Trends in Pharmacological Sciences

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Review

Pharmacological Targeting of the Host–Pathogen Interaction: Alternatives to Classical Antibiotics to Combat Drug-Resistant Superbugs

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The rise of multidrug-resistant pathogens and the dearth of new antibiotic development place an existential strain on successful infectious disease therapy. Breakthrough strategies that go beyond classical antibiotic mechanisms are needed to combat this looming public health catastrophe. Reconceptualizing antibiotic therapy in the richer context of the host–pathogen interaction is required for innovative solutions. By defining specific virulence factors, the essence of a pathogen, and pharmacologically neutralizing their activities, one can block disease progression and sensitize microbes to immune clearance. Likewise, host-directed strategies to boost phagocyte bactericidal activity, enhance leukocyte recruitment, or reverse pathogen-induced immunosuppression seek to replicate the success of cancer immunotherapy in the field of infectious diseases. The answer to the threat of multidrug-resistant pathogens lies 'outside the box' of current antibiotic paradigms.

Trends

To address the ever-increasing problem of multidrug-resistant pathogens, the narrow conceptualization of antibiotic pharmacology must be expanded to a holistic analysis of the host–pathogen interaction.

Pharmacologically targeting bacterial virulence factors is an alternative strategy to halt disease progression and enhance immune clearance. By 'disarming' specific pathogens, instead of killing them with broad-spectrum antibiotics, the normal microbiome and its crucial functions are preserved.

Host-directed therapies to boost the resilience and microbicidal capacities of immune cells may enhance clearance of antibiotic-resistant pathogens, mirroring successful cancer immunotherapeutics.

When pharmacological activities are studied in the richer context of bacterial immune systems interactions, antibiotics deemed to be inactive in standard laboratory testing show hidden activities, and drugs approved for other clinical indications may be repurposed to expand our antimicrobial armamentarium.

Antimicrobial Resistance

An alarming and persistent rise in antibiotic resistance among many important pathogenic bacterial species poses one of the greatest contemporary challenges to public health. The 2014 UK Government *Review on Antimicrobial Resistance* performed in collaboration with the Wellcome Trust concluded that, without a dramatic change in our response to the crisis, the true cost of antimicrobial resistance will be 300 million premature deaths and up to $100 trillion lost to the global economy by 2050, at which point it will exceed cancer as a cause of human mortality [1]. As World Health Organization Director-General Margaret Chan recently addressed the United Nations General Assembly: 'Antimicrobial resistance is a global crisis – a slow motion tsunami. The situation is bad, and getting worse. With few replacement products in the pipeline, the world is heading towards a post-antibiotic era in which common infections, especially those caused by Gram-negative bacteria, will once again kill' [2].

The effects of the antibiotic resistance epidemic are particularly distressing in hospitals and chronic care facilities where such infections tend to strike the most vulnerable patient groups with chronic diseases and weakened immune systems. Highest-risk populations include the

elderly, cancer patients, diabetics, premature newborns, surgical patients, and those fighting for their lives in the intensive care unit. Antibiotic resistance also disproportionately impacts on developing countries with poor public health infrastructure that cannot deploy costly last-line antibiotic treatments.

The roots of the current dilemma are multifactorial. Ill-considered overprescription of antibiotics for self-resolving conditions, physician reliance on unnecessarily broad-spectrum treatment regimens, widespread use of antibiotics in agricultural feed for growth promotion, an innovation gap because of the exodus of most major pharmaceutical companies from antibiotic R&D that they judge to be unprofitable, and pure Darwinian evolution of bacteria subjected to life-or-death selective pressures all contribute deeply. Although conventional antibiotics have cured more disease than all other drug classes combined, this 'golden era' is coming to a close, and increasingly complex patient conditions and multi-resistant pathogens are exacting high morbidity and mortality in the face of a monolithic and uncreative approach to therapy. Broadening the definition of 'antibiotic' is essential for innovation [3], and is a prerequisite to escape from the difficult situation that has emerged as a result of our unfortunate collective complacency and neglect.

A barrier to innovation exists because the conceptual and scientific basis for current antibiotics centers solely on the bacterium, but serious infection is more properly understood as a disease of the host–pathogen interaction. Most leading agents of human bacterial infection frequently colonize the skin or mucosal surfaces of healthy individuals without producing symptoms.

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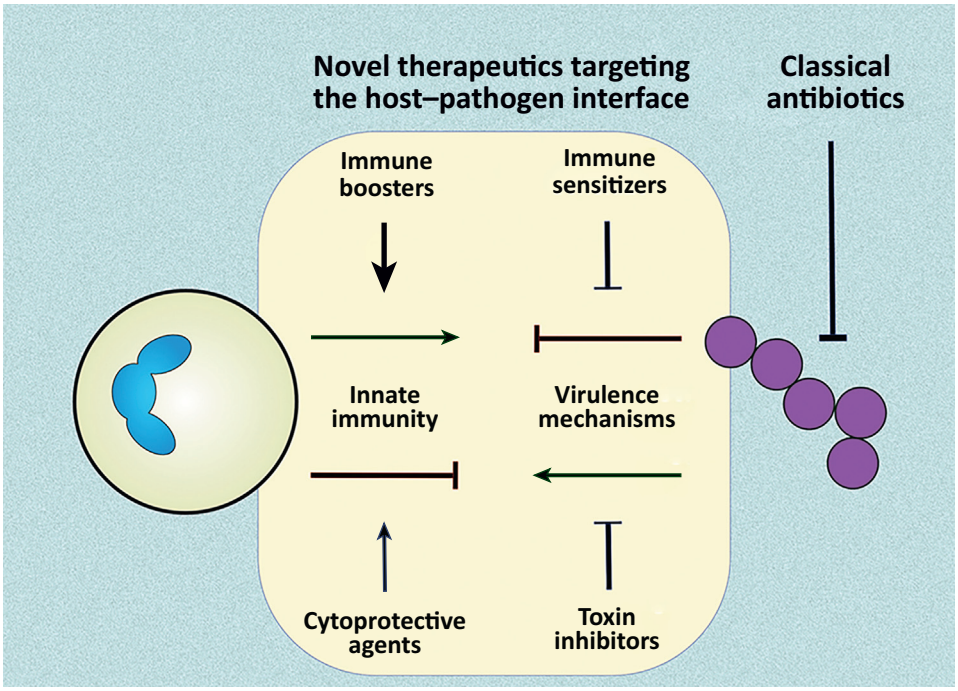

Figure 1. Potential for Novel Infectious Disease Therapeutics Targeting the Host–Pathogen Interface. Classical antibiotics, drugs that kill or suppress the growth of pathogens, have been the cornerstones of infectious disease therapy for decades. However, continual evolution of antibiotic resistance has eroded their once reliable efficacy. Considering serious bacterial infection as a perturbation of the host–pathogen interaction, novel therapeutic drug classes are under evaluation. These drugs seek to inhibit bacterial toxins and immune resistance factors, or stimulate immune cell resilience and the expression of antimicrobial effectors.

