018 (2002).
87. Saura, M. *et al.* An antiviral mechanism of nitric oxide: inhibition of a viral protease. *Immunity* **10**, 21–28 (1999).
88. Colasanti, M., Persichini, T., Venturini, G. & Ascenzi, P. *S*-nitrosylation of viral proteins: molecular bases for antiviral effect of nitric oxide. *IUBMB Life* **48**, 25–31 (1999).
89. Cao, W., Bao, C. & Lowenstein, C. J. Inducible nitric oxide synthase expression inhibition by adenovirus E1A. *Proc. Natl Acad. Sci. USA* **100**, 7773–7778 (2003).
90. Seyler, R. W. Jr, Olson, J. W. & Maier, R. J. Superoxide dismutase-deficient mutants of *Helicobacter pylori* are hypersensitive to oxidative stress and defective in host colonization. *Infect. Immun.* **69**, 4034–4040 (2001).
91. Fang, F. C. *et al.* Virulent *Salmonella typhimurium* has two periplasmic Cu, Zn-superoxide dismutases. *Proc. Natl Acad. Sci. USA* **96**, 7502–7507 (1999).
92. Wilson, T. M., de Lisle, G. W. & Collins, D. M. Effect of *inhA* and *katG* on isoniazid resistance and virulence of *Mycobacterium bovis*. *Mol. Microbiol.* **15**, 1009–1015 (1995).
93. Bishai, W. R., Howard, N. S., Winkelstein, J. A. & Smith, H. O. Characterization and virulence analysis of catalase mutants of *Haemophilus influenzae*. *Infect. Immun.* **62**, 4855–4860 (1994).
94. Poole, R. K. & Hughes, M. N. New functions for the ancient globin family: bacterial responses to nitric oxide and nitrosative stress. *Mol. Microbiol.* **36**, 775–783 (2000).
95. Pathania, R., Navani, N. K., Gardner, A. M., Gardner, P. R. & Dikshit, K. L. Nitric oxide scavenging and detoxification by the *Mycobacterium tuberculosis* haemoglobin, HbN in *Escherichia coli*. *Mol. Microbiol.* **45**, 1303–1314 (2002).
96. Gardner, A. M., Helmick, R. A. & Gardner, P. R. Flavorubredoxin, an inducible catalyst for nitric oxide reduction and detoxification in *Escherichia coli*. *J. Biol. Chem.* **277**, 8172–8177 (2002).
97. Liu, L. *et al.* A metabolic enzyme for *S*-nitrosothiol conserved from bacteria to humans. *Nature* **410**, 490–494 (2001).
98. Bryk, R., Griffin, P. & Nathan, C. Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* **407**, 211–215 (2000).
99. de Jesus-Berrios, M. *et al.* Enzymes that counteract nitrosative stress promote fungal virulence. *Curr. Biol.* **13**, 1963–1968 (2003).
100. Newton, G. L. *et al.* Distribution of thiols in microorganisms: mycothiol is a major thiol in most actinomycetes. *J. Bacteriol.* **178**, 1990–1995 (1996).
101. Thomson, L., Denicola, A. & Radi, R. The trypanothione–thiol system in *Trypanosoma cruzi* as a key antioxidant mechanism against peroxynitrite-mediated cytotoxicity. *Arch. Biochem. Biophys.* **412**, 55–64 (2003).
102. Brenot, A., King, K. Y., Janowiak, B., Griffith, O. & Caparon, M. G. Contribution of glutathione peroxidase to the virulence of *Streptococcus pyogenes*. *Infect. Immun.* **72**, 408–413 (2004).
103. St John, G. *et al.* Peptide methionine sulfoxide reductase from *Escherichia coli* and *Mycobacterium tuberculosis* protects bacteria against oxidative damage from reactive nitrogen intermediates. *Proc. Natl Acad. Sci. USA* **98**, 9901–9906 (2001).
104. De Groote, M. A., Testerman, T., Xu, Y., Stauffer, G. & Fang, F. C. Homocysteine antagonism of nitric oxide-related cytostasis in *Salmonella typhimurium*. *Science* **272**, 414–417 (1996).
Shows that homocysteine antagonizes the actions of NO• in bacteria as well as in blood vessels.
