Kunkel Lecture: Fundamental immunodeficiency and its correction

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"Fundamental immunodeficiency" is the inability of the encoded immune system to protect an otherwise healthy host from every infection that could threaten its life. In contrast to primary immunodeficiencies, fundamental immunodeficiency is not rare but nearly universal. It results not from variation in a given host gene but from the rate and extent of variation in the genes of other organisms. The remedy for fundamental immunodeficiency is "adopted immunity," not to be confused with adaptive or adoptive immunity. Adopted immunity arises from four critical societal contributions to the survival of the human species: sanitation, nutrition, vaccines, and antimicrobial agents. Immunologists have a great deal to contribute to the development of vaccines and antimicrobial agents, but they have focused chiefly on vaccines, and vaccinology is thriving. In contrast, the effect of antimicrobial agents in adopted immunity, although fundamental, is fragile and failing. Immunologists can aid the development of sorely needed antimicrobial agents, and the study of antimicrobial agents can help immunologists discover targets and mechanisms of host immunity.

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Introduction

It was a privilege to give the Kunkel Lecture at the Kunkel Society meeting on Primary Immunodeficiencies at The Rockefeller University on March 30, 2017. This essay expands on that talk. I first met Henry Kunkel when I joined the editorial board of *The Journal of Experimental Medicine* 36 years ago. He greeted me with a challenge: "What do *you* know?" I suppose he meant, "What papers are *you* fit to review?" I could not answer him and was still mulling the question over when he passed away 2 years later. Since then, I have seen that the more I learn, the less I know, but at least I can share what I have been thinking about in the past few years: the potentially rewarding, but relatively neglected, conceptual space where immunology comes together with the development of drugs for infectious disease.

Two immune systems: Encoded and adopted

Metchnikoff, a visionary founder of immunology, conceived of the discipline as the study of cells that fight infection, remodel tissues during organismal development, and preserve homeostasis (Vikhanski, 2016). Notwithstanding his preoccupation with phagocytes to the exclusion of soluble factors, his view of the scope of the immune system was so broad that for years only the first postulate was accepted; the latter two were ignored. By the mid-twentieth century, studies by Medawar and others on the rejection of allografts and xenografts and the emergence of transplant rejection as a major clinical concern led to a different view: immunology was considered a system for distinguishing "self" from "nonself."

In 1992, Irun Cohen objected that this was too narrow: "…[T]he evolutionary aim of the immune system is not to distinguish between self and nonself. …[T]he aim of the immune system…is to enhance fitness" (Cohen, 1992). Recently, immunologists who share Cohen's view have embraced all three of Metchnikoff's postulates, extending them to include both cellular and soluble factors of the immune system. For example, complement guides microglia to sculpt synapses in the developing brain (Schafer et al., 2012), IL-17 regulates synaptic responses in *Caenorhabditis elegans* (Chen et al., 2017), and inflammation and immunity profoundly affect metabolism (Brestoff and Artis, 2015; Kotas and Medzhitov, 2015; Hotamisligil, 2017).

Immunity, however, has an even wider scope than this enlarged version of Metchnikoff's vision. Cohen's definition was published 3 years before the first genome of an organism was sequenced. Today, with tens of thousands of species' genomes sequenced, the perspective has shifted. Here is a contemporary definition: The immune system of higher animals is a cellular and humoral network that controls interactions among the five genomes that do or may inhabit the host, in such a way as to favor the host's opportunity to transmit the germline genome to its descendants and protect them until they achieve reproductive maturity—that is, to propagate the species (Fig. 1). To do so requires the germline genome to encode a means of mediating its own beneficial interaction with the somatic, mitochondrial, and microbiotal genomes and, in pregnant mammals, the genome of the fetal allograft.

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Abbreviations used: AMR, antimicrobial resistance; iNOS, inducible nitric oxide synthase; MIC, minimum inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; RNS, reactive nitrogen species; ROS, reactive oxygen species; TB, tuberculosis; tRNA, transfer RNA.

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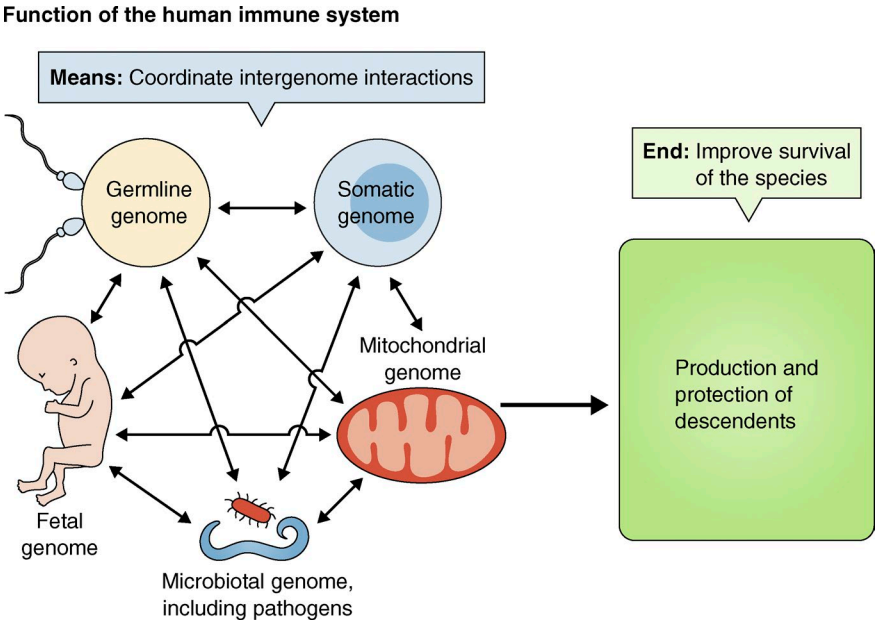

Figure 1. **Function of the human immune system.** The immune system is the set of cellular and soluble factors by which the germline genome interacts with the somatic, mitochondrial, fetal, and microbiotal genomes to protect the individual's ability to propagate the species. Arrows between genomes signify epigenetic, transcriptional, and posttranscriptional influences on expression of genes in one genome of an individual by the alleles present in the individual's other genomes.

The somatic genome, coding and noncoding, is shaped not only by inheritance but also by X-chromosome inactivation, monoallelic expression, other epigenetic controls, and the neoantigens of diversified antigen receptors and aging or neoplastic cells. The present discussion views the microbiotal genome as the genomes of all viral, bacterial, archaeal, fungal, protist, and helminthic organisms, commensal or pathogenic, that chronically or transiently occupy the host. Collectively, these four genomes (five in a pregnant female) comprise the metagenome of an individual.

As expansive a definition of the immune system as the foregoing is, it describes only one of our two immune systems, the encoded one. Collective actions that improve the ability of a species' members to survive infection constitute a second immune system. This second, societal system is genetically encoded only insofar as genes govern how individuals can communicate and cooperate. There is no way to predict specific volitional behaviors of a group over evolutionarily short periods of time from the sequences of its members' genomes. Therefore, the immune system that a group builds for its members through collective behaviors can be described as "adopted" rather than "encoded" (Fig. 2).

Fundamental immunodeficiency

The main causes of death that shape evolution are the difficulty of acquiring resources and the difficulty of not serving as a resource for others. With respect to the latter challenge, we call the nonhuman life forms that threaten humans "predators," "parasites," or "microbes," depending on their size. For simplicity, I will use the term "microbes" to include all life forms that can live within us after entering in a form too small to see unaided. I will use the term "pathogen" not to classify species of microbes but to refer to a microbe in the act of causing disease, a context-dependent event.

The encoded immune system is largely shaped by the challenges of resisting exploitation by pathogens. The main challenges to immunity arise from pathogens' functional and genetic diversity. What limits a species' success is in large part the inability of its encoded immune system to meet all these challenges.

Functional diversity among pathogens is exemplified by the variety of their life cycles, in particular, their routes of passage from one host to another and the implications for the host. For example, for *Bacillus anthracis*, decomposition of the host is important for the pathogen to get from the blood and other organs to the soil, where it forms spores, its means of transmission. Hence, a race: the vegetative form of the bacterium must commit the host to decomposition before the host kills the bacterium. For *Bordetella pertussis*, the host's death is incidental and inconsequential, given that transmission by exhalation has usually occurred well before death of some hosts, typically from a secondary infection. For *Mycobacterium tuberculosis* (*Mtb*), early death of the host from systemic disease is catastrophic, given that transmission requires a breathing person whose inflammatory and immune responses have lasted long enough and been strong enough to liquefy lung, erode into an airway, and provoke the expulsion of an infectious aerosol. From these three examples alone, the host needs mechanisms to forestall or withstand syndromes as diverse as sepsis, asphyxia, and cachexia that evolve over hours, days, or years, respectively.

The genetic diversity of pathogens presents another sort of challenge, a numerical one that is essentially insoluble for the encoded immune system unaided. The problem is inherent in the difference between organ systems that are governed

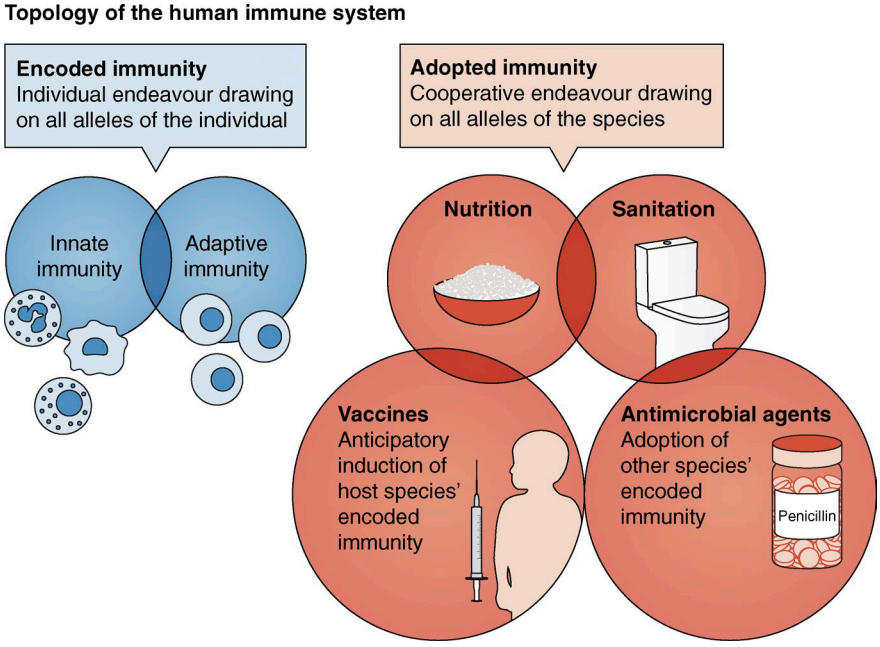

Figure 2. **Encoded and adopted immunity together comprise the contemporary human immune system.** The alleles in an individual's metagenome (see Fig. 1) determine his or her encoded immune response. The genes in the societal groups to which an individual belongs influence the groups' potential for cooperative endeavors. Society can adopt behaviors that help the encoded immune system compensate for fundamental immunodeficiency, such as provision of nutrition (including vitamins), sanitation (including shelter, potable water, hygiene, and disposal of waste), vaccines, and antimicrobial agents. Such forms of immunity were only recently adopted and are not automatically maintained. Some sectors of society have withdrawn from vaccines. Globally, society has unintentionally been on course to abandon antimicrobial agents.

by three of the four genomes in a nonpregnant individual's metagenome or by all four of them.

The function of most organs is fulfilled through use of information contained in the germline, somatic, and mitochondrial genomes, each of which begins with a fixed set of genes. With a fixed set of genes, evolution asymptotically approaches an ideal solution to problems presented by long-standing aspects of the environment within the constraints imposed by the gene pool and earlier solutions.

In contrast, the immune system deals not only with those sets of genes but also with an effectively unlimited number and variety of genes of microbes that evolve orders of magnitude faster than the hosts they may come to inhabit. Evolution in the encoded immune system can never approach an ideal solution to infection because the solution imposes selection on the problem, and the problem evolves faster than the host.

Within the encoded immune system, the innate branch has probably been evolving since the beginnings of cellular life. The adaptive branch evolved over an estimated 430 million years, inventing, as Koonin (2016) has put it, "certain molecular mechanisms [that] have evolved under specific selective pressure for increased evolvability." That is, the adaptive branch of the encoded immune system enables selection of antigen receptors that are diversified in a given individual. Nonetheless, that individual's solution to a specific infectious problem is not heritable. In contrast, evolution of microbes over a few hours or days leads to genomic changes that are not only heritable but also often horizontally transferrable within and between species.