However, if a bacterium has invaded into the bloodstream or deep tissues to sicken the patient, one can say that the sentinel defense functions of our innate immune system have failed. A more opportune definition of antibiotic therapy that centers on understanding correcting the dysfunctional host–pathogen interaction can unlock opportunities for therapeutic discovery (Figure 1). Such novel pharmacological concepts come at the question from both sides: (i) pathogen-directed therapeutics that target virulence factors to reduce bacterial toxicity and/or sensitize the pathogen to normal immune clearance; and (ii) host-directed therapeutics that boost the endogenous antimicrobial activity of host innate immune cells.

Neutralization of Virulence Factors: Disarming the Pathogen

Virulence factors are those characteristics that differentiate disease-causing bacteria from the hundreds of species of beneficial bacteria that comprise the normal flora of our intestine, mucosal surfaces, and skin. Virulence factors include bacterial surface structures or secreted molecules that promote mucosal/epithelial adherence, biofilm formation, and/or intracellular invasion to breech host cell barriers. Additional virulence determinants promote resistance to immunological clearance by host antimicrobial peptides, complement, or phagocytes, thereby allowing the pathogen to continue replication in normally sterile sites and produce a deep-seated or systemic infection. Finally, bacterial factors that directly injure host cells or membranes, impair crucial host cell functions, or elicit exaggerated and deleterious proinflammatory responses, collectively referred to as 'toxins', are important contributors to virulence.

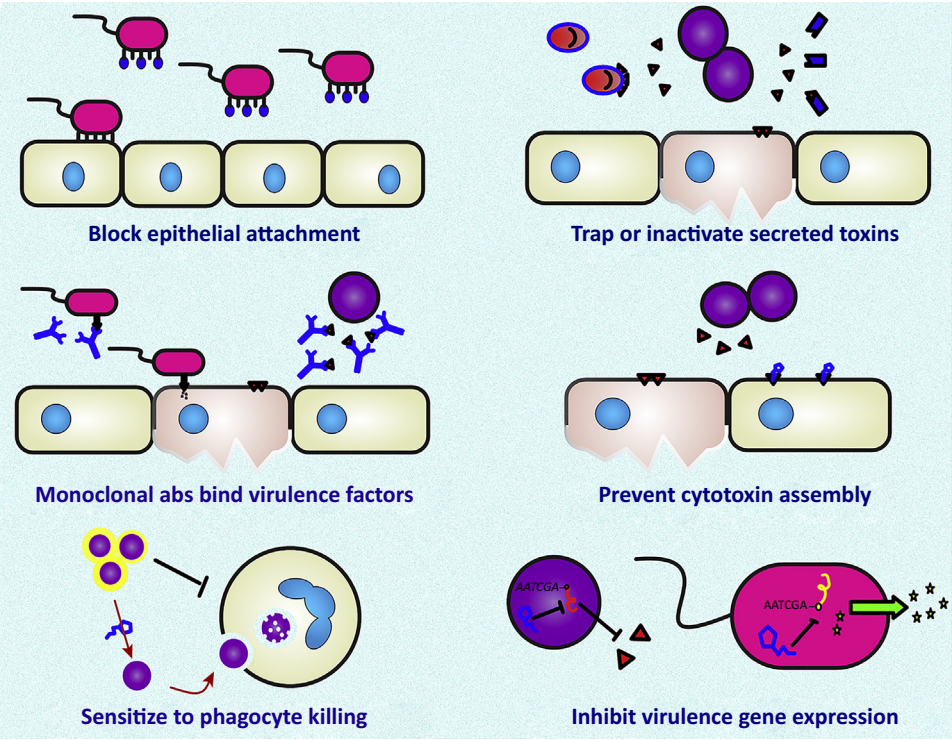

Figure 2. Potential Targets for Pathogen-Directed Anti-virulence Therapies against Multidrug-Resistant Bacterial Pathogens. Small molecules, nanoparticles, engineered proteins, and monoclonal antibodies are under investigation to block the expression or function of bacterial toxins and other virulence factors. Anti-virulence therapies hold promise of being more specific to the infectious bacterium while sparing the healthy microbiome, and can be used as adjuncts to classical antibiotics for difficult infections.

The basic pharmacological concept at play here is that, instead of identifying drugs than directly kill or suppress the growth (classical bactericidal or bacteriostatic antibiotic activities), one could screen or design drugs to target a virulence factor of the pathogen, thereby rendering the pathogen 'harmless' or readily susceptible to immune clearance (Figure 2). The ideal virulence factor target should be universally or widely expressed among disease-associated strains of the pathogenic bacterial species, and play an essential role in disease pathophysiology. An express advantage of the virulence factor neutralization concept is that the therapy is envisaged to be very specific for the pathogen in question, avoiding extensive 'collateral damage' to the normal flora that accompanies courses of broad-spectrum antibiotic therapy and increases the risk of opportunistic infection or metabolic dysregulation. Virulence factor inhibitor therapy can also be contemplated as a companion or adjunct to effective classical antibiotic therapy (when available) to improve clinical outcome in severe or recalcitrant infections, or as a prophylactic therapy for patients entering a high-risk window for nosocomial infections (e.g., chemotherapy, major surgery).