105. van Guldener, C. & Stehouwer, C. D. Hyperhomocysteinemia, vascular pathology, and endothelial dysfunction. *Semin. Thromb. Hemost.* **26**, 281–289 (2000).
106. Wang, Y., Aisen, P. & Casadevall, A. *Cryptococcus neoformans* melanin and virulence: mechanism of action. *Infect. Immun.* **63**, 3131–3136 (1995).
107. Chaturvedi, V., Wong, B. & Newman, S. L. Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. *J. Immunol.* **156**, 3836–3840 (1996).
108. Simpson, J. A., Smith, S. E. & Dean, R. T. Scavenging by alginate of free radicals released by macrophages. *Free Radic. Biol. Med.* **6**, 347–353 (1989).
109. Andrews, S. C. Iron storage in bacteria. *Adv. Microb. Physiol.* **40**, 281–351 (1998).
110. Almiron, M., Link, A. J., Furlong, D. & Kolter, R. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. *Genes Dev.* **6**, 2646–2654 (1992).
111. Hantke, K. Iron and metal regulation in bacteria. *Curr. Opin. Microbiol.* **4**, 172–177 (2001).
112. Chen, C. Y. & Morse, S. A. *Neisseria gonorrhoeae* bacterioferritin: structural heterogeneity, involvement in iron storage and protection against oxidative stress. *Microbiology* **145**, 2967–2975 (1999).
113. Wai, S. N., Nakayama, K., Umene, K., Moriya, T. & Amako, K. Construction of a ferritin-deficient mutant of *Campylobacter jejuni*: contribution of ferritin to iron storage and protection against oxidative stress. *Mol. Microbiol.* **20**, 1127–1134 (1996).
114. Halsey, T. A., Vazquez-Torres, A., Gravdahl, D. J., Fang, F. C. & Libby, S. J. The ferritin-like Dps protein is required for *Salmonella enterica* serovar *typhimurium* oxidative stress resistance and virulence. *Infect. Immun.* **72**, 1155–1158 (2004).
115. D'Autreaux, B., Touati, D., Bersch, B., Latour, J. M. & Michaud-Soret, I. Direct inhibition by nitric oxide of the transcriptional ferric uptake regulation protein via nitrosylation of the iron. *Proc. Natl Acad. Sci. USA* **99**, 16619–16624 (2002).
116. Lobysheva, I. I., Stupakova, M. V., Mikoyan, V. D., Vasilieva, S. V. & Vanin, A. F. Induction of the SOS DNA repair response in *Escherichia coli* by nitric oxide donating agents: dinitrosyl iron complexes with thiol-containing ligands and *S*-nitrosothiols. *FEBS Lett.* **454**, 177–180 (1999).
117. Christman, M. F., Morgan, R. W., Jacobson, F. S. & Ames, B. N. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. *Cell* **41**, 753–762 (1985).
118. Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D. & Demple, B. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **87**, 6181–6185 (1990).
119. Zheng, M., Aslund, F. & Storz, G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718–1721 (1998).
120. Paget, M. S., Kang, J. G., Roe, J. H. & Buttner, M. J. σR, an RNA polymerase σ-factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). *EMBO J.* **17**, 5776–5782 (1998).
121. Helmann, J. D. *et al.* The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. *J. Bacteriol.* **185**, 243–253 (2003).
122. Boylan, J. A., Posey, J. E. & Gherardini, F. C. *Borrelia* oxidative stress response regulator, BosR: a distinctive Zn-dependent transcriptional activator. *Proc. Natl Acad. Sci. USA* **100**, 11684–11689 (2003).
123. Sak, B. D., Eisenstark, A. & Touati, D. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. *Proc. Natl Acad. Sci. USA* **86**, 3271–3275 (1989).
124. Mukhopadhyay, P., Zheng, M., Bedzyk, L. A., LaRossa, R. A. & Storz, G. Prominent roles of the NorR and Fur regulators in the *Escherichia coli* transcriptional response to reactive nitrogen species. *Proc. Natl Acad. Sci. USA* **101**, 745–750 (2004).
The authors argue that, despite some overlap, there seem to be important differences in the microbial transcriptional responses to oxidative and nitrosative stress.
125. Cruz-Ramos, H. *et al.* NO sensing by FNR: regulation of the *Escherichia coli* NO-detoxifying flavohaemoglobin, Hmp. *EMBO J.* **21**, 3235–3244 (2002).