So it happens that the encoded immune system routinely fails before old age, in the following sense: Before they have offspring who are independent, most members of the species will experience one or more infections that impair function to an extent that would materially increase the individual's risk of death if he or she were living in the presocietal wild, subject to resource scarcity, predation, and exposure. As impressive as the encoded immune system is, it is the only organ system that routinely leaves infants, children, and young adults at risk of death.

The human adopted immune system

Many species have evolved the ability to borrow or invent tools and to develop social structures that enable cooperative endeavors. Among them, only humans evolved the ability to transmit newly created knowledge to others at a distance in space and time. Collectively, these evolved skills have allowed humans to compensate for fundamental immunodeficiency better than any other species.

This compensation is only partial. Infectious diseases remain a leading cause of death of people before their reproductive age (World Health Organization, 2016). Nonetheless, the ability to deploy an adopted immune system to complement the encoded immune system has played a large role in the expansion of the human species and accounts for much of the striking increase in human life expectancy over the past century.

Adopted immunity came about because we found, invented, tested, rationalized, mass produced, and distributed four kinds of tools that collectively make up an adopted immune system: nutrition, including vitamins; sanitation, including shelter, potable water, hygiene, and disposal of waste; vaccines; and antimicrobial agents. These tools are interdependent. For example, use of vaccines diminishes the need for antimicrobial agents. Deficiency of vitamins impairs the effectiveness of vaccines. Disposal into the environment of wastes containing antimicrobial agents reduces their effectiveness by selecting for microbes resistant to them.

The following discussion focuses on antimicrobial agents as a fundamental aspect of adopted immunity to which immunologists could productively contribute.

Adopting immunity from other species

Vaccines mobilize innate and adaptive mechanisms encoded in the germline of the recipient in advance of their natural expression in the course of infection. Antimicrobial agents likewise mobilize innate and adaptive immune mechanisms independently of their natural expression but do so from the genomes of other life forms: bacteria, fungi, corals, and plants (Fig. 2).

Bacteria, like humans, use innate and adaptive immune mechanisms to protect against infection and to preserve fitness in their competition for resources. Innate immune mechanisms in bacteria include phage-blocking surface proteins, restriction enzymes, and death of an infected cell to prevent spread of infection. *CRISPR-Cas* systems provide many bacteria with adaptive immunity (Marraffini, 2015).

Like humans, bacteria have both cell-bound effectors of immunity and secreted, soluble effectors. As in animals, the soluble effectors include proteins (bacteriocins, such as colicins and microcins) and small, chemical compounds. Bacteria use these products to compete with and protect themselves from others and perhaps to send other intercellular messages (Davies and Ryan, 2012; Kommineni et al., 2015; Sassone-Corsi et al., 2016). Most of the antimicrobial agents deployed by humans consist in, are derived from, or are modeled on microbes' soluble immune effectors.

Some other synthetic antimicrobials do not mimic a bacterial effector structurally but instead mimic a bacterial, cell-bound immunity mechanism. For example, fluoroquinolone antibiotics bind and inhibit bacterial DNA gyrase; so do certain stable phage-encoded toxins whose inhibition by unstable, phage-encoded antitoxins forces a bacterium to retain the phage (Gupta et al., 2016). By mounting a so-called stringent response that reduces synthesis of most proteins, a given bacterium can refuse to propagate a phage by allowing itself to succumb to the toxin, forestalling the infection of others of its species. The process is mimicked in humans when infected cells commit apoptosis or flag themselves to invite lysis by T cells or NK cells.

Yet another form of antimicrobial agent is the commensal microbiota itself, whether acquired by intent—for example, to treat enteritis caused by overgrowth of *Clostridium difficile*—or acquired without intent, such as during vaginal delivery of the newborn, and whether acting to suppress growth of a pathogen, as in the first example, or to foster maturation of the encoded immune system, as in the second.

Unique features of antimicrobial agents among medicines

Over the past six generations, humans have found or invented several thousand medicines. Among them, the antimicrobial agents discovered over the past four generations are unique in two respects. First, until recently, antimicrobial agents were the only medicines that cured large numbers of the sick, and they remain the only medicines that do so routinely. Within the last two generations, some antineoplastic regimens have been curative, including some that are immunity-based, and corticosteroids sometimes cure temporal arteritis. Second, antimicrobial agents are the only medicines whose use hastens their loss of usefulness for people who have not yet taken them.

The first claim hinges on using "cure" in the true sense. Administration of an appropriately chosen antimicrobial agent has the routine capacity to restore an individual to the state of wellness that prevailed before the onset of an illness that would not otherwise have resolved, that would not otherwise have resolved as quickly, or whose unaided resolution would not restore the individual to their prior state of wellness. In contrast, when the administration of most other medicines stops, the individual returns to the state of illness that invited intervention, unless the illness had resolved spontaneously or from a change in contributory factors, such as diet. Some other medicines help prevent the onset of illness rather than treating it. Recently, some forms of antineoplastic chemotherapy and immunotherapy have also been curative in the strict sense.

The definition of "cure" given above is admittedly idealized. Clinical cure can be ambiguous. "Cure" does not return the patient to a previous state of health if tissue damage caused by the pathogen or the host's reaction to the pathogen is irreparable, as is often the case in successfully treated tuberculosis (TB). Finally, cures achieved with broad-spectrum antimicrobial agents often come at the cost of long-lasting perturbation of the microbiota, and in that sense, one of the host's genomes has not returned to its preexistent state. Nonetheless, within the bounds of these ambiguities and qualifications, antimicrobial agents stand out among medicines for their ability to cure large numbers of people routinely.

Unfortunately, the ability of antimicrobial agents to cure most patients for whom such drugs are appropriately prescribed is handicapped by a second unique feature of this class of medicines: their use eventually selects for resistance. The resistant pathogens are eventually shared among hosts, or the determinants of resistance are eventually shared among pathogens. Thus, we are all likely to need antimicrobial agents, yet the more a given agent is used, the nearer it comes to being useless.

To summarize, antimicrobial agents are among the most important achievements of medicine, an indispensable branch of adopted immunity, and both products and victims of collective behaviors.

Rising stakes: The growing reach and recognition of antimicrobial resistance (AMR)

Beginning with the use of penicillin in civilian populations in the mid-1940s, physicians, scientists, and much of the public quickly came to regard antimicrobial agents as both indispensable and invincible (Nathan, 2015). Beginning just

20 years later, taking antimicrobial agents for granted put us on a path to losing them.

Over the past few decades, a declining rate of success in discovering new antimicrobial agents discouraged much of the pharmaceutical industry from continuing the search (Payne et al., 2007). Meanwhile, levels of AMR continue to rise. These respectively falling and rising curves have crossed in recent years for one pathogen after another, in the sense that antimicrobial agents are now lacking to treat a significant proportion of formerly curable infections caused by nearly a dozen different bacterial species. As the remaining agents become less often useful, elective surgery and cancer chemotherapy may become prohibitively risky, trauma care ineffective, premature babies nonviable, and incidental wounds potentially lethal.

To imagine what it might be like to return ourselves to the preantibiotic era, consider the reaction to the introduction of penicillin for public use after World War II. Alexander Fleming "was showered with gifts of carnations. [P]eople whose lives had been saved by penicillin…now knelt before him to kiss his hands" (Brown, 2013). In 1964, the city of Madrid installed statues of Fleming and of a bullfighter saluting him outside the municipal bullring because antibiotics had so greatly reduced the lethality of matadors' wounds.

One of the first postwar effects of penicillin was the cure of gonorrhea with a single injection. Yet *Neisseria gonorrhoeae* is one of the bacterial pathogens some of whose clinical isolates are now resistant to most antibiotics. Others include *Enterococcus faecium*; *Staphylococcus aureus*; *Klebsiella pneumonia*; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; *Enterobacter* species; some *Salmonella* species, including invasive, nontyphoidal strains; some *Shigella* species; and *Mtb*. Leaving out the single most-prevalent instance of AMR—drug-resistant TB—it is estimated that drug-resistant bacterial pathogens now kill some 700,000 people a year, and if present trends continue, the toll will rise to 10 million deaths per year by 2050 (O'Neill, 2014). Authorities seem reluctant to factor drug-resistant TB into this tally, perhaps fearing that its unfamiliarity to the citizenry of economically advanced countries might blunt their concern. Nearly 500,000 people a year develop drug-resistant TB; >50% of them will die from it as matters now stand.

After decades of advocacy by scientists and physicians, beginning with Fleming himself in his Nobel Prize acceptance speech in 1945, acknowledgment of the gravity of AMR has finally come from leaders in business and government, as voiced by the World Health Organization, the World Economic Forum, the G20, and the G7. In 2015, President Obama issued a National Action Plan for Combating Antibiotic-Resistant Bacteria (White House, 2015). In May 2016, a panel commissioned by the British government issued cogent recommendations for coordinated global action (O'Neill, 2016). That same month, the Drugs for Neglected Diseases Initiative announced that it had raised over 2 million euros to fund a Global Antibiotic Research and Development Partnership (2016) initiative. In July 2016, the US National Institutes of Health (NIH), the US Department of Defense's Biomedical Advanced Research and Development Authority, the Wellcome Trust, the California Life Sciences Institute, the Massachusetts Biotechnology Council, and the AMR Centre in the UK announced that Kevin Outterson, a Boston University law professor interested in incentives to overcome AMR, will oversee the award of $350 million in grants via a consortium called the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X; Outterson et al., 2016; Bagley and Outterson, 2017). In September 2016, the NIH announced a $20 million Antimicrobial Resistance Diagnostic Challenge, and the government of China announced a national initiative to counter antimicrobial misuse and to find new antimicrobials (McLaughlin, 2016). Also in September 2016, the United Nations General Assembly declared AMR to be a risk to global health security, placing it alongside HIV/AIDS, noncommunicable diseases, and Ebola virus as only the fourth global health issue prioritized for discussion and action in the history of the General Assembly. The United Nations' 193 member nations agreed to develop an action plan (United Nations, 2016). In October 2016, the US Centers for Disease Control and Prevention announced the award of $14 million in grants to assess how antibiotics affect the microbiome, the consequences of AMR, and the implications for antibiotic husbandry (Centers for Disease Control and Prevention, 2016). Also in October 2016, the journal *Science* listed six major "science lessons for the next president," the first of which was that "pathogens change faster than our defenses…. And the new administration will have to find way to create incentives for drug companies to develop new antibiotics, which have little profit potential, to replace ineffective drugs" (Malakoff and Mervis, 2016). Sensing the coming change in momentum, between 2013 and the first quarter of 2016, investors plowed some $2.6 billion into 106 companies working on antimicrobial agents or infectious disease diagnostics (Mancini, 2016).

As funding swells, there is an opportunity for microbiologists and immunologists to join forces with medicinal chemists and other pharmaceutical scientists—the few who have remained engaged in developing antibiotics and the many who may now return to the task or join it anew. Such cooperation would reverse a historical trend, discussed next.

Historical disconnects between immunology and antimicrobial drug discovery

Once antimicrobial agents are seen as an element of the adopted human immune system based on the immune systems of other organisms, it seems striking that the academic fields of immunology and antimicrobial chemotherapy, which began a few decades apart, took separate paths. Those studying immunology and those developing antimicrobial agents have generally ignored each other. The faculties that teach those subjects, the students who study them, and their course materials, conferences, and journals barely overlap.

This mutual indifference is all the more regrettable when one considers the productive engagement of immunology with oncology and the parallels between the biology and treatment of cancers and infections. Cancers and infectious diseases both involve cells whose genomes differ from the encoded genome of the host and that invade, damage, and metastasize. Contagious infectious diseases metastasize not only within hosts but between them. In both cases, some of the disease-causing cells are replicating at the time of treatment and some are not. Mechanisms of resistance to chemotherapeutics are shared by pathogens and tumor cells, including the form of resistance displayed by nonreplicating cancer cells and nonreplicating bacteria. Many antibiotics have antitumor activity, and many antitumor agents have antimicrobial activity. The encoded immune mechanisms deployed against cancer are the same ones that evolved in competition with infectious agents. The occasionally curative checkpoint blockers do not kill malignant cells but enable the immune system to do so. Some antimicrobial agents could be designed with the same goal in mind (Bryk et al., 2008; Nathan et al., 2008).