Block Epithelial Adherence or Biofilm Formation

An essential first step in disease pathogenesis for many mucosal pathogens is to gain a secure foothold on the epithelium of the target organ mediated by specific adhesin-mediated interactions with a host epithelial cell receptor, indirect binding to mucus or extracellular matrix components, or by the formation of a polymeric self-produced matrix of extracellular substances known as a biofilm. The opportunistic and frequently multidrug-resistant Gram-negative bacterial pathogen *Pseudomonas aeruginosa* binds to glycostructures on mucosal surfaces via two carbohydrate-binding proteins (lectins), PA-IL and PA-IIL. D-galactose or peptides mimicking human natural killer-1 antigen (HNK-1), polysialic acid, or fucose blocked PA-IL-mediated airway cell binding, while L-fucose and pHNK-1 blocked PA-IIL-mediated binding, restoring normal ciliary beat frequency [4]. *P. aeruginosa* also produces a fucose-specific lectin, LecB, which participates in tissue attachment and the formation of biofilms. High-affinity LecB ligands obtained by screening combinatorial libraries of multivalent fucosyl-peptide dendrimers induced total dispersion of established biofilms of several clinical *P. aeruginosa* isolates [5]. Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infections, and its type I fimbrial (FimH) lectin is a key factor in bladder cell adherence and colonization. A small molecular weight orally bioavailable 'mannoside' inhibitor that targets FimH in its mannose-binding pocket showed good therapeutic efficacy against UPEC infections, including antibiotic-resistant strains [6]. In Gram-positive pathogens such as *Staphylococcus aureus*, many surface proteins, including the fibronectin-binding protein A (FbpA) that is crucial for epithelial adherence, are anchored to the cell envelope by the action of transpeptidase enzymes, termed sortases, which recognize a motif (LPXTG) in the target protein and catalyze covalent linkage to cell-wall peptidoglycan. Small molecules with potent inhibitory activity against *S. aureus* sortases A and B *in vitro* blocked adherence of the living pathogen to fibronectin [7].

Monoclonal Antibodies (mAbs)

The antibiotic resistance crisis has spurred renewed interest in mAb therapeutics against specific antigens expressed by key pathogens. Many such passive immunotherapies bind to conserved surface targets of the pathogen, which themselves may or may not be virulence factors, to promote opsonization and phagocytic clearance of the organism, as recently reviewed [8,9]. We highlight here some novel mAb therapeutic concepts that target toxins or other virulence factors of pathogens to improve disease outcome without directly promoting opsonophagocytic clearance/killing of the pathogen.

α-Hemolysin ('α toxin') of *S. aureus* is a small β-barrel pore-forming toxin with a broad range of cellular specificities that binds to its receptor (ADAM10) on target cells to trigger membrane

disruption [10]. α-Toxin is an important virulence factor, as demonstrated through targeted mutagenesis of the encoding *hla* gene in strains of the globally disseminated epidemic USA300 clone of methicillin-resistant *S. aureus* (MRSA), which markedly reduces host cell injury and virulence potential in murine models of infection, particularly pneumonia [11]. mAbs targeting α-toxin prevent assembly of its stable oligomer on the target cell and protect against lethal *S. aureus* pneumonia in mice [12]. Treatment with such a mAb provided dose-dependent increases in murine survival against challenge with a variety of different *S. aureus* clinical isolates, reduced proinflammatory cytokine and chemokine release, improved lung function independent of Fc activities, and showed synergistic or additive effects with concurrent antibiotics [13]. Two anti-α-toxin mAbs (MEDI4982/Medimmune and AR301/Aridis) have now entered Phase I/II human clinical trials for prevention of staphylococcal pneumonia [9]. *S. aureus* also produces a family of related bi-component leukocidins that contribute to disease severity: Panton–Valentine leukocidin (PVL), γ-hemolysin (HlgABC), and leukocidin ED (LukED). A recent study described a single human mAb capable of recognizing a conformational epitope shared by the bi-component staphylococcal toxins plus α-toxin that prevented lysis of human phagocytes and provided high-level protection in murine pneumonia and sepsis models [14].

Another key aspect of *S. aureus* pathogenesis is subversion or dysregulation of host coagulation through specific virulence determinants that bind to or activate key clotting factors and platelets to promote pathogen clumping, endovascular clot formation, and/or the development of tissue abscesses. A mAb against *S. aureus* surface protein clumping factor A (ClfA) has been evaluated in a Phase II trial of hospitalized patients with documented *S. aureus* bacteremia (www.clinicaltrials.gov #NCT00198302). In a recent study, mice systemically challenged with *L. lactis* expressing ClfA died within 24 h; passive immunization of such mice with an anti-ClfA mAb antibody to block fibrinogen binding dramatically increased survival compared to a control anti-ClfA mAb antibody that allowed fibrinogen binding [15]. This result suggests that inhibition of virulence functions (fibrinogen binding) and not simply antibody-mediated opsonization contributed to the efficacy of the passive immunization. Coagulase A (CoA) and von Willebrand factor binding protein (vWbp) are two additional *S. aureus* proteins that synergize with ClfA to allow the pathogen to create fibrin cables *in vivo*. In a murine endocarditis model, passive immunization against all three virulence factors (CoA, vWbp, ClfA) reduced the development of heart lesions and increased survival [16]. A combination of prothrombin inhibitors plus anti-ClfA mAb also prolonged time to death in *S. aureus* sepsis [16].

Newer mAbs target bacterial toxins with mechanisms of action other than membrane pore formation and also improve specific bacterial disease outcomes. The virulence potential of *P. aeruginosa* is associated with a type III secretion system (TTSS), a 'molecular syringe' that directly injects cytotoxins into host cells, inducing cell death. Protein PcrV makes an indispensable contribution to *P. aeruginosa* TTSS toxin translocation, and an engineered humanized anti-PcrV IgG antigen-binding fragment, KB001, has been developed for clinical use in ventilator-associated pneumonia and the chronic pneumonitis of cystic fibrosis [17]. Other mAbs targeting PcrV likewise show efficacy in pre- and post-challenge animal models of *P. aeruginosa* infection [18]. Obiltoxaximab, a mAb targeting protective antigen (PA), a component of anthrax lethal toxin (LT) and edema toxin (ET) required for toxin entry, prevents dissemination of *Bacillus anthracis* and reduces mortality in pre- and post-exposure rabbit or cynomolgus macaque models of inhalational anthrax, a foremost biodefense concern [19]. A cocktail of one fully human MAb specific to the receptor-binding domain of *Clostridium difficile* toxin A and two fully human MAbs specific to non-overlapping regions of the glucosyltransferase domain of *C. difficile* toxin B reduced the severity and duration of diarrhea and mortality upon challenge with highly virulent *C. difficile* strains [20]. Lastly, an interesting derivative of mAb therapeutics was reported in the treatment of difficult *S. aureus* infections, where a slow-growing intracellular reservoir of the pathogen is often resistant to antibiotic clearance. An anti-*S. aureus* mAb was

conjugated to an antibiotic (rifalogue) activated only after it is released in the proteolytic environment of the phagolysosome [21]. This unique mAb–drug conjugate showed superior efficacy versus vancomycin in a mouse model of MRSA bacteremia.