126. Zumft, W. G. Nitric oxide signaling and NO dependent transcriptional control in bacterial denitrification by members of the FNR-CRP regulator family. *J. Mol. Microbiol. Biotechnol.* **4**, 277–286 (2002).
127. Suvarnapunya, A. E., Lagasse, H. A. & Stein, M. A. The role of DNA base excision repair in the pathogenesis of *Salmonella enterica* serovar *typhimurium*. *Mol. Microbiol.* **48**, 549–559 (2003).
128. Darwin, K. H., Ehrt, S., Gutierrez-Ramos, J. C., Weich, N. & Nathan, C. F. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* **302**, 1963–1966 (2003).
129. Buchmeier, N. A., Lipps, C. J., So, M. Y. & Heffron, F. Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. *Mol. Microbiol.* **7**, 933–936 (1993).
130. O'Rourke, E. J. *et al.* Pathogen DNA as target for host-generated oxidative stress: role for repair of bacterial DNA damage in *Helicobacter pylori* colonization. *Proc. Natl Acad. Sci. USA* **100**, 2789–2794 (2003).
131. Buchmeier, N. A. *et al.* DNA repair is more important than catalase for *Salmonella* virulence in mice. *J. Clin. Invest.* **95**, 1047–1053 (1995).
132. Brawn, M. K. & Fridovich, I. Increased superoxide radical production evokes inducible DNA repair in *Escherichia coli*. *J. Biol. Chem.* **260**, 922–925 (1985).
133. Zheng, M. *et al.* DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* **183**, 4562–4570 (2001).
134. Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P. & Fenn, W. O. Oxygen poisoning and X-irradiation: a mechanism in common. *Science* **119**, 623–626 (1954).
135. Schneemann, M. *et al.* Nitric oxide synthase is not a constituent of the antimicrobial armature of human mononuclear phagocytes. *J. Infect. Dis.* **167**, 1358–1363 (1993).
136. Weinberg, J. B. Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. *Mol. Med.* **4**, 557–591 (1998).
Reviews compelling evidence for NO• production by human macrophages.
137. Wheeler, M. A. *et al.* Bacterial infection induces nitric oxide synthase in human neutrophils. *J. Clin. Invest.* **99**, 110–116 (1997).
138. Schneemann, M. & Schoedon, G. Species differences in macrophage NO production are important. *Nature Immunol.* **3**, 102 (2002).
139. Fang, F. C. & Vazquez-Torres, A. Nitric oxide production by human macrophages: there's NO doubt about it. *Am. J. Physiol. Lung Cell Mol. Physiol.* **282**, L941–L943 (2002).

140. Nicholson, S. *et al.* Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *J. Exp. Med.* **183**, 2293–2302 (1996).
141. Annane, D. *et al.* Compartmentalised inducible nitric-oxide synthase activity in septic shock. *Lancet* **355**, 1143–1148 (2000).
142. Pham, T. N. *et al.* Elevated serum nitric oxide levels in patients with inflammatory arthritis associated with co-expression of inducible nitric oxide synthase and protein kinase C-η in peripheral blood monocyte-derived macrophages. *J. Rheumatol.* **30**, 2529–2534 (2003).
143. Nauseef, W. M. & Malech, H. L. Analysis of the peptide subunits of human neutrophil myeloperoxidase. *Blood* **67**, 1504–1507 (1986).
144. Daugherty, A., Dunn, J. L., Rateri, D. L. & Heinecke, J. W. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J. Clin. Invest.* **94**, 437–444 (1994).
145. Foote, C. S., Goyne, T. E. & Lehrer, R. I. Assessment of chlorination by human neutrophils. *Nature* **301**, 715–716 (1983).
146. Eiserich, J. P. *et al.* Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* **391**, 393–397 (1998).
147. Klebanoff, S. J. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **126**, 1063–1078 (1967).
148. Rosen, H. & Klebanoff, S. J. Bactericidal activity of a superoxide anion-generating system. A model for the polymorphonuclear leukocyte. *J. Exp. Med.* **149**, 27–39 (1979).
149. Rosen, H., Orman, J., Rakita, R. M., Michel, B. R. & VanDevanter, D. R. Loss of DNA–membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **87**, 10048–10052 (1990).