Below, I discuss ways that immunologists and developers of antibiotics could benefit from closer interactions and shared ideas. Then, the discussion returns to the topic of AMR to drill down on the critical contribution of host immunity to a form of AMR called "phenotypic tolerance," in which host immunity antagonizes the action of anti-infectives that were developed without taking immunity into account.

Opportunities for collaboration between immunologists and drug developers to treat infectious disease and advance our understanding of immunity

Medicinal chemists commonly laud natural products, including antibiotics, as the handiwork of evolution, to be matched, if possible, by new drugs. We could profitably regard the immune system in the same light: an evolved source of wisdom in how to combat infectious disease. However, principles of host defense that have emerged in immunology have historically been disavowed in drug discovery, such that many of the core precepts of industrial antimicrobial drug development run directly counter to those of human immune mechanisms (Table 1). These precepts are held up as constraints on antimicrobial drug development, even though they have often been violated in practice to good effect. When dealing with infectious diseases that are both life-threatening and contagious, those precepts could be violated more often to confer on antimicrobial drug development more of the evolved effectiveness of immune mechanisms.

For example, the immune system benefits from combining different kinds of specificity and selectivity. The antimicrobial chemistries of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are narrow spectrum with respect to the types of atoms they modify and the contexts in which those atoms are vulnerable, but have a broad spectrum in the molecules they affect and the pathogens they control (Nathan, 2003; Nathan and Cunningham-Bussel, 2013). Such chemistries are mobilized, along with antibodies and cytolytic T cells, which, individually, are narrow spectrum in the molecules they recognize and the pathogens they control. Contrary to these precepts, conventional antibiotic development has simultaneously demanded high molecular selectivity and a broad microbiologic spectrum. This has markedly limited the number of targets and has produced drugs whose wide spectrum increases morbidity and whose wide use hastens resistance. Further, with a few recent exceptions, bacterial targets considered suitable for antibiotics have been confined to enzymes involved in the synthesis of nucleic acids, proteins, cell walls, and folate. This focus has excluded a wide variety of other molecular pathways in microbes that contribute to the morbidity, mortality, and contagiousness of infectious disease, pathways that the immune system targets routinely (Nathan, 2011).

The immune system's targets often include host molecules. The body typically copes well with bystander damage during an infection and repairs it (Nathan and Ding, 2010). In contrast, antibiotic candidates are often rejected if their target has a human homologue, despite the success of antibiotics that violate this rule by taking advantage of opportunities to achieve a high degree of species selectivity. For example, many antibiotics inhibit bacterial ribosomes and spare mammalian cytosolic ribosomes. Moreover, some bacterial ribosome inhibitors, such as linezolid, do have toxicity related to inhibition of a host homologue—in this case, mito-

Table 1. **Contrast between principles of encoded immunity and conventional precepts of antimicrobial drug development**

Issue	Principle of encoded immunity	Pharmaceutical precept constraining development of antimicrobial agents as an element of adopted immunity
Target essentiality	Targets essential to the pathogen under conditions pertaining in the host	Targets essential under standard growth conditions in the laboratory
Target selectivity	Multiple targets of ROS, RNS, CO, AMPs	Single target
Target conservation	Targets often present in host as well as in pathogen	Target absent in host
Suitable target classes	Many: synthesis of nucleic acids, proteins, cell walls, or folate, but also induction, synthesis, secretion, or action of virulence factors; energy generation; ion gradients; transport; signaling; processing; repair; degradation; sequestration	Few: Synthesis of nucleic acids, proteins, cell walls, or folate
Microbial species specificity	Combination of broad spectrum and narrow spectrum	Broad spectrum
Host toxicity	Inescapable; almost always manageable and reparable	Forbidden when predictable
Genotoxicity	Inescapable; very rarely consequential	Forbidden

chondrial ribosomes—but have saved many tens of thousands of lives (Leach et al., 2011).

Of course, it is preferable for a drug to be utterly nontoxic, but insistence on nontoxicity, even for agents used in the treatment of contagious, life-threatening, infectious diseases can make "the perfect, the enemy of the good." For example, genotoxicity is accepted in cancer chemotherapy and can arise from host immune responses. In contrast, genotoxicity is forbidden in antibiotic candidates, without distinction among the infectious diseases that a given antibiotic might treat. Some infectious diseases have a mortality rate comparable to that of malignancies, and unlike cancers, pose a risk of morbidity and mortality to other people besides those being treated. The risk–benefit ratio would be markedly reduced if it took into account the otherwise secondary hosts who benefit from treatment of a primary host and face no risk from treatment.

In addition to recommending changes in the criteria used for evaluating candidate antimicrobial agents for contagious, life-threatening infections, immunologists could help drug developers both make (Cohen et al., 2016) and deliver (Lehar et al., 2015) antimicrobial agents. For example, monoclonal antibodies against virulence factors can help control infection (Cohen et al., 2016). Targeting a virulence factor is unlikely to select for resistance because its inhibition does not impair bacterial viability. Knowledge that *S. aureus* is not solely an extracellular pathogen led to recognition that the bacteria in host cell phagolysosomes can hide from antibiotics that fail to enter that compartment. This led, in turn, to development of a monoclonal antibody conjugate that allowed delivery of an antibiotic to phagolysosomes. The conjugate outperformed vancomycin in the treatment of staphylococcal bacteremia in mice (Lehar et al., 2015).

Knowledge of the immune system can contribute to antimicrobial chemotherapy not only by suggesting what to target in bacteria and how to target them but also by nominating targets in the host. Just as extensive efforts are underway to find synergistic combinations of immuno-oncologic therapy with cancer chemotherapy, more effort is needed to explore host-directed therapy of infectious disease as an adjunct to the use of direct-acting antimicrobial agents (Mayer-Barber et al., 2014). Understanding of host–pathogen interactions offers additional opportunities for adjunctive therapy of infectious diseases aimed at reducing immunopathology (Ayres and Schneider, 2012).

Finally, compounds with direct antimicrobial activity could help immunologists discover pathways that the immune system targets and mechanisms by which it does so. For example, studies of the antimicrobial activity of itaconic acid led to the discovery that IFN-γ–induced interferon-regulated gene 1 (IRG1) is a host cis-aconitate decarboxylase that produces itaconic acid and that itaconic acid inhibits bacterial isocitrate lyases (Michelucci et al., 2013). *Mtb* needs its isocitrate lyases to survive in macrophages and mice (McKinney et al., 2000) and to defend itself against rifampin, streptomycin, and isoniazid (Nandakumar et al., 2014). Some bacteria encode enzymes that degrade itaconic acid (Sasikaran et al., 2014). Agents that inhibit the homologous enzymes in *Mtb* might help the host recover from TB, even though such a target would not meet the standard antibiotic developer's criterion of essentiality under standard laboratory conditions. Thus, a chemical biology inquiry that began with an antimicrobial compound led to identification of a new host immune chemistry, new ideas about how the IFN-γ–dependent host immune response may attack *Mtb*, new hypotheses about how host immunity may synergize with conventional antimicrobial agents, and a new potential drug target.

Antagonism between immunity and antimicrobial agents: Further opportunities for intervention

The foregoing example of potential synergy—between the product of an IFN-γ–induced gene on the one hand and antimicrobial agents on the other—illustrates a reasonable presumption underlying the general indifference for each other's work on the part of those studying immunity and those developing antimicrobial agents. Because a primary function of the encoded immune system is to protect the host from infection and the purpose of administering antimicrobial agents is the same, then encoded and adopted immunity can be expected to exert additive or synergistic effects, and no special effort should be necessary to take advantage of their common actions.

Indeed, it is sometimes difficult to cure an infection with antibiotics in someone whose encoded immune system is dysfunctional. For example, most patients with nontuberculous mycobacterial infections who are discovered to have autoantibodies that neutralize IFN-γ fail to clear the pathogen in response to treatment with antimicrobial agents (Lin et al., 2016). Besides cis-aconitate decarboxylase, another IFN-γ–induced gene is inducible nitric oxide synthase (iNOS; Xie et al., 1992). TB can be cured in most mice with isoniazid and pyrazinamide (McCune et al., 1966), but the apparent cure is quickly followed by relapse if the mice are deficient in iNOS (Nathan, 2009). Such observations indicate that antimicrobial agents not only synergize with host immunity but can depend on host immunity to effect a clinical cure.

At the same time, immune mechanisms often act at cross-purpose with antimicrobial agents. When antibiotics are selected for their ability to kill replicating bacteria, as is almost always the case, they usually work best—or only—against replicating bacteria. When encoded immunity serves to halt the replication of some infecting bacteria but fails to kill all of them, as is often the case at the time that an infection manifests as clinically apparent disease, then encoded immunity can antagonize the antibiotics of adopted immunity. Such antagonism has been demonstrated in axenic culture (Gold et al., 2012), in cultured macrophages (Helaine et al., 2014), in rabbits (Tuomanen, 1986), and in mice (Liu et al., 2016).

In fact, some of the foregoing examples underscore that the same antibiotic and the same element of host immunity

can work both for and against each other in the same disease. As noted, the apparent clinical cure of TB in mice with isoniazid and pyrazinamide was sustained in most WT mice (McCune et al., 1966) but was rapidly followed by relapse in all mice that lacked iNOS (Nathan, 2009). However, the action of isoniazid in *Mtb*-infected mice was partially impaired by iNOS (Liu et al., 2016) because products of iNOS block replication of *Mtb* and, at least in vitro, isoniazid only kills *Mtb* when the bacteria are replicating. There may be diverse mechanisms for such antagonisms. For example, RNS target cytochromes involved in electron transport; the reduction in energy generation can block uptake of aminoglycoside antibiotics (Zemke et al., 2015). Bacteria themselves can generate RNS that induce their own antioxidant defenses, covalently modify antibiotics, and confer resistance (Gusarov et al., 2009). Host-derived RNS may do the same.

Similar to the generation of RNS, generation of ROS is a major element of host immunity against infection. Genetic deficiency in the primary ROS-generating enzyme of phagocytes, NADPH oxidase 2 (NOX2), predisposes a host to life-threatening bacterial and fungal infections (The International Chronic Granulomatous Disease Cooperative Study Group, 1991), including by *S. aureus*. However, the autotoxicity of NOX2-derived ROS for host myeloid cells can impair the ability of antibiotics to cure *S. aureus* pneumonia (Sun et al., 2016).

When immunity adversely affects the action of antimicrobial agents, it creates a form of AMR. The more we understand about the mutual antagonism between antimicrobial chemotherapy and partially effective host immunity, the more opportunity we have to identify drug targets in the bacterial pathogen whose inhibition may convert a noncurative response to chemotherapy into a cure (Nathan et al., 2008; Nathan, 2012).

A fuller appreciation of the opportunity for immunologists to contribute to solving this problem will be aided by a deeper discussion of AMR, addressed next.

AMR as a scientific challenge; TB as a case in point

There is now a cross-sector consensus that to preserve antibiotics as a mainstay of human medicine will require overcoming four kinds of obstacles—scientific, regulatory, economic, and political (Nathan, 2004, 2012, 2015; Nathan and Cars, 2014). Among the several scientific challenges confronting the development of new antimicrobial agents (Nathan, 2011), one stands out as most needful of fresh thinking: the nature of AMR itself. The problem straddles microbiology and immunology, both encoded and adopted.

The discussion that follows deals only with bacterial infections and antibacterial agents, now generally called "antibiotics" without regard to whether they are of microbial origin, as the term was originally used. This focus is for purposes of illustration; it is not meant to discount the urgency of developing antimicrobial agents for viral, fungal, protist, and helminthic infections.

Mtb serves as a further focus, for the following reasons (Nathan, 2009). That these four points are all true reveals serious shortcomings in existing approaches to antibiotic development and use: (1) *Mtb* is now the single leading cause of death from infectious disease; (2) despite causing a curable infection; (3) one that is now becoming progressively incurable because of AMR (among potentially lethal bacterial pathogens displaying AMR, *Mtb* is estimated to account for the most cases, even though most cases of drug-resistant TB go undiagnosed, given that drug sensitivity testing is lacking in many endemic areas; the fate of people whose TB displayed extensive AMR was recently monitored: 5% were cured; 73% died; 10% failed all efforts to treat them and were discharged into the community in a contagious state; Pietersen et al., 2014; Coscolla et al., 2015); and (4) even in its drug-sensitive form, TB takes longer to cure than almost any other bacterial infection.