An overarching advantage of mAb therapeutics for the treatment of bacterial infections is the opportunity for specific targeting of an individual pathogen or molecular virulence factor, thereby allowing narrow-spectrum precision and sparing the healthy microbiome. Their long half-life may allow prophylactic administration to high-risk patients, and newer antibody engineering technologies to enhance Fc effector functions or create bi-specific platforms can provide additional benefit. Limitations of mAbs include constraints on tissue penetration owing to their large size, inability to enter cells to access intracellular pathogens and pathways, and the requirement for parenteral, and not oral, administration.

Strategies for Toxin Neutralization

Beyond specific mAbs, other approaches have been devised to prevent the action of bacterial toxins by their physical sequestration or by interfering with their key initial interactions with host cell receptors or cellular trafficking pathways.

The essential mechanism of action of bacterial pore-forming toxins reflects their natural affinity for the lipid bilayer of host cell membranes, wherein they assemble to disrupt the integrity of the membrane and promote cell death through hypo-osmotic lysis. By designing pharmacological agents that mimic structural features or biochemical properties of the host cell membrane, a 'decoy' strategy can be used to sequester toxin away from the target cells and protect the host from pathogenic insult. For example, cholesterol, packaged within methyl-β-cyclodextrin (CD), was used as a topical therapy to sequester α-toxin and prevent corneal erosions in a rabbit model of ocular keratitis with live *S. aureus* or purified toxin [22]. Another modified CD compound, IB201, blocked α-toxin-induced lysis of human alveolar epithelial cells by preventing formation of the lytic pore, and prevented or treated pulmonary infections with highly virulent *S. aureus* strains in mice [23]. Another group engineered artificial liposomes composed exclusively of naturally occurring lipids tailored to compete effectively with host cells for toxin binding. Liposome-bound toxins such as α-toxin or pneumolysin from *Streptococcus pneumoniae* did not lyse mammalian cells *in vitro*, and administration of such liposomes up to 10 h after infectious challenge rescued mice from fatal septicemia caused by *S. aureus* and *S. pneumoniae* [24]. Another innovative approach derives biomimetic 'nanosponges' consisting of a polymeric nanoparticle core surrounded by freshly isolated red blood cell membranes. These functioned as toxin decoys *in vivo* to scavenge *S. aureus* α-toxin and improve survival in mice challenged with the toxin [25], and reduced lesion size in a skin infection model [26]. Platelet-membrane derived nanosponge mimics also bind to pathogens and enhance the therapeutic efficacy of vancomycin in systemic *S. aureus* infection [27].

Blocking host receptors and entry pathways used by toxins is a promising therapeutic concept because extensive adaptations by the microbe would be necessary to enable it to switch to a new receptor that can still support pathogenesis. For example, phage display was used to select a peptide that binds to both natural host cell receptors for anthrax toxins (ANTXR1 and ANTXR2), and polyvalent presentation of this peptide on a synthetic scaffold neutralized anthrax toxin action upon intravenous administration in rats [28]. The cell-surface metalloprotease ADAM 10 is a receptor for *S. aureus* α-toxin, and a small-molecule ADAM 10 inhibitor protects against α-toxin-mediated disease pathogenesis in murine staphylococcal pneumonia [11] and abscess formation [29]. Upon receptor binding, a common mechanism for cellular entry of bacterial toxins requires trafficking to an acidified endosome, promoting translocation across the host membrane. The most active compound identified in a 30 000 compound screen against anthrax lethal toxin activity, 4-bromobenzaldehyde *N*-(2,6-dimethylphenyl)

semicarbazone (EGA), effectively blocked entry of lethal toxin and other acid-dependent bacterial toxins into mammalian cells [30].

Direct inhibition of bacterial protease toxins has also been explored therapeutically. For example, a small-molecule hydroxamate compound that inhibits the metalloprotease activity of anthrax lethal toxin provided a significant survival advantage to mice given a lethal challenge of vegetative bacilli and to rabbits given a lethal inhalation challenge of spores [31]. Likewise, ebselen inhibits the cysteine protease activity of *C. difficile* toxins TcdA and TcdB at nanomolar concentrations. Ebselen administration increased the survival of mice and reduced pathological injury to the gastrointestinal mucosa in toxin or *C. difficile* gastrointestinal infection [32]. Several bacterial pathogens with pore-forming toxins induce macrophage necroptosis, a proinflammatory mode of cell death regulated by receptor-interacting protein kinases RIP1 and RIP3, and mediated by the effector mixed-lineage kinase domain-like protein MLKL; macrophages deficient in MLKL are consequently resistant to pore-forming toxin-induced cell death. Treatment of mice with necrostatin-5, an inhibitor of RIP1 and/or GW806742X, an inhibitor of MLKL, reduced the severity of *Serratia marcescens* pneumonia in mice, protected alveolar macrophages from cell death, and reduced bacterial burden [33]; necrostatin-5 also reduced pneumolysin-mediated macrophage necroptosis and cardiac damage following pneumococcal bacteremia and myocardial invasion [34].

Reducing Bacterial Virulence Factor Gene Expression

In most medically important human pathogens, transcription of genes encoding key virulence determinants or their biosynthetic pathways is regulated by complex, intersecting pathways to allow fine tuning of expression in response to environmental cues encountered at each stage of colonization and disease progression. Virstatin is a virulence inhibitor drug against *Vibrio cholerae* disease that inhibits dimerization of transcriptional activator, ToxT. When ToxT is blocked, transcription of the genes encoding cholera toxin, which triggers the hallmark watery diarrhea, and of the toxin-coregulated pilus (Tcp), which promotes intestinal colonization by the pathogen, are both shut off [35]. Virstatin also inhibits pilus biogenesis, motility, and biofilm formation in the multidrug-resistant nosocomial pathogen *Acinetobacter baumannii* [36]. Regacin is a drug that inhibits the ability of an AraC-like virulence regulator, RegA, to bind to DNA and activate target promoters in the genome of the model gastrointestinal pathogen *Citrobacter rodentium.* Regacin reduced *C. rodentium* toxicity to intestinal epithelial cells, and treatment of infected mice with the drug reduced colonization and bacterial virulence [37]. Similar results have been observed with small-molecule inhibitors of VirF, an AraC-like transcriptional regulator in the foodborne pathogen *Shigella flexneri*; the inhibitors blocked the expression of numerous virulence genes and inhibited intestinal epithelial cell invasion [38].