150. Thomas, E. L. & Aune, T. M. Oxidation of *Escherichia coli* sulfhydryl components by the peroxidase–hydrogen peroxide–iodide antimicrobial system. *Antimicrob. Agents Chemother.* **13**, 1006–1010 (1978).
151. Rakita, R. M., Michel, B. R. & Rosen, H. Myeloperoxidase-mediated inhibition of microbial respiration: damage to *Escherichia coli* ubiquinol oxidase. *Biochemistry* **28**, 3031–3036 (1989).
152. Rosen, H., Crowley, J. R. & Heinecke, J. W. Human neutrophils use the myeloperoxidase–hydrogen peroxide–chloride system to chlorinate but not nitrate bacterial proteins during phagocytosis. *J. Biol. Chem.* **277**, 30463–30468 (2002).
153. Chapman, A. L., Hampton, M. B., Senthilmohan, R., Winterbourn, C. C. & Kettle, A. J. Chlorination of bacterial and neutrophil proteins during phagocytosis and killing of *Staphylococcus aureus*. *J. Biol. Chem.* **277**, 9757–9762 (2002).
154. Saito, M. *et al.* H_2O_2-nonproducing *Streptococcus pyogenes* strains: survival in stationary phase and virulence in chronic granulomatous disease. *Microbiology* **147**, 2469–2477 (2001).
155. Kottilil, S., Malech, H. L., Gill, V. J. & Holland, S. M. Infections with *Haemophilus* species in chronic granulomatous disease: insights into the interaction of bacterial catalase and H_2O_2 production. *Clin. Immunol.* **106**, 226–230 (2003).
156. Parry, M. F. *et al.* Myeloperoxidase deficiency: prevalence and clinical significance. *Ann. Intern. Med.* **95**, 293–301 (1981).
Shows that humans with MPO deficiency have little or no immunocompromise, in contrast to individuals with CGD.
157. Aratani, Y. *et al.* Severe impairment in early host defense against *Candida albicans* in mice deficient in myeloperoxidase. *Infect. Immun.* **67**, 1828–1836 (1999).
Presents a description of an MPO-deficient mouse model.
158. Brennan, M. L. *et al.* Increased atherosclerosis in myeloperoxidase-deficient mice. *J. Clin. Invest.* **107**, 419–430 (2001).
159. Eiserich, J. P. *et al.* Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* **296**, 2391–2394 (2002).
160. Brennan, M. L. *et al.* Prognostic value of myeloperoxidase in patients with chest pain. *N. Engl. J. Med.* **349**, 1595–1604 (2003).
161. Vorbach, C., Harrison, R. & Capecchi, M. R. Xanthine oxidoreductase is central to the evolution and function of the innate immune system. *Trends Immunol.* **24**, 512–517 (2003).
162. Takao, S. *et al.* Role of reactive oxygen metabolites in murine peritoneal macrophage phagocytosis and phagocytic killing. *Am. J. Physiol.* **271**, C1278–C1284 (1996).
163. Umezawa, K. *et al.* Induction of nitric oxide synthesis and xanthine oxidase and their roles in the antimicrobial mechanism against *Salmonella typhimurium* infection in mice. *Infect. Immun.* **65**, 2932–2940 (1997).
164. Segal, B. H. *et al.* Xanthine oxidase contributes to host defense against *Burkholderia cepacia* in the *p47$^{phox-/-}$* mouse model of chronic granulomatous disease. *Infect. Immun.* **68**, 2374–2378 (2000).
165. Simmonds, H. A., Goday, A. & Morris, G. S. Superoxide radicals, immunodeficiency and xanthine oxidase activity: man is not a mouse! *Clin. Sci. (Lond)* **68**, 561–565 (1985).
166. Aratani, Y. *et al.* Relative contributions of myeloperoxidase and NADPH-oxidase to the early host defense against pulmonary infections with *Candida albicans* and *Aspergillus fumigatus*. *Med. Mycol.* **40**, 557–563 (2002).
167. Miyakawa, H. *et al.* Effects of inducible nitric oxide synthase and xanthine oxidase inhibitors on SEB-induced interstitial pneumonia in mice. *Eur. Respir. J.* **19**, 447–457 (2002).