That an immunologic perspective might help derives from four additional points: (1) *Mtb* has no known naturally transmitting host, except humans. (2) As noted earlier, for its transmission, *Mtb* needs a live human whose immune response is vigorous enough to liquefy infected lung and erode into an airway. (This dependency probably accounts for the striking finding that the sequences most highly conserved among 1,226 clinical isolates of *Mtb* were those encoding human T cell epitopes; Coscolla et al., 2015.) (3) Untreated, the active disease has a fatality rate of 50% or more. (4) Nonetheless, after an estimated 70,000 yr of parasitism, neither species—*Mtb* nor human—has eliminated the other.

From these considerations, we can reach four conclusions: *Mtb* has evolved the ability to (1) incite, (2) titrate (Marakalala et al., 2016), (3) survive, and (4) exploit the human immune response.

To the degree that immunologists understand the host–pathogen relationship in TB, they should be able to contribute to devising an adopted immune response to *Mtb*. There are likely to be similar opportunities for immunologists studying other infectious diseases whose treatment is handicapped by rising AMR.

Heritable AMR

The best understood form of AMR is heritable. There are bacterial genes that encode resistance to antibiotics that were not invented or deployed at the time that the bacteria acquired those genes (Bhullar et al., 2012), and it is usually possible to isolate bacteria that have become heritably resistant to any new antibiotic as soon as there is enough of the antibiotic on hand to conduct a selection (Kling et al., 2015). Apparent exceptions (de Carvalho et al., 2009; Ling et al., 2015; Moreira et al., 2016) are likely to involve compounds with multiple targets or no specific target. Only a few such agents are sufficiently selective to be clinically useful. In general, the issue with heritable AMR is not whether, but when, the medical deployment of a given antibiotic will select for the emergence of heritable resistance in clinical settings.

Although the correct use of antibiotics will usually lead in time to heritable AMR, other forms of use hasten its emergence: misuse, overuse, and underuse.

Misuse is exemplified by feeding more than one half of the United States' antibiotic tonnage to healthy food animals and plants to accelerate their growth; the proportion is thought to be higher in China (Van Boeckel et al., 2015). Another form of misuse is the routine failure to account for individual variation in drug levels attained with standard dosing, although it is possible to conduct therapeutic drug monitoring with finger-prick blood tests (Alsultan and Peloquin, 2014). Without dose adjustment, peak rifampin levels in the blood can vary by nearly two orders of magnitude in people treated for TB (Wilkins et al., 2008), with some 40–70% being undertreated (Um et al., 2007). Undertreatment fosters the emergence of resistance.

Overuse results from lack of rapid, point-of-care diagnostics. An estimated 30% of antibiotic prescriptions in the United States are written for the wrong indication, typically a viral infection (Fleming-Dutra et al., 2016). Overuse is also fostered in settings in which the prescribers are the purveyors or the consumers, that is, where doctors sell the drugs or patients purchase them without recourse to doctors.

Underuse is a problem when the drugs are diluted by inexpert manufacture or fraudulent intent or when patients discontinue them prematurely because they feel better, feel worse, or cannot afford to buy more of them.

Mechanisms of heritable AMR are still being discovered. They include mutation or posttranslational modification of the target, so that it continues to support the viability of the organism but no longer binds the antibiotic; increased expression of the target, so that it titrates the antibiotic; expression of a pathway that compensates for the impairment caused by the antibiotic; inactivation of the antibiotic (Warrier et al., 2016); decreased activation of a prodrug form of the antibiotic; and decreased uptake or increased export of the antibiotic.

Discovery of mechanisms of AMR has profoundly affected both basic science and clinical care. In basic science, studies of heritable AMR had a prominent role in introducing the concept that small chemical compounds can have specific macromolecular targets in biological systems and can serve as tools to identify the targets' functions (Gold and Nathan, 2017). Clinically, mechanistic understanding of heritable AMR allowed the design of combination chemotherapy with agents that thwart resistance. For example, the World Health Organization's list of essential medicines includes the combination of amoxicillin, a β-lactam, with clavulanate, an inhibitor of some bacterial β-lactamases. Moreover, mechanistic understanding of heritable AMR allows combination chemotherapy with agents to which bacteria manifest resistance by different mechanisms. Combination chemotherapy was introduced to the practice of medicine in the 1950s with the discovery that there was no other way to avoid routine emergence of resistance in the treatment of TB (Fox and Sutherland, 1956). The practice was later adopted for the treatment of cancer and HIV/AIDS.

Nonheritable AMR: phenotypic tolerance and its subtypes

In contrast to the situation with heritable AMR, we have a very limited understanding of nonheritable AMR, also called "phenotypic tolerance," a term introduced by Tuomanen (1986). Phenotypic tolerance can be defined as conditional drug resistance that is not attributable to changes in the nucleic acid sequence of the pathogen's genome. Phenotypic tolerance gives rise to bacterial persistence: survival of bacteria during treatment of a host with a drug to which the same strain of pathogen is sensitive under standard laboratory conditions at concentrations achieved in the host. Phenotypic tolerance predisposes bacteria to the emergence of mutants with heritable resistance (Levin-Reisman et al., 2017).

The first two studies of phenotypic tolerance hold such important lessons for today that they deserve detailed discussion. The purification of penicillin was reported in 1942 (Abraham and Chain, 1942). That same year, Gladys Hobby and her colleagues reported that, at 37°C, ∼1 *Streptococcus* bacterium remained viable after 48 h of exposure to penicillin for every 10^6 bacteria present in the control culture at the end of that period. The authors did not comment on that but drew attention to the survival of nearly all the penicillin-treated streptococci if the exposure took place at 4°C, conditions in which there was no increase in bacterial number in the untreated control culture. The authors concluded, "It is apparent that penicillin is capable of destroying bacteria only if multiplication takes place" (Hobby et al., 1942).

Joseph Bigger (1944) repeated and extended the experiments using staphylococci. He introduced the term "persisters" to stress the observation that ∼1 in 10^6 staphylococci survived the treatment of logarithmically replicating cultures at body temperature. He inferred that persisters to penicillin must be "cocci…[which] happen to be, when exposed to it, in a phase in which they are insusceptible to its action," because "[i]f persisters had an abnormally high resistance, either natural [i.e., heritable and existing before the experiment] or acquired [i.e., heritable but acquired during the experiment], it is probable that their descendants would also possess abnormally high resistance. The descendants of several persisters which had survived contact with 1 unit per c.c. penicillin for 3–5 days were found to be killed by 1/8 unit per c.c. within 46 hours and to have no greater tendency than normal forms to produce persisters" (Bigger, 1944).

Bigger went on to confirm the observation of Hobby et al. (1942) that cooling the bacteria elevated the frequency of persisters to nearly 100%, that is, by six orders of magnitude. He demonstrated the same effect by acidifying the medium or lowering its tonicity. He concluded that persisters are cocci that "are believed to survive contact with penicillin because they are [in a] dormant (non-dividing)" phase (Bigger, 1944).

In fact, within 2 years of the publication of the study on penicillin, the two groups mentioned above, working on two continents with two different pathogens, had each observed two different classes of phenotypic tolerance, but without distinguishing them. It took another 70 yr before the distinction

was made, driven by the recognition that the two classes have different implications for drug discovery (Nathan, 2012).

Class I phenotypic tolerance can be viewed as a form of bacterial bet-hedging manifested by a few members of a population in conditions permissive for growth. The upper limit of the size of the minority population that can display class I phenotypic tolerance is set by the precision of the assay used to determine the minimum inhibitory concentration (MIC) of the antibiotic. If the MIC is defined as the concentration that inhibits growth by 90%, then 10% of the population could be phenotypically tolerant without changing the population's MIC. Typically, in a WT population, the frequency of class I phenotypic tolerance is ∼1 in 10^6. Certain mutations can increase the frequency of class I phenotypic tolerance by orders of magnitude without changing the MIC and without conferring heritable AMR. The phenotypically tolerant few may be nonreplicating at the time, as Hobby et al. (1942) and Bigger (1944) inferred, and others then assumed and asserted, or it may be replicating, as documented in later studies. The key feature is that a population of class I persisters, once expanded in the absence of the antibiotic, succumbs in the same proportion to the same concentration of antibiotic as did the population from which the persisters were recovered.

Heritable AMR can emerge more readily after antibiotics select for a mutation that increases the frequency of class I phenotypically tolerant bacteria in the population. Such mutations can arise in diverse genes, including those encoding antitoxins or enzymes that catalyze metabolic processes (Levin-Reisman et al., 2017). Mutations that augment class I phenotypic tolerance increase the proportion of bacteria that survive one exposure to antibiotic, providing a larger population in which mutants may arise that confer heritable resistance to a subsequent exposure (Levin-Reisman et al., 2017).

In contrast, class II phenotypic tolerance is a bacterial response to exogenous stress, including nonsterilizing immunity. It is imposed by conditions that impair growth and pertains to all of the bacteria whose growth is impaired, which may be most or all of the bacterial population at a given site at the time that chemotherapy is administered. Conditions that impair growth can be imposed by the host environment, by host immune chemistries, or by exposure to sublethal levels of other antibiotics (Table 2). The stresses that lead to class II phenotypic tolerance can foster the emergence of heritable AMR by increasing the frequency of mutation (Boshoff et al., 2003; Kohanski et al., 2010).

A particularly challenging form of class II phenotypic tolerance is displayed by bacteria whose nonreplicative state is not reversed by plating them on a rich medium rendered semisolid with agar. That is, they are not CFUs, yet their viability is demonstrable by some other means, such as growth after limiting dilution in liquid culture or injection into an experimental host. More than 80 bacterial species have been shown to have the property of becoming what Rita Colwell and colleagues originally called "viable but non-culturable" (Xu et al., 1982). Strikingly, in two studies to date, most of the *Mtb* in the sputum of most treatment-naive patients with TB were unable to replicate as CFUs and were detected instead by limiting dilution (Mukamolova et al., 2010; Chengalroyen et al., 2016; Dartois et al., 2016).

To the extent that individual bacteria in an otherwise antibiotic-sensitive population manifest class I phenotypic tolerance to different antibiotics by various mechanisms, then those that are phenotypically tolerant to one antibiotic are likely to be susceptible to another. In such a case, to kill the whole population, it should suffice to combine antibiotics in such a way that no one bacterium is phenotypically tolerant to all of them, provided that each of the drugs in the combination reaches the bacteria in adequate concentrations at the same time. In contrast, if nearly all the bacteria in a population are phenotypically tolerant to several different antibiotics, then each individual bacterium must be tolerant to each of them, and combinations of those antibiotics are unlikely to be effective. Instead, it will be necessary to discover antibiotics that can kill nonreplicating bacteria.

The foregoing theses constitute a practical imperative for distinguishing classes of phenotypic tolerance (Table 2). Other classifications of nonheritable AMR are also useful, for example, to frame mechanistic questions (Brauner et al., 2016). A caveat of all classifications based on in vitro observations is that the relationship is complex and variable between the

Table 2. **Classes of phenotypic tolerance and their therapeutic implications**

Feature	Class I	Class II
Growth state of bacterial population	Most cells replicating	Most cells not replicating
Persistence phenotype	Small minority; different cells tolerate different antibiotics	Large majority; same cells tolerate many antibiotics
Inducers of persistence	Unknown; stochastic	Acidification, ROS, RNS, hypoxia, deprivation of C, N, P, or Fe; sublethal exposure to antibiotics
Speculative mechanisms	Epigenetic, transcriptional, translational, or posttranslational expression or suppression of any process for which genetic change can produce heritable resistance	Decreased uptake, increased export, or increased catabolism of drug; metabolic stress leading to oxidative stress and adaptation; increase in proteostasis pathways; preferential transcription and translation; alternate respiratory pathways and electron acceptors
Therapeutic implications	Combine different drugs that each reach the sites of infection	Include new kinds of drugs active on nonreplicating cells that reach the sites of infection

Based on Nathan (2012) and modified from Nathan and Barry (2015).

MIC measured in low-protein, host cell–free medium over short periods and the dosing regimens of antibiotics required for clinical cure (Frimodt-Møller, 2002; Mueller et al., 2004).

Mechanisms of class I phenotypic tolerance

Class I phenotypic tolerance can theoretically arise by any mechanism that confers heritable AMR, from epigenetic regulation to posttranslational modification, as long as the mechanism does not depend on a change in the pathogen's coding sequence. As noted earlier, the size of the tolerant subpopulation may be affected by a change in coding sequence, as long as the tolerant subpopulation remains such a minority that the overall population does not manifest an increase in the antibiotic's MIC.