S. aureus virulence has been targeted using enol-acyl carrier protein inhibitor AFN-1252. AFN-1252 treatment rapidly increased *S. aureus* expression of fatty acid synthesis genes, thereby perturbing membrane dynamics to influence signaling through the SaeRS two-component regulator and repressing the expression of virulence genes including α-toxin, β- and γ-hemolysins, and two fibrinogen-binding adhesins. Oral treatment of mice with AFN-1252 in an *S. aureus* air-pouch infection model reduced viable bacteria recovered [39]. Two small-molecule compounds (CCG-2979 and its analog CCG-102487) were identified in a high-content screen for reduced expression of streptokinase (SK), a virulence factor of *Streptococcus pyogenes* that coopts and activates host plasminogen to facilitate systemic spread of the pathogen during necrotizing fasciitis ('flesh-eating disease'). Microarray analysis of group A streptococci (GAS) grown in the presence of these inhibitors showed downregulation of other important *S. pyogenes* virulence factors in addition to SK, including the antiphagocytic surface-anchored M protein and the cytolytic toxins streptolysin O and S, consistent with

disruption of a general virulence gene regulatory network. Drug treatment increased neutrophil phagocytosis and killing of *S. pyogenes*, protecting mice from mortality in a systemic infection model [40].

Interference with Bacterial Quorum Sensing

Quorum sensing is an important regulatory principle in numerous bacterial species in which the organisms produce and release chemical signal molecules, called autoinducers, that increase in concentration as a function of cell density. Many bacterial pathogens use quorum sensing to coordinate gene expression according to the density of their local population within host tissues. The natural product 6-gingerol, a pungent oil derived from fresh ginger, competitively inhibited the binding of two related homoserine lactone autoinducers of *P. aeruginosa* to their cognate receptors, reducing the expression of known virulence factors including elastase and pyocyanin, and blocking biofilm formation. Pretreatment with 6-gingerol reduced mouse mortality from *P. aeruginosa* infection in a dose-dependent manner [41]. Another strategy for competitive inhibition and disruption of *P. aeruginosa* quorum sensing utilized meta-bromothiolactone (mBTL), which resulted in reduced biofilm formation, pyocyanin production, and increased survival of *Caenorhabditis elegans* and human lung epithelial cells at low μM levels after *P. aeruginosa* infection [42]. Likewise, the antimetabolite compound *S*-phenyl-L-cysteine sulfoxide inhibited the kynurenine pathway implicated in *P. aeruginosa* alkyl quinolone autoinducer production and quorum sensing, thereby reducing the production of pyocyanin and other toxins [43].

In *S. aureus*, the accessory gene regulator (*agr*) quorum-sensing system decreases expression of several cell-surface proteins and increases the expression of many secreted toxins in the transition from late-exponential growth to stationary phase. Because the Agr system, and in particular its response regulator AgrA, are required for full *S. aureus* virulence, they have been explored as a therapeutic target. Treatment with naringenin, a flavonoid present in grapefruits and tomatoes, reduced transcription of *S. aureus* genes encoding AgrA and α-toxin, attenuating α-toxin-mediated injury to alveolar epithelial cells, and reduced pulmonary inflammation and injury in a mouse pneumonia model [44]. Very recently, the nonsteroidal anti-inflammatory drug diflunisal was shown to block *S. aureus* AgrA transcriptional regulation of small peptide toxin virulence factors known as phenol-soluble modulins (PSMs); diflunisal treatment reduced PSM production and diminished bone destruction in a murine osteomyelitis model of infection [45].

Sensitization of the Pathogen to Host Innate Immune Clearance

When a patient is suffering a serious infection with a multidrug-resistant pathogen, it is important to consider not only last-line antibiotic options but also that the pathogen is being combated by multiple endogenous molecular and cellular effectors of microbial clearance, such as phagocytic cells, reactive oxygen species (ROS), and cationic host defense peptides. One emerging concept in alternative infectious disease therapeutics is to identify genes that deprive the pathogen of the virulence factors it uses to defend itself against host defense mechanisms, thus resensitizing it to innate immune destruction. For example, the polysialic acid capsule of K1 serotype *E. coli* defends against complement- and phagocyte-mediated immune clearance, and plays a role in invasive disease potential (e.g., urosepsis). Small-molecule inhibitors identified in a screen for inhibition of *E. coli* capsule biogenesis render the pathogen highly sensitive to active human serum and provided near-total protection to mice in lethal systemic infection with a virulent K1 serotype isolate [46]. Recent research has also shown that particular antibiotics that have no demonstrable activity against a particular pathogen in standard laboratory minimum inhibitory concentration (MIC) testing do nevertheless sensitize the pathogen to killing by endogenous host defense peptides such as human cathelicidin LL-37. Examples include (i) the use of the β-lactam antibiotic nafcillin against MRSA, which promotes

LL-37 killing and bacterial clearance in human whole blood, in a murine necrotizing skin infection model as well as in human patients with recalcitrant bacteremia [47]; and (ii) the use of azithromycin to sensitize highly multidrug-resistant Gram-negative bacterial pathogens such as *P. aeruginosa*, *A. baumannii*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* to LL-37 killing and human serum, as well as to clearance in murine models of pneumonia with each pathogen [48,49].

Phagocytic cells, including neutrophils and macrophages, generate ROS including hydrogen peroxide, hypochlorite, and singlet oxygen through the NADPH oxidase-mediated 'oxidative burst', a key component of phagolysosomal killing of pathogens after their engulfment. *S. aureus* expresses a hallmark golden carotenoid pigment, staphyloxanthin, with antioxidant properties, which promotes resistance to ROS and phagocyte killing, increasing virulence in mouse infection models [50]. Interestingly, a high degree of similarity exists between a key enzyme required for production of the staphylococcal pigment (CrtM, dehydrosqualene synthase) and a human enzyme (squalene synthase) in the cholesterol biosynthesis pathway. Phosphonosulfate drug BPH-652, a squalene synthase inhibitor in clinical development for its cholesterol-lowering properties, blocked *S. aureus* pigment production, sensitizing the pathogen to ROS, neutrophil killing, and reducing the bacterial burden in kidneys of mice following systemic infection [51]. Interestingly, the next enzyme in the *S. aureus* carotenoid pigment synthesis pathway is the diapophytoene desaturase, CrtN, which is inhibited by nanomolar concentrations by the FDA-approved antifungal drug, naftifine. Pigment inhibition by naftidine treatment decreased bacterial burden and increased mouse survival in a systemic *S. aureus* challenge model [52]. Blocking carotenoid synthesis also increased cell membrane fluidity to sensitize *S. aureus* to the antimicrobial action of cationic host defense peptides [53].