168. Moorhouse, P. C., Grootveld, M., Halliwell, B., Quinlan, J. G. & Gutteridge, J. M. Allopurinol and oxypurinol are hydroxyl radical scavengers. *FEBS Lett.* **213**, 23–28 (1987).
169. Frayha, R. A., Salti, I. S., Arnaout, A., Khatchadurian, A. & Uthman, S. M. Hereditary xanthinuria: report on three patients and short review of the literature. *Nephron* **19**, 328–332 (1977).
170. Vorbach, C., Scriven, A. & Capecchi, M. R. The housekeeping gene xanthine oxidoreductase is necessary for milk fat droplet enveloping and secretion: gene sharing in the lactating mammary gland. *Genes Dev.* **16**, 3223–3235 (2002).
171. Wang, J. *et al.* Serum xanthine oxidase: origin, regulation, and contribution to control of trypanosome parasitemia. *Antioxid. Redox Signal.* **4**, 161–178 (2002).
172. Sengelov, H., Nielsen, M. H. & Borregaard, N. Separation of human neutrophil plasma membrane from intracellular vesicles containing alkaline phosphatase and NADPH oxidase activity by free flow electrophoresis. *J. Biol. Chem.* **267**, 14912–14917 (1992).
173. Badwey, J. A. *et al.* Comparative aspects of oxidative metabolism of neutrophils from human blood and guinea pig peritonea: magnitude of the respiratory burst, dependence upon stimulating agents, and localization of the oxidases. *J. Cell. Physiol.* **105**, 541–545 (1980).
174. Ohno, Y., Hirai, K., Kanoh, T., Uchino, H. & Ogawa, K. Subcellular localization of H_2O_2 production in human neutrophils stimulated with particles and an effect of cytochalasin-B on the cells. *Blood* **60**, 253–260 (1982).
175. Heyworth, P. G. *et al.* Neutrophil nicotinamide adenine dinucleotide phosphate oxidase assembly. Translocation of p47-phox and p67-phox requires interaction between p47-phox and cytochrome b_{558}. *J. Clin. Invest.* **87**, 352–356 (1991).
176. Allen, L. A. *et al.* Transient association of the nicotinamide adenine dinucleotide phosphate oxidase subunits p47phox and p67phox with phagosomes in neutrophils from patients with X-linked chronic granulomatous disease. *Blood* **93**, 3521–3530 (1999).
177. Kobayashi, T., Robinson, J. M. & Seguchi, H. Identification of intracellular sites of superoxide production in stimulated neutrophils. *J. Cell Sci.* **111**, 81–91 (1998).
Shows that active NADPH oxidase might assemble in vesicles that subsequently move to the plasma membrane or phagosomal compartments.
178. Seguchi, H. & Kobayashi, T. Study of NADPH oxidase-activated sites in human neutrophils. *J. Electron Microsc. (Tokyo)* **51**, 87–91 (2002).
179. Lang, M. L. & Kerr, M. A. Neutrophil NADPH oxidase does not assemble on macropinocytic vacuole membranes. *Immunol. Lett.* **72**, 1–6 (2000).
180. Kobayashi, T. *et al.* A simple approach for the analysis of intracellular movement of oxidant-producing intracellular compartments in living human neutrophils. *Histochem. Cell Biol.* **113**, 251–257 (2000).
181. Johansson, A. *et al.* Different subcellular localization of cytochrome b and the dormant NADPH-oxidase in neutrophils and macrophages: effect on the production of reactive oxygen species during phagocytosis. *Cell. Immunol.* **161**, 61–71 (1995).
182. Badwey, J. A. *et al.* Comparative biochemical and cytochemical studies on superoxide and peroxide in mouse macrophages. *J. Cell. Physiol.* **115**, 208–216 (1983).
183. Calafat, J. *et al.* Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b_{558} and the adhesion molecule CD11b/CD18. *Blood* **81**, 3122–3129 (1993).
184. Teixeira, C. F., Azevedo, N. L., Carvalho, T. M., Fuentes, J. & Nagao, P. E. Cytochemical study of *Streptococcus agalactiae* and macrophage interaction. *Microsc. Res. Tech.* **54**, 254–259 (2001).