Much of the research in this field has wrestled with a descriptive question, whether class I phenotypic tolerance is as tightly linked with nonreplication as Hobby et al. (1942) and Bigger (1944) inferred. In short, the answer is "no."

The first study to use time-lapse photomicroscopy of bacteria in microfluidic chambers to study phenotypic tolerance at the single-cell level (Balaban et al., 2004) revealed that, in an otherwise replicating population of *Escherichia coli*, most of the few cells that survived ampicillin were nonreplicating at the time of exposure to the drug. However, some of the other surviving *E. coli* had been replicating. This study was rendered feasible by using *E. coli* with compound mutations in *hipA*, which raised the frequency of the class I phenotypically tolerant *E. coli* by several orders of magnitude without changing the MIC of the overall population.

Nine years later, a study of similar design reached a different conclusion when Wakamoto et al. (2013) studied the action of isoniazid on *Mtb*. Isoniazid is a prodrug whose activation depends on the *Mtb* catalase–peroxidase. The investigators showed that stochastic extinction of catalase–peroxidase expression conferred resistance to isoniazid. Growth rate had nothing to do with it (Wakamoto et al., 2013).

The same year, Orman and Brynildsen (2013) showed that *E. coli* persisters to ampicillin and fluoroquinolones are enriched among the nonreplicating subpopulation but are not confined to it or highly prevalent in it. Natural clinical and veterinary isolates of *E. coli* each showed the same MICs to a given antibiotic, yet each showed different levels of persistence to different sets of antibiotics (Stewart and Rozen, 2012). This suggested that different individual cells were phenotypically tolerant to different antibiotics, meaning that nonreplication of a given cell could not be a universal explanation for phenotypic tolerance.

Working with *Mtb*, Su et al. (2016) discovered a growth rate–independent form of class I phenotypic tolerance to rifampin and defined its molecular mechanism. Individual *Mtb* cells mistranslate different proportions of individual copies of rifampin's target, RNA polymerase subunit B. The basis of the mistranslation is the propensity of *Mtb*'s glutaminyl transfer RNA (tRNA) synthetase to charge tRNA not only with glutamine but also with glutamate and the ability of *Mtb*'s asparaginyl tRNA synthetase to charge tRNA not only with asparagine but also with aspartate. The errors are corrected by a glutamine amidotransferase, but not perfectly. If a given cell's collection of RNA polymerase subunit B molecules includes sufficient copies in which Asn170 has been replaced with Asp, the cell can survive a dose of rifampin that kills genetically identical siblings. Heritable mutations in the gene encoding a subunit of the amidotransferase increased the frequency of class I phenotypically tolerant *Mtb* in a population, but, similar to *hipA* mutations in *E. coli* discussed at the beginning of this section, they did not allow the persisters, when grown up without antibiotic, to display a higher MIC than the population from which they were recovered (Su et al., 2016).

Some researchers view class I phenotypic tolerance as an outcome of noise: random variation arising from imperfect execution of one or more processes. In contrast, the high value of class I phenotypic tolerance for survival of a replicating population in the face of emergent stress and its susceptibility to genetic regulation argue for the existence of specific, evolved mechanisms. Both views are likely to be correct.

Mechanisms of class II phenotypic tolerance

One of the most important challenges for antibiotic research and, therefore, for adopted immunity, is to understand mechanisms of class II phenotypic tolerance, a state for which incompletely effective immunity bears much of the responsibility.

We have a long way to go. We do not know whether a given bacterial species that enters a nonreplicating state in response to different host conditions manifests class II phenotypic tolerance to the same antibiotic by different mechanisms or whether a given bacterial species that enters a nonreplicating state in response to the same host conditions manifests class II phenotypic tolerance to different antibiotics by different mechanisms.

Consistent with the reasoning that Bigger (1944) advanced three quarters of a century ago, some scientists today argue that nonreplicating bacteria are phenotypically tolerant to inhibitors of biosynthetic processes because they are "dormant," where dormancy is inferred from the cells' survival of exposure to inhibitors of biosynthetic processes. For example, it was recently stated said that "Tolerance is a property of dormant, nongrowing bacterial cells in which antibiotic targets are inactive, allowing bacteria to survive" (Lewis and Shan, 2017).

Such reasoning is circular. Although class II phenotypic tolerance is associated with nonreplication, by definition, nonreplication does not constitute a mechanistic explanation of class II phenotypic tolerance. In fact, nonreplication offers bacteria no blanket reprieve from the need for biosynthetic processes, such as generation of energy to maintain membrane potential. Generation of energy requires the action of enzymes. Stresses associated with imposition of nonreplication cause damage to macromolecules. Sometimes, such damage is reparable; most repair requires energy. Some damage is irreparable. Replacement of irreparably damaged molecules requires synthesis, which, again, requires energy and usually

requires transcription as well. Indeed, nonreplicating *Mtb* maintains its membrane potential (de Carvalho et al., 2011; Venugopal et al., 2011; Darby et al., 2013) and a large, altered transcriptome (Schnappinger et al., 2003;Voskuil et al., 2003).

In short, nonreplication is a state associated with class II phenotypic tolerance but is not a mechanism accounting for it. Only recently have underlying mechanisms begun to come into focus. Nonreplicating states can lead to reduced antibiotic uptake (Sarathy et al., 2013) or reduced retention (Adams et al., 2011) and, perhaps, to altered drug catabolism. Stress can lead to up-regulation of antioxidant pathways. To the extent that antibiotic action is augmented by generation of ROS secondary to disordered metabolism (Kohanski et al., 2007), the increase in antioxidant defenses may contribute to phenotypic tolerance (Nandakumar et al., 2014), and the induction of proteostasis pathways for macromolecular preservation and repair may contribute as well. Nonreplicating bacteria may switch to alternate respiratory pathways and use alternate electron acceptors. During nonreplication, an essential process may occur so slowly that its corruption by the antibiotic only leads to death after the conventional period of observation. Condition-dependent changes in gene essentiality may lead to prioritization of the transcription and translation of newly essential genes in the face of partial inhibition of overall transcription or translation.

It is a separate question how stresses suppress replication. Some stresses limit the supply of exogenous precursors for an increase in biomass. Many stresses activate the stringent response, leading to inactivation of antitoxins in toxin–antitoxin modules, of which *Mtb* has >80 (Harms et al., 2016). The activated toxins can cleave specific tRNAs, mRNAs, or ribosomal RNAs; phosphorylate and inhibit specific tRNA synthetases; interfere with DNA gyrase; ADP-ribosylate DNA (Jankevicius et al., 2016); and reduce the proton motive force (Harms et al., 2016). Although it is clear how these actions could suppress replication, as noted above, suppression of replication does not, by itself, constitute an explanation for phenotypic tolerance.

Is it possible to find new antibiotics that can kill bacteria displaying class II phenotypic tolerance to existing antibiotics?

TB illustrates the importance of answering this question. A central hypothesis is that class II phenotypic tolerance to existing TB drugs is a major contributor to the failure of those drugs to reduce the time it takes to cure TB to less than 6 mo for >86% of individuals with drug-sensitive disease. If most of the *Mtb* at a given site in the host are nonreplicating because of conditions they encounter at that site, such as hypoxia, nutritional restriction, acidity, or ROS or RNS, and in association with those conditions, are phenotypically tolerant to every antibiotic that reaches the site, then chemotherapy that combines those drugs is not likely to be effective.

The following considerations illustrate one way that immunologic thinking can suggest new targets for unconventional antibiotics against *Mtb* to complement the action of conventional antibiotics.

Mechanisms by which *Mtb* survives host immunity can be understood in terms of successive lines of resistance. First, *Mtb* can suppress host immunity (e.g., Rath et al., 2013). Failing that, or in addition to that, *Mtb* can detoxify host effector molecules (e.g., Bryk et al., 2000, 2002, 2008;Venugopal et al., 2011; Maksymiuk et al., 2015). Next, the pathogen can adapt to effector molecules whose production it failed to block and whose level it failed to reduce (e.g., Vandal et al., 2008). If macromolecules are nonetheless damaged, the bacteria can repair them (e.g., Darwin and Nathan, 2005). If repair is inadequate, the bacteria can degrade damaged macromolecules to avoid their toxic gain of function (e.g., Darwin et al., 2003; Lin et al., 2009). Some macromolecules are too damaged to be repaired, such as irreversibly oxidized proteins that cannot be unfolded for degradation by chambered proteases. These can be sequestered (Vaubourgeix et al., 2015). If all else fails, some bacteria can survive long periods without replicating, awaiting the return of conditions in which replication can be sustained. In many cases, enzymes have been identified that mediate these microbial defenses, and compounds have been identified that inhibit these enzymes (Bryk et al., 2008, 2010, 2013; Lin et al., 2009, 2013). Where human homologues exist, it has been possible to identify *Mtb*-selective inhibitors that spare the corresponding human enzymes (Bryk et al., 2008, 2010, 2013; Lin et al., 2009, 2013).

Almost all antibiotics selected based on their ability to kill replicating bacteria have been found to be much less effective, or actually ineffective, against the same organisms when they are nonreplicating. Although rifampin, fluoroquinolones, and bedaquiline are active against nonreplicating *Mtb* in vitro, much of that effect appears to be an artifact of the carryover of antibiotic from the nonreplicating stage of the assay to the stage of the assay in which recovery is assessed under conditions that support replication (Gold et al., 2015). Rifampin has genuine bactericidal action on nonreplicating *Mtb* in vitro but at far greater concentrations than needed to kill replicating *Mtb*, and even then, the maximum extent of killing in vitro is far less (Gold et al., 2015). This is not meant to disparage the proven clinical utility of those drugs but, rather, to suggest that they do not represent an ideal solution to the problem of class II phenotypic tolerance.

Fortunately, compounds can be found that extensively kill bacteria in a state that confers class II phenotypic tolerance to conventional antibiotics. An early example was a thioxothiazolidine that killed *Mtb* only when the *Mtb* was nonreplicating, without regard to diverse conditions tested that imposed nonreplication (Bryk et al., 2008). Another target-based screen led to two chemically distinct classes of *Mtb*-selective proteasome inhibitors (Lin et al., 2009, 2013) that killed *Mtb* that was rendered nonreplicating by nitrosative stress (Lin et al., 2009, 2013) or by starvation (Russo et al., 2015). A whole-cell screen designed to identify compounds that kill nonreplicating *Mtb* identified oxyphenbuta-

zone (Gold et al., 2012) and other compounds (Warrier et al., 2015). Subsequently, >100 compounds have been reported to kill nonreplicating *Mtb* selectively, including novel cephalosporins (Gold et al., 2016). However, in only a few cases did the investigators exclude the possibility that carryover of compound into the replicative phase of the assay may have led to a false impression of activity in the preceding, nonreplicative phase of the assay (Gold and Nathan, 2017).

Why are some compounds only able to kill nonreplicating bacteria, sparing the same cells when they replicate? Barring compound modification under one of the two sets of assay conditions, and assuming equivalent uptake under both, the question becomes why some targets are nonessential under conditions that support replication but essential under conditions that do not. For example, at least four sets of *Mtb* enzymes involved in central carbon metabolism—hydroxyoxoadipate synthase, dihydrolipoamide acyltransferase, lipoamide dehydrogenase, and the isocitrate lyases—are dispensable for survival under nonstressed conditions but become essential for *Mtb* to withstand oxidative or nitrosative stresses that impose nonreplication (Bryk et al., 2008; Venugopal et al., 2011; Nandakumar et al., 2014; Maksymiuk et al., 2015). This invites the speculation that some pathways that would afford redundancy in a critical function targeted by the antibiotic are inactivated under nonreplicative conditions or that a singular, essential pathway incompletely inhibited by the antibiotic is further inhibited by the nonreplicative conditions.

Even more encouraging are antibiotics that can kill bacteria extensively, not only when they are replicating but also when they are not replicating and are phenotypically tolerant to other antibiotics. With respect to TB, this has been reported with 8-hydoxyquinolines (Darby and Nathan, 2010; Shah et al., 2016) and nitazoxanide, an antibiotic approved for other indications (de Carvalho et al., 2009). In vitro, the nitroimidazole PA-824 (pretomanid) kills both replicating and nonreplicating *Mtb* to comparable extents and at comparable concentrations (Singh et al., 2008; Gold and Nathan, 2017). Under nonreplicating conditions, the mechanism involves generation of RNS (Singh et al., 2008), a striking example of a synthetic antibiotic mimicking host immunity (Nathan, 2008).