Pharmacologically Boosting the Bactericidal Activity of Phagocytic Cells

Development of a serious bacterial infection, by definition, declares a functional failure of innate immune cells to execute their frontline antimicrobial defense function. Although there are legions of medicines used in clinical medicine today whose purpose is to dial down immune cell activity in the treatment of inflammatory disorders such as rheumatoid arthritis or multiple sclerosis (e.g., corticosteroids, anti-cytokine therapies), the management of serious acute bacterial infections is performed agnostic to immune cell function. However, because the activation states of all immune cells are controlled through intricate pathways that rapidly deploy but promptly counter-regulate inflammatory processes ('accelerators' and 'brakes'), the opportunity to pharmacologically target more powerful immune cell killing is gaining attention (Figure 3).

Manipulating Cytokines and Chemokines

One group of strategies being pursued in this vein envisage the administration of endogenous pro-immune cytokines or chemokine to augment clearance of the potentially antibiotic-resistant pathogen. For example, treating mice with macrophage-activating lipopeptide 2 (MIP-2), a C-X-C family cytokine akin to human interleukin (IL) 8, increased neutrophil and macrophage recruitment to the lung in S. *pneumoniae* lung infection, decreased bacterial counts, and promoted survival [54]. *S. aureus* resistance to lysozyme results suppresses the induction of IFN-β production from resident dendritic cells in skin, but addition of exogenous IFN-β can reverse this defect and promote bacterial clearance *in vivo* [55]. Intranasal administration of the mitogen keratinocyte growth factor (KGF) increased GM-CSF-dependent killing of *Mycobacterium tuberculosis* through enhanced macrophage phagolysosome fusion and nitric oxide production, improving clearance of the pathogen [56]. Similar results were found in *E. coli* pulmonary challenge, where KGF treatment protected animals by enhancing alveolar macrophage function and release of antimicrobial peptides into the broncheoalveolar fluid [57]. Finally, some pathogens, such as *K. pneumoniae,* can immunosuppress the host by stimulating the

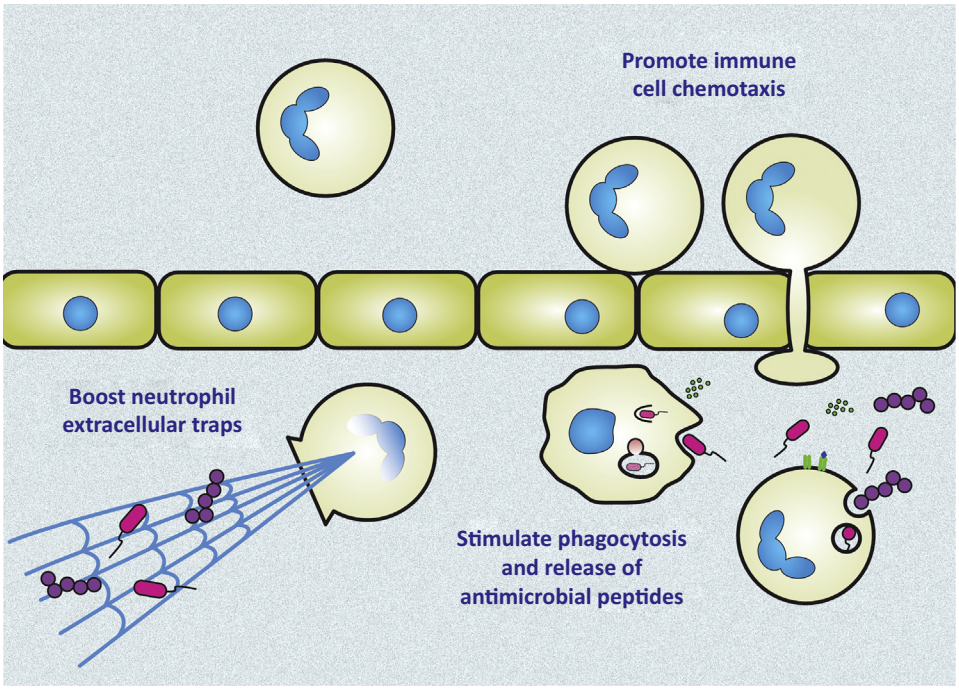

Figure 3. Potential Targets for Host-Directed Immune Boosting Therapies against Multidrug-Resistant Bacterial Pathogens. Taking advantage of evolving knowledge regarding chemokines and cytokines, pattern recognition receptors, and the regulatory programs they control, pharmacological approaches are being explored to boost the intrinsic antibacterial activity of macrophages and neutrophils.

production of anti-inflammatory cytokines such as IL-10. Pretreatment of *K. pneumoniae*-infected mice with anti-IL-10 serum boosted levels of TNF-α, MIP-2, and neutrophil-derived myeloperoxidase, reducing bacterial load and increasing survival [58].

Lipid Mediators of Innate Immunity to Bacterial Infection

Leukotrienes are a family of eicosanoid lipid inflammatory mediators arising from the oxidative metabolism of arachidonic acid. Treatment of macrophages with leukotriene B4 (LTB4) enhanced NADPH oxidase-dependent production of ROS and killing of *S. pyogenes* [59]. Aerosolized administration of LTB4 to mice improved bacterial clearance and protected against mortality in pneumonia challenge models with *S. pneumoniae* or *Klebsiella pneumoniae* [60,61]. Other specialized endogenous mediators (SPMs) explored for proimmune properties are derived from the ω3 fatty acid docosahexaenoic acid (DHA), including molecules termed protectins, resolvins, and maresins. Protectins, naturally produced by human M2 macrophages, enhance the resolution of inflammation caused by infection. Administration of a synthetic protectin enhanced macrophage recruitment and phagocytosis of *E. coli*, while decreasing neutrophil infiltration, promoting efferocytosis of dead cells, and blunting the production of proinflammatory lipid mediators [62]. Likewise, maresins and resolvins promote the resolution of experimental murine *E. coli* or *S. aureus* infections, respectively, by increasing macrophage phagocytosis and efferocytosis while limiting neutrophil and eicosanoid production [63,64]. Additional compounds, termed 13-series resolvins, were recently identified from neutrophil–endothelial co-cultures as a product of cyclooxygenase-2 (COX-2) activity, further induced by atorvastatin via *S*-nitrosylation of COX-2, and these are present in human tissues after sterile inflammation or infection. Atorvastatin and 13-series resolvins had additive therapeutic effects to accelerate resolution of inflammation and protect against mortality in *E. coli*

peritonitis [65]. Moreover, dietary modulations may skew lipid profiles in a beneficial manner because mice fed a high-fat diet containing the ω3 unsaturated fatty acid (HFD-ω3) survived fivefold better upon intravenous *S. aureus* challenge than mice fed with a control high-fat diet with saturated fatty acids (HFD-S). Mice fed with HFD-ω3 had reduced bacterial loads in the kidney, better neutrophil stores in their bone marrow, and enhanced phagocytic activity of their neutrophils versus *S. aureus* [66].