185. Vazquez-Torres, A., Fantuzzi, G., Edwards, C. K., Dinarello, C. A. & Fang, F. C. Defective localization of the NADPH phagocyte oxidase to *Salmonella*-containing phagosomes in tumor necrosis factor p55 receptor-deficient macrophages. *Proc. Natl Acad. Sci. USA* **98**, 2561–2565 (2001).
186. Vodovotz, Y., Russell, D., Xie, Q. W., Bogdan, C. & Nathan, C. Vesicle membrane association of nitric oxide synthase in primary mouse macrophages. *J. Immunol.* **154**, 2914–2925 (1995).
187. Fang, F. & Vazquez-Torres, A. *Salmonella* selectively stops traffic. *Trends Microbiol.* **10**, 391–392 (2002).
188. Webb, J. L., Harvey, M. W., Holden, D. W. & Evans, T. J. Macrophage nitric oxide synthase associates with cortical actin but is not recruited to phagosomes. *Infect. Immun.* **69**, 6391–6400 (2001).
189. Brunelli, L., Crow, J. P. & Beckman, J. S. The comparative toxicity of nitric oxide and peroxynitrite to *Escherichia coli*. *Arch. Biochem. Biophys.* **316**, 327–334 (1995).
190. Hickman-Davis, J., Gibbs-Erwin, J., Lindsey, J. R. & Matalon, S. Surfactant protein A mediates mycoplasmacidal activity of alveolar macrophages by production of peroxynitrite. *Proc. Natl Acad. Sci. USA* **96**, 4953–4958 (1999).
191. Vazquez-Torres, A., Jones-Carson, J. & Balish, E. Peroxynitrite contributes to the candidacidal activity of nitric oxide-producing macrophages. *Infect. Immun.* **64**, 3127–3133 (1996).
192. Darrah, P. A., Hondalus, M. K., Chen, Q., Ischiropoulos, H. & Mosser, D. M. Cooperation between reactive oxygen and nitrogen intermediates in killing of *Rhodococcus equi* by activated macrophages. *Infect. Immun.* **68**, 3587–3593 (2000).
193. De Groote, M. A. *et al.* Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase. *Proc. Natl Acad. Sci. USA* **94**, 13997–14001 (1997).
194. Reeves, E. P. *et al.* Killing activity of neutrophils is mediated through activation of proteases by K^+ flux. *Nature* **416**, 291–297 (2002).
This report proposes a new mechanism for the synergy between NADPH phagocyte oxidase and neutrophil granule proteases.
195. Tkalcevic, J. *et al.* Impaired immunity and enhanced resistance to endotoxin in the absence of neutrophil elastase and cathepsin G. *Immunity* **12**, 201–210 (2000).
196. Ahluwalia, J. *et al.* The large-conductance Ca^{2+}-activated K^+ channel is essential for innate immunity. *Nature* **427**, 853–858 (2004).
197. Roos, D. & Winterbourn, C. C. Immunology. Lethal weapons. *Science* **296**, 669–671 (2002).
198. Staudinger, B. J., Oberdoerster, M. A., Lewis, P. J. & Rosen, H. mRNA expression profiles for *Escherichia coli* ingested by normal and phagocyte oxidase-deficient human neutrophils. *J. Clin. Invest.* **110**, 1151–1163 (2002).
Presents evidence that the bacteria in neutrophils experience oxidative stress and that their ability to respond is crucial for their intracellular survival.
199. Belaaouaj, A. *et al.* Mice lacking neutrophil elastase reveal impaired host defense against Gram-negative bacterial sepsis. *Nature Med.* **4**, 615–618 (1998).
200. MacIvor, D. M. *et al.* Normal neutrophil function in cathepsin G-deficient mice. *Blood* **94**, 4282–4293 (1999).
201. Murray, H. W. & Cartelli, D. M. Killing of intracellular *Leishmania donovani* by human mononuclear phagocytes. Evidence for oxygen-dependent and -independent leishmanicidal activity. *J. Clin. Invest.* **72**, 32–44 (1983).
202. Mandell, G. L. & Hook, E. W. Leukocyte bactericidal activity in chronic granulomatous disease: correlation of bacterial hydrogen peroxide production and susceptibility to intracellular killing. *J. Bacteriol.* **100**, 531–532 (1969).