Conclusion

Better understanding of the encoded immune system's targets and chemistries can help guide the development of new antibiotics for adopted immunity. Reciprocally, learning how more of the antimicrobial agents work could generate hypotheses regarding bacterial targets that the encoded human immune system attacks and the chemistry it uses to do so. One of the pioneers in antibiotic discovery, H. Boyd Woodruff, died on January 19, 2017. In an autobiographic essay, he made the same point in broader terms: "…I realized the unity of biology and chemistry, that each biological observation has an underlying chemical cause, that in unraveling the latter, one could understand the other" (Woodruff, 1981).

In his Kunkel Lecture of 2016, Michel Nussenzweig illustrated the progress that follows when academic immunobiologists join forces with other vaccine scientists, including those with industrial experience and resources (Escolano et al., 2017). As summarized in Table 3, I have tried to make the case that there is also good reason for academic immunobiologists to join forces with scientists who develop antimicrobial agents, including those in industry—adopted immunity depends on it, and with it, the opportunity to correct fundamental immunodeficiency.

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Table 3. **Examples of opportunities for collaboration between immunobiologists and developers of antimicrobial agents**

Identify chemistries and molecular targets of host immunity: use this information when developing drugs that target the pathogen	Identify mechanisms of host–pathogen interactions: use this information when developing drugs that target the pathogen	Identify mechanisms of host–pathogen interactions: use this information when developing drugs that target the host or to better understand host immunity
Consider evolved principles of host immunity when setting criteria for chemical properties and when selecting targets of antimicrobial agents that will be used to treat contagious, life-threatening diseases (e.g., desirability of multiple targets, potential acceptability of low-level genotoxicity)	Identify and target pathways in the pathogen that allow it to evade host immunity or resist or repair the damage it inflicts	Bolster host immunity
Design antimicrobial agents that mimic or reproduce host immune chemistry (e.g., generation of reactive nitrogen species from nitroimidazoles)	Identify and target mechanisms of phenotypic tolerance displayed by bacteria in response to conditions in the host, including host immunity	Target pathways in the host that counteract effective host immunity or allow pathogens to evade it
Use elements of host immunity as antimicrobial agents (e.g., mAbs; members of the commensal microbiota)		Suppress immunopathology
Use elements of immunity to help deliver antimicrobial agents (e.g., mAb–drug conjugates)		Use antimicrobial agents as tool compounds to identify new mechanisms of host immunity

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REFERENCES

Abraham, E.P., and E. Chain. 1942. Purification of penicillin. *Nature.* 149:328. http://dx.doi.org/10.1038/149328b0

Adams, K.N., K. Takaki, L.E. Connolly, H. Wiedenhoft, K. Winglee, O. Humbert, P.H. Edelstein, C.L. Cosma, and L. Ramakrishnan. 2011. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. *Cell.* 145:39–53. http://dx.doi.org/10.1016/j.cell.2011.02.022

Alsultan, A., and C.A. Peloquin. 2014. Therapeutic drug monitoring in the treatment of tuberculosis: an update. *Drugs.* 74:839–854. (published erratum appears in *Drugs.* 2014. http://dx.doi.org/10.1007/s40265-014-0237-1) http://dx.doi.org/10.1007/s40265-014-0222-8

Ayres, J.S., and D.S. Schneider. 2012. Tolerance of infections. *Annu. Rev. Immunol.* 30:271–294. http://dx.doi.org/10.1146/annurev-immunol-020711-075030

Bagley, N., and K. Outterson. January 19, 2017. How to avoid a post-antibiotic world. *The New York Times.* Op-Ed section. p. A27.

Balaban, N.Q., J. Merrin, R. Chait, L. Kowalik, and S. Leibler. 2004. Bacterial persistence as a phenotypic switch. *Science.* 305:1622–1625. http://dx.doi.org/10.1126/science.1099390

Bhullar, K., N. Waglechner, A. Pawlowski, K. Koteva, E.D. Banks, M.D. Johnston, H.A. Barton, and G.D. Wright. 2012. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One.* 7:e34953. http://dx.doi.org/10.1371/journal.pone.0034953

Bigger, J. 1944. Treatment of staphylococcal infections with penicillin by intermittent sterilisation. *Lancet.* 244:497–500. http://dx.doi.org/10.1016/S0140-6736(00)74210-3

Boshoff, H.I., M.B. Reed, C.E. Barry III, and V. Mizrahi. 2003. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell.* 113:183–193. http://dx.doi.org/10.1016/S0092-8674(03)00270-8

Brauner, A., O. Fridman, O. Gefen, and N.Q. Balaban. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol.* 14:320–330. http://dx.doi.org/10.1038/nrmicro.2016.34

Brestoff, J.R., and D. Artis. 2015. Immune regulation of metabolic homeostasis in health and disease. *Cell.* 161:146–160. http://dx.doi.org/10.1016/j.cell.2015.02.022

Brown, K. 2013. Penicillin Man: Alexander Fleming and the Antibiotic Revolution. History Press, Stroud, UK. 368 pp.

Bryk, R., P. Griffin, and C. Nathan. 2000. Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature.* 407:211–215. http://dx.doi.org/10.1038/35025109

Bryk, R., C.D. Lima, H. Erdjument-Bromage, P. Tempst, and C. Nathan. 2002. Metabolic enzymes of mycobacteria linked to antioxidant defense by a thioredoxin-like protein. *Science.* 295:1073–1077. http://dx.doi.org/10.1126/science.1067798

Bryk, R., B. Gold, A. Venugopal, J. Singh, R. Samy, K. Pupek, H. Cao, C. Popescu, M. Gurney, S. Hotha, et al. 2008. Selective killing of nonreplicating mycobacteria. *Cell Host Microbe.* 3:137–145. http://dx.doi.org/10.1016/j.chom.2008.02.003

Bryk, R., N. Arango, A. Venugopal, J.D. Warren, Y.H. Park, M.S. Patel, C.D. Lima, and C. Nathan. 2010. Triazaspirodimethoxybenzoyls as selective inhibitors of mycobacterial lipoamide dehydrogenase. *Biochemistry.* 49:1616–1627. http://dx.doi.org/10.1021/bi9016186

Bryk, R., N. Arango, C. Maksymiuk, A. Balakrishnan, Y.T. Wu, C.H. Wong, T. Masquelin, P. Hipskind, C.D. Lima, and C. Nathan. 2013. Lipoamide channel-binding sulfonamides selectively inhibit mycobacterial lipoamide dehydrogenase. *Biochemistry.* 52:9375–9384. http://dx.doi.org/10.1021/bi401077f

Centers for Disease Control and Prevention. 2016. CDC funds 34 innovative projects to combat antibiotic resistance. Available at: http://www.cdc.gov/media/releases/2016/p1006-cdc-antibiotic-resistance-research.html.

Chen, C., E. Itakura, G.M. Nelson, M. Sheng, P. Laurent, L.A. Fenk, R.A. Butcher, R.S. Hegde, and M. de Bono. 2017. IL-17 is a neuromodulator of *Caenorhabditis elegans* sensory responses. *Nature.* 542:43–48. http://dx.doi.org/10.1038/nature20818

Chengalroyen, M.D., G.M. Beukes, B.G. Gordhan, E.M. Streicher, G. Churchyard, R. Hafner, R. Warren, K. Otwombe, N. Martinson, and B.D. Kana. 2016. Detection and quantification of differentially culturable tubercle bacteria in sputum from patients with tuberculosis. *Am. J. Respir. Crit. Care Med.* 194:1532–1540. http://dx.doi.org/10.1164/rccm.201604-0769OC

Cohen, I.R. 1992. The cognitive principle challenges clonal selection. *Immunol. Today.* 13:441–444. http://dx.doi.org/10.1016/0167-5699(92)90071-E

Cohen, T.S., J.J. Hilliard, O. Jones-Nelson, A.E. Keller, T. O'Day, C. Tkaczyk, A. DiGiandomenico, M. Hamilton, M. Pelletier, Q. Wang, et al. 2016. *Staphylococcus aureus* α toxin potentiates opportunistic bacterial lung infections. *Sci. Transl. Med.* 8:329ra31. http://dx.doi.org/10.1126/scitranslmed.aad9922

Coscolla, M., R. Copin, J. Sutherland, F. Gehre, B. de Jong, O. Owolabi, G. Mbayo, F. Giardina, J.D. Ernst, and S. Gagneux. 2015. *M. tuberculosis* T cell epitope analysis reveals paucity of antigenic variation and identifies rare variable TB antigens. *Cell Host Microbe.* 18:538–548. http://dx.doi.org/10.1016/j.chom.2015.10.008

Darby, C.M., and C.F. Nathan. 2010. Killing of non-replicating *Mycobacterium tuberculosis* by 8-hydroxyquinoline. *J. Antimicrob. Chemother.* 65:1424–1427. http://dx.doi.org/10.1093/jac/dkq145

Darby, C.M., H.I. Ingólfsson, X. Jiang, C. Shen, M. Sun, N. Zhao, K. Burns, G. Liu, S. Ehrt, J.D. Warren, et al. 2013. Whole cell screen for inhibitors of pH homeostasis in *Mycobacterium tuberculosis*. *PLoS One.* 8:e68942. (published erratum appears in *PLoS One.* 2013. http://dx.doi.org/10.1371/annotation/760b5b07-4922-42c4-b33a-162c1e9ae188) http://dx.doi.org/10.1371/journal.pone.0068942

Dartois, V., K. Saito, T. Warrier, and C. Nathan. 2016. Editorial: New evidence for the complexity of the population structure of *Mycobacterium tuberculosis* increases the diagnostic and biologic challenges. *Am. J. Respir. Crit. Care Med.* 194:1448–1451. http://dx.doi.org/10.1164/rccm.201607-1431ED

Darwin, K.H., and C.F. Nathan. 2005. Role for nucleotide excision repair in virulence of *Mycobacterium tuberculosis*. *Infect. Immun.* 73:4581–4587. http://dx.doi.org/10.1128/IAI.73.8.4581-4587.2005

Darwin, K.H., S. Ehrt, J.C. Gutierrez-Ramos, N. Weich, and C.F. Nathan. 2003. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science.* 302:1963–1966. http://dx.doi.org/10.1126/science.1091176

Davies, J., and K.S. Ryan. 2012. Introducing the parvome: bioactive compounds in the microbial world. *ACS Chem. Biol.* 7:252–259. http://dx.doi.org/10.1021/cb200337h

de Carvalho, L.P., G. Lin, X. Jiang, and C. Nathan. 2009. Nitazoxanide kills replicating and nonreplicating *Mycobacterium tuberculosis* and evades resistance. *J. Med. Chem.* 52:5789–5792. http://dx.doi.org/10.1021/jm9010719

de Carvalho, L.P., C.M. Darby, K.Y. Rhee, and C. Nathan. 2011. Nitazoxanide disrupts membrane potential and intrabacterial pH homeostasis of *Mycobacterium tuberculosis*. *ACS Med. Chem. Lett.* 2:849–854. http://dx.doi.org/10.1021/ml200157f

Escolano, A., P. Dosenovic, and M.C. Nussenzweig. 2017. Progress toward active or passive HIV-1 vaccination. *J. Exp. Med.* 214:3–16. http://dx.doi.org/10.1084/jem.20161765

Fleming-Dutra, K.E., A.L. Hersh, D.J. Shapiro, M. Bartoces, E.A. Enns, T.M. File Jr., J.A. Finkelstein, J.S. Gerber, D.Y. Hyun, J.A. Linder, et al. 2016. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. *JAMA.* 315:1864–1873. http://dx.doi.org/10.1001/jama.2016.4151

Fox, W., and I. Sutherland. 1956. A five-year assessment of patients in a controlled trial of streptomycin, para-aminosalicylic acid, and streptomycin plus para-aminosalicylic acid, in pulmonary tuberculosis. *Q. J. Med.* 25:221–243.

Frimodt-Møller, N. 2002. Correlation between pharmacokinetic/pharmacodynamic parameters and efficacy for antibiotics in the treatment of urinary tract infection. *Int. J. Antimicrob. Agents.* 19:546–553. http://dx.doi.org/10.1016/S0924-8579(02)00105-X

Global Antibiotic Research and Development Partnership. 2016. Global Antibiotic Research and Development Partnership (GARDP) garners key financial support for launch [press release]. Drugs for Neglected Disease initiative, Geneva, Switzerland. Available at: https://www.dndi.org/2016/media-centre/press-releases/gard-garners-key-support-for-launch/

Gold, B., and C. Nathan. 2017. Targeting phenotypically tolerant *Mycobacterium tuberculosis*. In Tuberculosis and the Tubercle Bacillus. Second edition. W.R. Jacobs, Jr., H. McShane, V. Mizrahi, and I. Orme, editors. American Society of Microbiology Press, Washington, DC.