Identifying and Targeting Immune Regulatory Pathways in Phagocytes

Studies of altered infectious disease susceptibility in knockout mice have also inspired pharmacological approaches to host-directed innate immune boosting. While studying the role of leptin receptor (LepR)/STAT3 signaling, it was found that mutant mice deficient in leptin-induced STAT3 phosphorylation had improved bacterial clearance and survival when challenged with *S. pneumoniae.* Treatment of normal mice with a pharmacologic cysteinyl-leukotriene receptor antagonist increased resistance to pneumococcal infection [67]. Similarly, macrophages and neutrophils deficient in the transcriptional regulator hypoxia-inducible factor 1 (HIF-1) have defects in glycolysis and ATP generation, leading to impaired microbicidal function and increased susceptibility of myeloid-specific HIF-1 knockout mice to infection [68]. Pharmacological stabilization of HIF-1 with a prolyl hydroxylase inhibitor drug (AKB-4924) to block its degradation pathway increased macrophage and neutrophil killing of MRSA and several Gram-negative bacterial pathogens, and provided protection against skin and urinary tract infection in mouse challenge models [69,70]. Mice in which deletion of C/EBPε mimics neutrophil-specific granule deficiency are hypersusceptible to *S. aureus* infection. Vitamin B3 (nicotinamide) pretreatment of human whole blood increased C/EBPε levels and clearance of *S. aureus* (as well as of *P. aeruginosa* and *K. pneumoniae*); this was attributable to enhanced phagocytic cell function. Treating mice with vitamin B3 also decreased *S. aureus* bacterial loads in kidneys and spleens [71]. Finally, in cystic fibrosis, mutations in the CFTR ion transporter lead to abnormal mucus dynamics, persistent bacterial pneumonia, and chronic debilitating loss of pulmonary function. CFTR-deficient macrophages have impaired autophagy-mediated killing of *Burkholderia cenocepacia* in association with exaggerated proinflammatory cytokine IL-1β production; stimulation of autophagy with rapamycin in CFTR-deficient macrophages and mice reduced bacterial burden and IL-1β secretion [72].

Immunomodulatory Peptides

Therapeutic strategies are emerging in which particular immune-stimulatory peptides are derived or modified from endogenous molecules to exploit key host signaling pathways. For example, a natural fragment of lactoferrin, the peptide HLR1r, alters cytokine production and antimicrobial responses in macrophages, facilitating their recognition and effector killing functions against *S. aureus* and *Candida albicans*, and providing protection in *ex vivo* and *in vivo* skin infection models [73,74]. Erythropoietin (Epo) participates in the stress responses to dampen proinflammatory signaling. An Epo analog (ARA290) that selectively binds to a tissue-protective receptor comprising one subunit of the Epo receptor disulfide linked to CD131 caused an altered response to uropathogenic *E. coli* (UPEC) infection, modulating IL-8 secretion and reducing UPEC cellular invasion by dampening β1-integrin signaling [75]. A novel peptide derived from the endogenous bovine neutrophil peptide bactenecin, named IDR-1002, induced potent chemokine production by human and murine macrophages, and, when injected locally at the site of infection, enhanced neutrophil recruitment to clear both Gram-positive and Gram-negative bacterial infections [76].

Enhancing Toll-like Receptor (TLR) Signaling Pathways

TLRs recognize 'pathogen-associated microbial patterns' (PAMPs; e.g., lipopolysaccharide, peptidoglycan, flagellin) and host-derived 'danger-associated molecular patterns' (DAMPs; e.g., HMGB1, serum amyloid A) to initiate signaling cascades that increase the expression of

innate immune response genes. Stimulating TLR4 with a synthetic lipid A mimetic induced macrophage and dendritic cell proinflammatory cytokine production, leading to an IFN-γ-dependent reduction in organ bacterial burdens and increased survival in *Francisella tularensis* infection [77]. Stimulating TLR5 with flagellin protected mice from lethal pneumococcal pulmonary challenge, increasing proinflammatory cytokine expression and neutrophil recruitment to the lung [78]. Stimulation of TLR9 with CpG motif-containing oligodeoxynucleotides reduced bacterial burden in mice infected with *Listeria monocytogenes*; immunological correlates were consistent with an effect mediated by IFN-γ-dependent IL-12 production [79]. Injecting mice with CpG motif-rich *F. tularensis* DNA also showed protective efficacy in this model, and promoted long-term pathogen-specific B cell-mediated responses against the *Listeria* [80]. Finally, targeting TLR coreceptor CD14 with leucine-rich repeat peptides enhanced TLR2- and TLR4-dependent proinflammatory responses to bacteria, increasing phagocyte recruitment and accelerating bacterial clearance in mouse models of Gram-negative Gram-positive bacterial peritonitis. This CD14-directed strategy also rescued the proinflammatory response of peripheral blood mononuclear cells from immunosuppressed sepsis patients *ex vivo* [81].

Current Drugs with Unanticipated Immune-Boosting Effects

Imatinib (Gleevec) is a breakthrough drug targeting the ATP-binding pocket of ABL tyrosine kinase with remarkable efficacy as a 'magic bullet' in the treatment of chronic myelogenous leukemia and some other malignancies. Several intracellular pathogens including *M. tuberculosis* manipulate imatinib-sensitive kinases during cellular entry and phagolysosomal trafficking. Imatinib treatment of mice infected with *Mycobacterium marinum* reduced bacterial burden and liver pathology, and synergy with the antibiotic rifampicin further decreased bacterial load [82]. The antimicrobial efficacy of imatinib may in part be attributed to increased expression of vATPase leading to better phagolysosomal acidification [83]. The nonsteroidal anti-inflammatory drug (NSAID) celecoxib is used to treat pain or inflammation. A celecoxib derivative (AR-12) has off-target autophagy-inducing properties that promote macrophage clearance of intracellular pathogens *Francisella* and *Salmonella*, prolonging survival in murine challenge models [84,85]. Finally, the estrogen receptor antagonist tamoxifen is a mainstay of treatment for many forms of breast cancer. Treating human neutrophils with tamoxifen increased chemotaxis and the formation of neutrophil 'extracellular traps' (NETs), a special type of cell death wherein nuclear DNA is released in a sticky meshwork to ensnare bacteria, exposing them to high concentrations of antimicrobial peptides and histones [86]. Tamoxifen neutrophil boosting was independent of estrogen receptor antagonism, but instead depended upon PKCζ-dependent increases in intracellular ceramide levels. Tamoxifen NET induction boosted neutrophil bacterial killing, and treatment of mice with tamoxifen decreased bacterial burden in multiple organs and protected against mortality in systemic MRSA infection [86].