203. Kim, Y. M., Hong, S. J., Billiar, T. R. & Simmons, R. L. Counterprotective effect of erythrocytes in experimental bacterial peritonitis is due to scavenging of nitric oxide and reactive oxygen intermediates. *Infect. Immun.* **64**, 3074–3080 (1996).
204. Speert, D. P., Bond, M., Woodman, R. C. & Curnutte, J. T. Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. *J. Infect. Dis.* **170**, 1524–1531 (1994).
205. Dinauer, M. C., Gifford, M. A., Pech, N., Li, L. L. & Emshwiller, P. Variable correction of host defense following gene transfer and bone marrow transplantation in murine X-linked chronic granulomatous disease. *Blood* **97**, 3738–3745 (2001).
206. Harrison, R. E., Touret, N. & Grinstein, S. Microbial killing: oxidants, proteases and ions. *Curr. Biol.* **12**, R357–R359 (2002).
207. Wentworth, P. Jr *et al.* Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. *Science* **298**, 2195–2199 (2002).
Suggests that ozone is an antimicrobial mediator that is formed by antibodies.
208. Takeuchi, K. & Ibusuki, T. Quantitative determination of aqueous-phase ozone by chemiluminescence using indigo-5,5′-disulfonate. *Anal. Chem.* **61**, 619–623 (1989).

209. Kettle, A. J., Clark, B. M. & Winterbourn, C. C. Superoxide converts indigo carmine to isatin sulfonic acid: implications for the hypothesis that neutrophils produce ozone. *J. Biol. Chem.* **279**, 18521–18525 (2004).
210. Parren, P. W., Leusen, J. H. & van de Winkel, J. G. Antibody-catalyzed water oxidation: state-of-the-art immunity or ancient history? *Trends Immunol.* **24**, 467–469 (2003).
211. Forman, H. J. & Torres, M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am. J. Respir. Crit. Care Med.* **166**, S4–S8 (2002).
212. Kim, S. & Ponka, P. Role of nitric oxide in cellular iron metabolism. *Biometals* **16**, 125–135 (2003).
213. Nathan, C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.* **111**, 769–778 (2003).
214. Ehrt, S. *et al.* Reprogramming of the macrophage transcriptome in response to interferon-γ and *Mycobacterium* tuberculosis: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. *J. Exp. Med.* **194**, 1123–1140 (2001). **Describes the profound effects of NADPH oxidase and iNOS on gene expression.**
215. Kobayashi, S. D. *et al.* Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. *J. Immunol.* **172**, 636–643 (2004).
216. Cooper, A. M., Adams, L. B., Dalton, D. K., Appelberg, R. & Ehlers, S. IFN-γ and NO in mycobacterial disease: new jobs for old hands. *Trends Microbiol.* **10**, 221–226 (2002).
217. Alam, M. S. *et al.* Role of nitric oxide in host defense in murine salmonellosis as a function of its antibacterial and antiapoptotic activities. *Infect. Immun.* **70**, 3130–3142 (2002).
218. Sadikot, R. T. *et al.* $p47^{phox}$ deficiency impairs NF-κB activation and host defense in *Pseudomonas* pneumonia. *J. Immunol.* **172**, 1801–1808 (2004).
219. Huang, J. *et al.* The quantity of nitric oxide released by macrophages regulates *Chlamydia*-induced disease. *Proc. Natl Acad. Sci. USA* **99**, 3914–3919 (2002).
220. van der Veen, R. C. *et al.* Superoxide prevents nitric oxide-mediated suppression of helper T lymphocytes: decreased autoimmune encephalomyelitis in nicotinamide adenine dinucleotide phosphate oxidase knockout mice. *J. Immunol.* **164**, 5177–5183 (2000).
221. Li, H. & Forstermann, U. Nitric oxide in the pathogenesis of vascular disease. *J. Pathol.* **190**, 244–254 (2000).