Gold, B., M. Pingle, S.J. Brickner, N. Shah, J. Roberts, M. Rundell, W.C. Bracken, T. Warrier, S. Somersan, A. Venugopal, et al. 2012. Nonsteroidal anti-inflammatory drug sensitizes *Mycobacterium tuberculosis* to endogenous and exogenous antimicrobials. *Proc. Natl. Acad. Sci. USA.* 109:16004–16011. http://dx.doi.org/10.1073/pnas.1214188109

Gold, B., J. Roberts, Y. Ling, L.L. Quezada, J. Glasheen, E. Ballinger, S. Somersan-Karakaya, T. Warrier, J.D. Warren, and C. Nathan. 2015. Rapid, semiquantitative assay to discriminate among compounds with activity against replicating or nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 59:6521–6538. http://dx.doi.org/10.1128/AAC.00803-15

Gold, B., R. Smith, Q. Nguyen, J. Roberts, Y. Ling, L. Lopez Quezada, S. Somersan, T. Warrier, D. Little, M. Pingle, et al. 2016. Novel cephalosporins selectively active on nonreplicating *Mycobacterium tuberculosis*. *J. Med. Chem.* 59:6027–6044. http://dx.doi.org/10.1021/acs.jmedchem.5b01833

Gupta, M., N. Nayyar, M. Chawla, R. Sitaraman, R. Bhatnagar, and N. Banerjee. 2016. The chromosomal *parDE2* toxin-antitoxin system of *Mycobacterium tuberculosis* H37Rv: Genetic and functional characterization. *Front. Microbiol.* 7:886. http://dx.doi.org/10.3389/fmicb.2016.00886

Gusarov, I., K. Shatalin, M. Starodubtseva, and E. Nudler. 2009. Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. *Science.* 325:1380–1384. http://dx.doi.org/10.1126/science.1175439

Harms, A., E. Maisonneuve, and K. Gerdes. 2016. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science.* 354:aaf4268. http://dx.doi.org/10.1126/science.aaf4268

Helaine, S., A.M. Cheverton, K.G. Watson, L.M. Faure, S.A. Matthews, and D.W. Holden. 2014. Internalization of *Salmonella* by macrophages induces formation of nonreplicating persisters. *Science.* 343:204–208. http://dx.doi.org/10.1126/science.1244705

Hobby, G.L., K. Meyer, and E. Chaffee. 1942. Observations on the mechanism of action of penicillin. *Proc. Soc. Exp. Biol. Med.* 50:281–285. http://dx.doi.org/10.3181/00379727-50-13773

Hotamisligil, G.S. 2017. Inflammation, metaflammation and immunometabolic disorders. *Nature.* 542:177–185. http://dx.doi.org/10.1038/nature21363

Jankevicius, G., A. Ariza, M. Ahel, and I. Ahel. 2016. The toxin-antitoxin system DarTG catalyzes reversible ADP-ribosylation of DNA. *Mol. Cell.* 64:1109–1116. http://dx.doi.org/10.1016/j.molcel.2016.11.014

Kling, A., P. Lukat, D.V. Almeida, A. Bauer, E. Fontaine, S. Sordello, N. Zaburannyi, J. Herrmann, S.C. Wenzel, C. König, et al. 2015. Antibiotics. Targeting DnaN for tuberculosis therapy using novel griselimycins. *Science.* 348:1106–1112. http://dx.doi.org/10.1126/science.aaa4690

Kohanski, M.A., D.J. Dwyer, B. Hayete, C.A. Lawrence, and J.J. Collins. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell.* 130:797–810. http://dx.doi.org/10.1016/j.cell.2007.06.049

Kohanski, M.A., M.A. DePristo, and J.J. Collins. 2010. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. *Mol. Cell.* 37:311–320. http://dx.doi.org/10.1016/j.molcel.2010.01.003

Kommineni, S., D.J. Bretl, V. Lam, R. Chakraborty, M. Hayward, P. Simpson, Y. Cao, P. Bousounis, C.J. Kristich, and N.H. Salzman. 2015. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature.* 526:719–722. http://dx.doi.org/10.1038/nature15524

Koonin, E.V. 2016. Big questions in evolution. *Cell.* 166:528–529. http://dx.doi.org/10.1016/j.cell.2016.07.021

Kotas, M.E., and R. Medzhitov. 2015. Homeostasis, inflammation, and disease susceptibility. *Cell.* 160:816–827. http://dx.doi.org/10.1016/j.cell.2015.02.010

Leach, K.L., S.J. Brickner, M.C. Noe, and P.F. Miller. 2011. Linezolid, the first oxazolidinone antibacterial agent. *Ann. N.Y. Acad. Sci.* 1222:49–54. http://dx.doi.org/10.1111/j.1749-6632.2011.05962.x

Lehar, S.M., T. Pillow, M. Xu, L. Staben, K.K. Kajihara, R. Vandlen, L. DePalatis, H. Raab, W.L. Hazenbos, J.H. Morisaki, et al. 2015. Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. *Nature.* 527:323–328. http://dx.doi.org/10.1038/nature16057

Levin-Reisman, I., I. Ronin, O. Gefen, I. Braniss, N. Shoresh, and N.Q. Balaban. 2017. Antibiotic tolerance facilitates the evolution of resistance. *Science.* 355:826–830. http://dx.doi.org/10.1126/science.aaj2191

Lewis, K., and Y. Shan. 2017. Why tolerance invites resistance. *Science.* 355:796. http://dx.doi.org/10.1126/science.aam7926

Lin, C.H., C.Y. Chi, H.P. Shih, J.Y. Ding, C.C. Lo, S.Y. Wang, C.Y. Kuo, C.F. Yeh, K.H. Tu, S.H. Liu, et al. 2016. Identification of a major epitope by anti-interferon-γ autoantibodies in patients with mycobacterial disease. *Nat. Med.* 22:994–1001. http://dx.doi.org/10.1038/nm.4158

Lin, G., D. Li, L.P. de Carvalho, H. Deng, H. Tao, G. Vogt, K. Wu, J. Schneider, T. Chidawanyika, J.D. Warren, et al. 2009. Inhibitors selective for mycobacterial versus human proteasomes. *Nature.* 461:621–626. http://dx.doi.org/10.1038/nature08357

Lin, G., T. Chidawanyika, C. Tsu, T. Warrier, J. Vaubourgeix, C. Blackburn, K. Gigstad, M. Sintchak, L. Dick, and C. Nathan. 2013. N,C-Capped dipeptides with selectivity for mycobacterial proteasome over human proteasomes: role of S3 and S1 binding pockets. *J. Am. Chem. Soc.* 135:9968–9971. http://dx.doi.org/10.1021/ja400021x

Ling, L.L., T. Schneider, A.J. Peoples, A.L. Spoering, I. Engels, B.P. Conlon, A. Mueller, T.F. Schäberle, D.E. Hughes, S. Epstein, et al. 2015. A new antibiotic kills pathogens without detectable resistance. *Nature.* 517:455–459. http://dx.doi.org/10.1038/nature14098

Liu, Y., S. Tan, L. Huang, R.B. Abramovitch, K.H. Rohde, M.D. Zimmerman, C. Chen, V. Dartois, B.C. VanderVen, and D.G. Russell. 2016. Immune activation of the host cell induces drug tolerance in *Mycobacterium tuberculosis* both in vitro and in vivo. *J. Exp. Med.* 213:809–825. http://dx.doi.org/10.1084/jem.20151248

Maksymiuk, C., A. Balakrishnan, R. Bryk, K.Y. Rhee, and C.F. Nathan. 2015. E1 of α-ketoglutarate dehydrogenase defends *Mycobacterium*

tuberculosis against glutamate anaplerosis and nitroxidative stress. *Proc. Natl. Acad. Sci. USA.* 112:E5834–E5843. (published erratum appears in *Proc. Natl. Acad. Sci. USA.* 2015. http://dx.doi.org/10.1073/pnas.1519907112) http://dx.doi.org/10.1073/pnas.1510932112

Malakoff, D., and J. Mervis. 2016. Science lessons for the next president. *Science.* 354:274–279 (editorial). http://dx.doi.org/10.1126/science.354.6310.274

Mancini, A. 2016. Infectious disease companies catch more investors. Pink Sheet. June 14, 2016.

Marakalala, M.J., R.M. Raju, K. Sharma, Y.J. Zhang, E.A. Eugenin, B. Prideaux, I.B. Daudelin, P.Y. Chen, M.G. Booty, J.H. Kim, et al. 2016. Inflammatory signaling in human tuberculosis granulomas is spatially organized. *Nat. Med.* 22:531–538. http://dx.doi.org/10.1038/nm.4073

Marraffini, L.A. 2015. CRISPR-Cas immunity in prokaryotes. *Nature.* 526:55–61. http://dx.doi.org/10.1038/nature15386

Mayer-Barber, K.D., B.B. Andrade, S.D. Oland, E.P. Amaral, D.L. Barber, J. Gonzales, S.C. Derrick, R. Shi, N.P. Kumar, W. Wei, et al. 2014. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature.* 511:99–103. http://dx.doi.org/10.1038/nature13489

McCune, R.M., F.M. Feldmann, H.P. Lambert, and W. McDermott. 1966. Microbial persistence. I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. *J. Exp. Med.* 123:445–468. http://dx.doi.org/10.1084/jem.123.3.445

McKinney, J.D., K. Höner zu Bentrup, E.J. Muñoz-Elías, A. Miczak, B. Chen, W.T. Chan, D. Swenson, J.C. Sacchettini, W.R. Jacobs Jr., and D.G. Russell. 2000. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature.* 406:735–738. http://dx.doi.org/10.1038/35021074

McLaughlin, K.2016. China Tackles Antimicrobial Resistance. Available at: http://scim.ag/Chinaresistance.

Michelucci, A., T. Cordes, J. Ghelfi, A. Pailot, N. Reiling, O. Goldmann, T. Binz, A. Wegner, A. Tallam, A. Rausell, et al. 2013. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc. Natl. Acad. Sci. USA.* 110:7820–7825. http://dx.doi.org/10.1073/pnas.1218599110

Moreira, W., D.B. Aziz, and T. Dick. 2016. Boromycin kills mycobacterial persisters without detectable resistance. *Front. Microbiol.* 7:199. http://dx.doi.org/10.3389/fmicb.2016.00199

Mueller, M., A. de la Peña, and H. Derendorf. 2004. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. *Antimicrob. Agents Chemother.* 48:369–377. http://dx.doi.org/10.1128/AAC.48.2.369-377.2004

Mukamolova, G.V., O. Turapov, J. Malkin, G. Woltmann, and M.R. Barer. 2010. Resuscitation-promoting factors reveal an occult population of tubercle bacilli in sputum. *Am. J. Respir. Crit. Care Med.* 181:174–180. http://dx.doi.org/10.1164/rccm.200905-0661OC

Nandakumar, M., C. Nathan, and K.Y. Rhee. 2014. Isocitrate lyase mediates broad antibiotic tolerance in *Mycobacterium tuberculosis*. *Nat. Commun.* 5:4306. http://dx.doi.org/10.1038/ncomms5306

Nathan, C. 2003. Specificity of a third kind: Reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.* 111:769–778. http://dx.doi.org/10.1172/JCI200318174

Nathan, C. 2004. Antibiotics at the crossroads. *Nature.* 431:899–902. http://dx.doi.org/10.1038/431899a

Nathan, C. 2008. Microbiology. An antibiotic mimics immunity. *Science.* 322:1337–1338. http://dx.doi.org/10.1126/science.1167452

Nathan, C. 2009. Taming tuberculosis: A challenge for science and society. *Cell Host Microbe.* 5:220–224. http://dx.doi.org/10.1016/j.chom.2009.02.004

Nathan, C. 2011. Making space for anti-infective drug discovery. *Cell Host Microbe.* 9:343–348. http://dx.doi.org/10.1016/j.chom.2011.04.013

Nathan, C. 2012. Fresh approaches to anti-infective therapies. *Sci. Transl. Med.* 4:140sr2. http://dx.doi.org/10.1126/scitranslmed.3003081

Nathan, C. 2015. Cooperative development of antimicrobials: Looking back to look ahead. *Nat. Rev. Microbiol.* 13:651–657. http://dx.doi.org/10.1038/nrmicro3523

Nathan, C., and C.E. Barry III. 2015. TB drug development: immunology at the table. *Immunol. Rev.* 264:308–318. http://dx.doi.org/10.1111/imr.12275

Nathan, C., and O. Cars. 2014. Antibiotic resistance--problems, progress, and prospects. *N. Engl. J. Med.* 371:1761–1763. http://dx.doi.org/10.1056/NEJMp1408040

Nathan, C., and A. Cunningham-Bussel. 2013. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat. Rev. Immunol.* 13:349–361. http://dx.doi.org/10.1038/nri3423

Nathan, C., and A. Ding. 2010. Nonresolving inflammation. *Cell.* 140:871–882. http://dx.doi.org/10.1016/j.cell.2010.02.029

Nathan, C., B. Gold, G. Lin, M. Stegman, L.P. de Carvalho, O. Vandal, A. Venugopal, and R. Bryk. 2008. A philosophy of anti-infectives as a guide in the search for new drugs for tuberculosis. *Tuberculosis (Edinb.).* 88:S25–S33. http://dx.doi.org/10.1016/S1472-9792(08)70034-9

O'Neill, J., editor. 2014. Tackling Drug-Resistant Infections Globally: Tackling a Crisis for the Health and Wealth of Nations. Review on Antimicrobial Resistance. London, England, UK. 18 pp.