Statins: Repurposing a Leading Human Medication at the Host–Pathogen Interface

Statins bind to the active site of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. Owing to their oral availability, favorable pharmacokinetic properties, and relatively low level of associated side effects, these drugs have become a mainstay of low-density lipoprotein (LDL) cholesterol-lowering therapy that is now used to treat tens of millions of individuals with hyperlipidemia worldwide. Interestingly, recent meta-analyses have compiled a heterogeneous collection of retrospective and prospective cohort studies and randomized trials to conclude that statin use may provide a beneficial effect in reducing morbidity and mortality associated with different infectious disease conditions, including pneumonia, bacteremia, and sepsis [87,88]. Thus it has been hypothesized that statins may have pharmacological effects, either through cholesterol reduction itself or via an independent 'off-target' mechanism, to aid in pathogen resistance. Because these

drugs have limited direct *in vitro* antimicrobial activity against the leading pathogens, recent studies have focused on the effects of statins on both sides of the host–pathogen interaction.

Statin treatment modulates some key bacterial virulence phenotypes. At subinhibitory concentrations, statins reduce biofilm formation in *P. aeruginosa*, *S. aureus*, and *Staphylococcus epidermidis* [89–91], and suppress the production of *S. aureus* cytolysins α-toxin and Panton–Valentine leukocidin [90]. Statins may also block host epithelial or endothelial cell invasion by *P. aeruginosa*, *S. aureus*, or the neonatal pathogen group B *Streptococcus* [92–94]; the last study suggesting that mevastatin antagonism of Rho-family GTPases involved in endocytotic uptake might inhibit pathogen host cell entry.

Likewise, statins may augment defense against bacterial pathogens by mechanisms that work through the host cell. Reduction of cholesterol may reduce host cell susceptibility to lysis by bacterial pore-forming toxins such as pneumolysin, which utilize cholesterol as a receptor for engagement and assembly in the host cell membrane [95]. Killing of macrophages by anthrax lethal toxin is also reduced significantly by statins because they antagonize Rho-family GTPases [96]. Statin depletion of intracellular cholesterol also impacts on the formation of lipid rafts, plasma membrane subdomains that are important in cell signaling, and which can also be exploited by some intracellular pathogens for intracellular trafficking and survival, for example *Chlamydia pneumoniae* and *L. monocytogenes*. Cellular transmission of *C. pneumoniae* in macrophage co-culture experiments with vascular smooth muscle cells is reduced by statin treatment [97], which reduced disease severity in a murine *C. pneumoniae* pulmonary infection model [98]. Statins protect against *L. monocytogenes* infection by reducing membrane cholesterol in macrophages and by blocking the ability of the cholesterol-dependent cytolysin listeriolysin O to mediate escape of the bacterium from the phagosome to the cytoplasm [99]. Lastly, statins increased neutrophil and macrophage extracellular trap-based killing of *S. aureus*, protecting against pulmonary challenge with the pathogen and enhancing extracellular trap formation *in vivo* [100].

Concluding Remarks

Decreasing treatment options for antibiotic-resistant pathogens place an imperative both on the medical community and upon academic and industry drug discovery initiatives to discover innovative therapeutic approaches to reduce morbidity and mortality in the increasingly complex patient populations at greatest risk. Thinking beyond classical antibiotics to envisage strategies that carefully target the host–pathogen interaction offers opportunities to 'tip the balance' in favor of host immune clearance and preservation of cell and tissue integrity; this would represent a welcome and overdue paradigm shift with the potential for considerable upside. Virulence factor inhibitors can offer increased specificity and personalization of therapy to the infection at hand, reducing the undesired side effects of broad-spectrum antibiotics on the integrity of the normal host microflora. Defining the virulence determinants for targeting, however, becomes more challenging in immunocompromised patients where organisms with little *de novo* virulence potential in normal hosts can sometimes produce severe infectious consequences, but much less is understood about their pathogenic mechanisms.

We should draw inspiration from host-directed immunotherapeutics that have revolutionized the field of cancer therapeutics, for example PDL-1 checkpoint inhibitors that boost antitumor T cells in melanoma leading to dramatically improved outcomes. These successes can inspire future precision medicines that modulate host targets to enhance antimicrobial function of innate immune cells such as macrophages and neutrophils for infectious disease therapeutics. In this manner, we may also theoretically help to cure or prevent deep tissue foci of antibiotic-resistant pathogens with minimal collateral damage on our microbiota. In our experience, promoting host-directed immune-boosting therapeutics has raised theoretical concerns

Outstanding Questions

Will a pharmaceutical agent that does not directly kill a bacterial pathogen be readily accepted into modern clinical dogma for treating multidrug-resistant infections?

How receptive will the FDA and other government agencies be to adopting novel clinical trial designs for pathogen-specific approaches in smaller targeted patient groups to aid the provision of innovative therapies to patients?

Can rapid, sensitive, and specific (culture-independent) diagnostic assays for bacterial identification be developed to allow prompt deployment of anti-virulence therapies in a cost-effective manner?

Will bacterial pathogens have the same potential for stepwise evolution of resistance to anti-virulence therapies as they do for classical antibiotics?

Is there a potential for undesired inflammatory or autoimmune side effects when innate immune cells are stimulated to fight off bacterial infections?

Will virulence factor inhibiting and immune cell boosting antibacterial therapies better preserve the normal microbial flora of the patient and their crucial roles in immune and metabolic homeostasis?

among some drug developers regarding the proinflammatory consequences of the therapy. We believe antibiotic-resistant pathogens themselves cause even more deleterious inflammatory effects on the host until effective medical cure is achieved, and that wise host immune pathway targeting can overcome such hurdles.

The many proofs of concept provided in this review are provided to illustrate that both screening-based and targeted approaches to the discovery of virulence factor inhibitors and immune boosters are feasible. Analysis of the biological action of current FDA-approved drugs, used for different indications but with known effects on cellular biochemistry and metabolism, offer the opportunity for repurposing as adjunctive antimicrobial agents to modulate virulence factor expression or fortify host immune function and resilience against bacterial infection.

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