222. Fang, F. C. *Nitric Oxide and Infection* 517 (Kluwer Academic/Plenum Publishers, New York, 1999). **A comprehensive examination of the many roles of $NO^{\bullet}$ in infection.**
223. Petros, A. *et al.* Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc. Res.* **28**, 34–39 (1994).
224. Cobb, J. P. Use of nitric oxide synthase inhibitors to treat septic shock: the light has changed from yellow to red. *Crit. Care Med.* **27**, 855–856 (1999). **Argues that although initial clinical studies of $NO^{\bullet}$ inhibition in sepsis have been disappointing, selective inhibitors await more extensive evaluation.**
225. Akaike, T. *et al.* Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. *Proc. Natl Acad. Sci. USA* **93**, 2448–2453 (1996). **Shows that the inhibition of ROS/RNS-mediated tissue damage can reduce mortality in certain infections.**
226. Adler, H. *et al.* Suppression of herpes simplex virus type 1 (HSV-1)-induced pneumonia in mice by inhibition of inducible nitric oxide synthase (iNOS, NOS2). *J. Exp. Med.* **185**, 1533–1540 (1997).
227. Davis, I. C. *et al.* Elevated generation of reactive oxygen/nitrogen species in hantavirus cardiopulmonary syndrome. *J. Virol.* **76**, 8347–8259 (2002).
228. Fujii, S., Akaike, T. & Maeda, H. Role of nitric oxide in pathogenesis of herpes simplex virus encephalitis in rats. *Virology* **256**, 203–212 (1999).
229. Suntres, Z. E., Omri, A. & Shek, P. N. *Pseudomonas aeruginosa*-induced lung injury: role of oxidative stress. *Microb. Pathog.* **32**, 27–34 (2002).
230. Auer, M., Pfister, L. A., Leppert, D., Tauber, M. G. & Leib, S. L. Effects of clinically used antioxidants in experimental pneumococcal meningitis. *J. Infect. Dis.* **182**, 347–350 (2000).
231. de Gans, J. & van de Beek, D. Dexamethasone in adults with bacterial meningitis. *N. Engl. J. Med.* **347**, 1549–1556 (2002).
232. Umeki, S. & Soejima, R. Hydrocortisone inhibits the respiratory burst oxidase from human neutrophils in whole-cell and cell-free systems. *Biochim Biophys. Acta* **1052**, 211–215 (1990).
233. Korhonen, R., Lahti, A., Hamalainen, M., Kankaanranta, H. & Moilanen, E. Dexamethasone inhibits inducible nitric-oxide synthase expression and nitric oxide production by destabilizing mRNA in lipopolysaccharide-treated macrophages. *Mol. Pharmacol.* **62**, 698–704 (2002).
234. Gao, X. P. *et al.* Role of NADPH oxidase in the mechanism of lung neutrophil sequestration and microvessel injury induced by Gram-negative sepsis: studies in *p47phox*$^{-/-}$ and *gp91phox*$^{-/-}$ mice. *J. Immunol.* **168**, 3974–3982 (2002).
235. Morgenstern, D. E., Gifford, M. A., Li, L. L., Doerschuk, C. M. & Dinauer, M. C. Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defense and inflammatory response to *Aspergillus fumigatus*. *J. Exp. Med.* **185**, 207–218 (1997). **Shows that NADPH oxidase might be important in the resolution of inflammatory lesions.**
236. Weiss, J., Kao, L., Victor, M. & Elsbach, P. Respiratory burst facilitates the digestion of *Escherichia coli* killed by polymorphonuclear leukocytes. *Infect. Immun.* **55**, 2142–2147 (1987).
237. Carson, M. J., Chadwick, D. L., Brubaker, C. A., Cleland, R. S. & Landing, B. H. Thirteen boys with progressive septic granulomatosis. *Pediatrics* **35**, 405–412 (1965).
238. Pignatelli, B. *et al. Helicobacter pylori* eradication attenuates oxidative stress in human gastric mucosa. *Am. J. Gastroenterol.* **96**, 1758–1766 (2001).
239. Blanchard, T. G., Yu, F., Hsieh, C. L. & Redline, R. W. Severe inflammation and reduced bacteria load in murine helicobacter infection caused by lack of phagocyte oxidase activity. *J. Infect. Dis.* **187**, 1609–1615 (2003).
240. Laroux, F. S. *et al.* Role of nitric oxide in the regulation of acute and chronic inflammation. *Antioxid. Redox Signal.* **2**, 391–396 (2000).
241. Anstey, N. M. *et al.* Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J. Exp. Med.* **184**, 557–567 (1996).
242. Matsushita, K. *et al.* Nitric oxide regulates exocytosis by *S*-nitrosylation of *N*-ethylmaleimide-sensitive factor. *Cell* **115**, 139–150 (2003).

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