O'Neill, J., editor. 2016. Tackling Drug Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance. London, England, UK. 80 pp.

Orman, M.A., and M.P. Brynildsen. 2013. Dormancy is not necessary or sufficient for bacterial persistence. *Antimicrob. Agents Chemother.* 57:3230–3239. http://dx.doi.org/10.1128/AAC.00243-13

Outterson, K., J.H. Rex, T. Jinks, P. Jackson, J. Hallinan, S. Karp, D.T. Hung, F. Franceschi, T. Merkeley, C. Houchens, et al. 2016. Accelerating global innovation to address antibacterial resistance: Introducing CARB-X. *Nat. Rev. Drug Discov.* 15:589–590. http://dx.doi.org/10.1038/nrd.2016.155

Payne, D.J., M.N. Gwynn, D.J. Holmes, and D.L. Pompliano. 2007. Drugs for bad bugs: Confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov.* 6:29–40. http://dx.doi.org/10.1038/nrd2201

Pietersen, E., E. Ignatius, E.M. Streicher, B. Mastrapa, X. Padanilam, A. Pooran, M. Badri, M. Lesosky, P. van Helden, F.A. Sirgel, et al. 2014. Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: A cohort study. *Lancet.* 383:1230–1239. http://dx.doi.org/10.1016/S0140-6736(13)62675-6

Rath, P., C. Huang, T. Wang, T. Wang, H. Li, R. Prados-Rosales, O. Elemento, A. Casadevall, and C.F. Nathan. 2013. Genetic regulation of vesiculogenesis and immunomodulation in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA.* 110:E4790–E4797. http://dx.doi.org/10.1073/pnas.1320118110

Russo, F., J. Gising, L. Åkerbladh, A.K. Roos, A. Naworyta, S.L. Mowbray, A. Sokolowski, I. Henderson, T. Alling, M.A. Bailey, et al. 2015. Optimization and evaluation of 5-styryl-oxathiazol-2-one *Mycobacterium tuberculosis* proteasome inhibitors as potential antitubercular agents. *ChemistryOpen.* 4:342–362. http://dx.doi.org/10.1002/open.201500001

Sarathy, J., V. Dartois, T. Dick, and M. Gengenbacher. 2013. Reduced drug uptake in phenotypically resistant nutrient-starved nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 57:1648–1653. http://dx.doi.org/10.1128/AAC.02202-12

Sasikaran, J., M. Ziemski, P.K. Zadora, A. Fleig, and I.A. Berg. 2014. Bacterial itaconate degradation promotes pathogenicity. *Nat. Chem. Biol.* 10:371–377. http://dx.doi.org/10.1038/nchembio.1482

Sassone-Corsi, M., S.P. Nuccio, H. Liu, D. Hernandez, C.T. Vu, A.A. Takahashi, R.A. Edwards, and M. Raffatellu. 2016. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature.* 540:280–283. http://dx.doi.org/10.1038/nature20557

Schafer, D.P., E.K. Lehrman, A.G. Kautzman, R. Koyama, A.R. Mardinly, R. Yamasaki, R.M. Ransohoff, M.E. Greenberg, B.A. Barres, and B. Stevens. 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron.* 74:691–705. http://dx.doi.org/10.1016/j.neuron.2012.03.026

Schnappinger, D., S. Ehrt, M.I. Voskuil, Y. Liu, J.A. Mangan, I.M. Monahan, G. Dolganov, B. Efron, P.D. Butcher, C. Nathan, and G.K. Schoolnik. 2003. Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: Insights into the phagosomal environment. *J. Exp. Med.* 198:693–704. http://dx.doi.org/10.1084/jem.20030846

Shah, S., A.G. Dalecki, A.P. Malalasekera, C.L. Crawford, S.M. Michalek, O. Kutsch, J. Sun, S.H. Bossmann, and F. Wolschendorf. 2016. 8-Hydroxyquinolines are boosting agents of copper-related toxicity in *Mycobacterium tuberculosis. Antimicrob. Agents Chemother.* 60:5765–5776. http://dx.doi.org/10.1128/AAC.00325-16

Singh, R., U. Manjunatha, H.I. Boshoff, Y.H. Ha, P. Niyomrattanakit, R. Ledwidge, C.S. Dowd, I.Y. Lee, P. Kim, L. Zhang, et al. 2008. PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO release. *Science.* 322:1392–1395. http://dx.doi.org/10.1126/science.1164571

Stewart, B., and D.E. Rozen. 2012. Genetic variation for antibiotic persistence in *Escherichia coli. Evolution.* 66:933–939. http://dx.doi.org/10.1111/j.1558-5646.2011.01467.x

Su, H.W., J.H. Zhu, H. Li, R.J. Cai, C. Ealand, X. Wang, Y.X. Chen, M.U. Kayani, T.F. Zhu, D. Moradigaravand, et al. 2016. The essential mycobacterial amidotransferase GatCAB is a modulator of specific translational fidelity. *Nat. Microbiol.* 1:16147. http://dx.doi.org/10.1038/nmicrobiol.2016.147

Sun, K., V.K. Yajjala, C. Bauer, G.A. Talmon, K.J. Fischer, T. Kielian, and D.W. Metzger. 2016. Nox2-derived oxidative stress results in inefficacy of antibiotics against post-influenza *S. aureus* pneumonia. *J. Exp. Med.* 213:1851–1864. http://dx.doi.org/10.1084/jem.20150514

The International Chronic Granulomatous Disease Cooperative Study Group. 1991. A controlled trial of interferon-γ to prevent infection in chronic granulomatous disease. *N. Engl. J. Med.* 324:509–516. http://dx.doi.org/10.1056/NEJM199102213240801

Tuomanen, E. 1986. Phenotypic tolerance: The search for β-lactam antibiotics that kill nongrowing bacteria. *Rev. Infect. Dis.* 8(Suppl 3):S279–S291. http://dx.doi.org/10.1093/clinids/8.Supplement_3.S279

Um, S.W., S.W. Lee, S.Y. Kwon, H.I. Yoon, K.U. Park, J. Song, C.T. Lee, and J.H. Lee. 2007. Low serum concentrations of anti-tuberculosis drugs and determinants of their serum levels. *Int. J. Tuberc. Lung Dis.* 11:972–978.

United Nations. 2016. Draft Political Declaration of the High-Level Meeting of the General Assembly on Antimicrobial Resistance. New York. Available at: https://www.un.org/pga/71/wp-content/uploads/sites/40/2016/09/DGACM_GAEAD_ESCAB-AMR-Draft-Political-Declaration-1616108E.pdf

Van Boeckel, T.P., C. Brower, M. Gilbert, B.T. Grenfell, S.A. Levin, T.P. Robinson, A. Teillant, and R. Laxminarayan. 2015. Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA.* 112:5649–5654. http://dx.doi.org/10.1073/pnas.1503141112

Vandal, O.H., L.M. Pierini, D. Schnappinger, C.F. Nathan, and S. Ehrt. 2008. A membrane protein preserves intrabacterial pH in intraphagosomal *Mycobacterium tuberculosis. Nat. Med.* 14:849–854. http://dx.doi.org/10.1038/nm.1795

Vaubourgeix, J., G. Lin, N. Dhar, N. Chenouard, X. Jiang, H. Botella, T. Lupoli, O. Mariani, G. Yang, O. Ouerfelli, et al. 2015. Stressed mycobacteria use the chaperone ClpB to sequester irreversibly oxidized proteins asymmetrically within and between cells. *Cell Host Microbe.* 17:178–190. http://dx.doi.org/10.1016/j.chom.2014.12.008

Venugopal, A., R. Bryk, S. Shi, K. Rhee, P. Rath, D. Schnappinger, S. Ehrt, and C. Nathan. 2011. Virulence of *Mycobacterium tuberculosis* depends on lipoamide dehydrogenase, a member of three multienzyme complexes. *Cell Host Microbe.* 9:21–31. http://dx.doi.org/10.1016/j.chom.2010.12.004

Vikhanski, L. 2016. Immunity. How Elie Metchnikoff Changed The Course of Modern Medicine. Chicago Review Press, Chicago. 322 pp.

Voskuil, M.I., D. Schnappinger, K.C. Visconti, M.I. Harrell, G.M. Dolganov, D.R. Sherman, and G.K. Schoolnik. 2003. Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J. Exp. Med.* 198:705–713. http://dx.doi.org/10.1084/jem.20030205

Wakamoto, Y., N. Dhar, R. Chait, K. Schneider, F. Signorino-Gelo, S. Leibler, and J.D. McKinney. 2013. Dynamic persistence of antibiotic-stressed mycobacteria. *Science.* 339:91–95. http://dx.doi.org/10.1126/science.1229858

Warrier, T., M. Martinez-Hoyos, M. Marin-Amieva, G. Colmenarejo, E. Porras-De Francisco, A.I. Alvarez-Pedraglio, M.T. Fraile-Gabaldon, P.A. Torres-Gomez, L. Lopez-Quezada, B. Gold, et al. 2015. Identification of novel anti-mycobacterial compounds by screening a pharmaceutical small-molecule library against nonreplicating *Mycobacterium tuberculosis. ACS Infect. Dis.* 1:580–585. http://dx.doi.org/10.1021/acsinfecdis.5b00025

Warrier, T., K. Kapilashrami, A. Argyrou, T.R. Ioerger, D. Little, K.C. Murphy, M. Nandakumar, S. Park, B. Gold, J. Mi, et al. 2016. N-methylation of a bactericidal compound as a resistance mechanism in *Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. USA.* 113:E4523–E4530. http://dx.doi.org/10.1073/pnas.1606590113

White House. 2015. National Action Plan for Combating Antibiotic-Resistant Bacteria. Washington, DC. 62 pp.

Wilkins, J.J., R.M. Savic, M.O. Karlsson, G. Langdon, H. McIlleron, G. Pillai, P.J. Smith, and U.S. Simonsson. 2008. Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption. *Antimicrob. Agents Chemother.* 52:2138–2148. http://dx.doi.org/10.1128/AAC.00461-07

Woodruff, H.B. 1981. A soil microbiologist's odyssey. *Annu. Rev. Microbiol.* 35:1–28. http://dx.doi.org/10.1146/annurev.mi.35.100181.000245

World Health Organization. 2016. The Top 10 Causes of Death. [Fact sheet]. Geneva, Switzerland. Available at: http://www.who.int/mediacentre/factsheets/fs310/en/

Xie, Q.W., H.J. Cho, J. Calaycay, R.A. Mumford, K.M. Swiderek, T.D. Lee, A. Ding, T. Troso, and C. Nathan. 1992. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science.* 256:225–228. http://dx.doi.org/10.1126/science.1373522

Xu, H.S., N. Roberts, F.L. Singleton, R.W. Attwell, D.J. Grimes, and R.R. Colwell. 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microb. Ecol.* 8:313–323. http://dx.doi.org/10.1007/BF02010671

Zemke, A.C., M.T. Gladwin, and J.M. Bomberger. 2015. Sodium nitrite blocks the activity of aminoglycosides against *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* 59:3329–3334. http://dx.doi.org/10.1128/AAC.00546-15