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PERSPECTIVES

Antibiotics and Innate Immunity: A Cooperative Effort Toward the Successful Treatment of Infections

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Despite the common ancestry of antimicrobial and immunological science, a divergence driven by artificially construed paradigms in microbiology has placed limits on how we understand the mechanisms of antibiotics in vivo. We summarize recent updates on data that shed light on how antibiotics interact with components of innate immunity.

Keywords. antibiotic; antimicrobial peptides; innate immunity, mechanism.

Despite common roots of antimicrobial therapy and immunology in the research efforts of legendary pioneers like Louis Pasteur and Paul Ehrlich, our divergent and hyperspecialized evolution of medicine has largely separated the pharmacology of antibiotics administered to patients from the functions of the patient's innate immune system [1]. Ehrlich's work in immunology won him the 1908 Nobel Prize in Medicine; his contributions to antimicrobial chemotherapy with salvarsan, an arsphenamine compound that was the first treatment for syphilis, should not be forgotten [2]. The great majority of current antibiotics are natural products produced by actinomycetes, fungi, or other microorganisms used in niche competition against other species, suggesting that antibiotics themselves may represent primeval precursors of the immune systems of higher organisms. Along those lines, the mechanisms of action of some current "exogenous" natural product antibiotics used in clinical medicine overlap considerably with the "endogenous" antimicrobial peptides of the mammalian innate immune system [3]. This process has implications for antimicrobial susceptibility, as the presence of host defense peptides in vivo during chronic infection is sufficient to select for cross-resistance to daptomycin, even in the treatment-naïve patient [4, 5].

ENDOGENOUS AND EXOGENOUS ANTIBIOTICS

There is abundant literature examining the pharmacodynamic relationships of exogenous antibiotics with one another, defining synergy, additivity, indifference, and antagonism. Conversely, studies of the pharmacodynamics of innate immune components and antibiotics are scarce and have been difficult to implement and replicate in the patient care setting. Serum inhibitory and bactericidal titers (SBTs) were devised as an investigational test to evaluate the bactericidal properties of antibiotic therapy in a more physiological context, namely the serum of the patient. Specifics for the performance of SBT testing have been published by the Clinical and Laboratory Standards Institute. This assay consists of collecting patient blood samples, ideally during serum peak and trough concentrations of the antibiotic, combining equally with bacterial growth medium and testing the bactericidal titer concentration against the patient's bacterial isolate. A high SBT may indicate that the dose chosen for the patient is sufficient for treatment [6]. However, significant variability in the medium, diluent, inoculum, incubation, and controls has impeded the widespread clinical application of SBT testing. In addition, use of only the serum excludes other critical components of the patient innate immune system, for example, leukocytes, and this may fail to recapitulate the full interaction of antibiotics with host immune defenses. For example, macrolide antibiotics induce formation of neutrophil extracellular traps (NETs) containing antimicrobial peptides and histones that can ensnare bacteria and attract other immune cells [7], while beta-lactams sensitize bacteria to neutrophils [8]. Anthropocentric debates about "monotherapy" vs "combination therapy" in the treatment of infection fail to consider the fact that the net antimicrobial effect in a patient receiving antibiotic pharmacotherapy is the collective summation of exogenous pharmacotherapy and innate immune defense factors. "Monotherapy" never really exists in a pure form.

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CONVENTIONAL BACTERIOLOGIC MEDIA: AN OBSTACLE TO OUR COMPLETE UNDERSTANDING OF ANTIMICROBIAL ACTIVITY

The conceptual divergence of antibiotic pharmacology and immunology disciplines may have been aggravated by the historical choice of media used to cultivate and examine microorganism susceptibility and resistance. From the beginnings of the human antibiotic era, antimicrobial susceptibility testing (AST) of pathogens has focused exclusively on media optimized for microbial growth, with the addition of antibiotics up to the point of growth inhibition defining the "minimum inhibitory concentration" (MIC), which serves as the centerpiece of clinical microbiology [9]. AST paradigms have somewhat arbitrarily converged on Mueller-Hinton broth (MHB) for most assays in the clinical microbiology lab. This medium, first developed 1941 for isolating pathogenic strains of *Neisseria* spp. [10], does not recapitulate in vitro the in vivo environment relevant for infection, nor is it a medium in which host antimicrobial peptide activity can be reliably assayed [9]. Recent work has shown considerable differences in MICs between standard bacteriological media and more physiological media such as mammalian tissue culture media [11, 12]. Concordance between AST performed on these medias is about 60%; however, 10%–20% of the time, an MIC difference of ≥8× is noted. Importantly, antimicrobial activity in vivo has been shown to be more concordant with activity in physiological media compared with standard bacteriological media [12]. Additional studies have shown that buffering with bicarbonate, the major anionic buffer in mammalian physiology, is a major contributor to the superior predictability of physiological media in vitro antibiotic susceptibility to in vivo efficacy [11]. For example, some strains of methicillin-resistant *Staphylococcus aureus* (MRSA) demonstrate a methicillin-susceptible phenotype in bicarbonate-buffered media, and such strains can be effectively treated in animal models of endocarditis with beta-lactam monotherapy [11, 13]. Azithromycin efficacy against multiple gram-negative bacterial species (eg, *Acinetobacter baumannii*, *Klebsiella pneumoniae*) depends on bicarbonate, and this activity translates to in vivo efficacy in murine infection models [14, 15]. MHB lacks bicarbonate buffer; thus standard clinical AST testing declares azithromycin inactive against these pathogens. Another recent study shows how hyperphysiological concentrations of zinc in MHB render metallo-beta-lactamase-producing Enterobacteriaceae resistant to carbapenems in such media. However, these organisms are susceptible to carbapenems in vivo, where nutritional immunity restricts zinc concentrations to much lower levels. Removal of zinc from MHB broth more reliably predicts in vivo activity of carbapenems against these organisms [16]. Rigid continuation of AST paradigms utilizing only standard bacteriological media overlooks potential useful activities of the currently available antibiotics against multidrug-resistant bacteria [14], but it also hinders the transition of pharmacodynamic analysis of innate immunity synergy with antibiotics to a more clinical mainstream.

BACTERICIDAL VS BACTERIOSTATIC IN THE CONTEXT OF INNATE IMMUNITY

The few studies on the pharmacodynamic interactions between innate immunity and exogenous antibiotics have revealed differences in the exposure–response relationship. Some antibiotics, including those that have been traditionally labeled "bacteriostatic," such as chloramphenicol and erythromycin, may antagonize the activity of endogenous host defense peptides [17]. In contrast, beta-lactam antibiotics, which are often touted for their overall bactericidal activities, have been shown to further synergize with cationic antimicrobial peptides produced by innate immunity [8]. This synergy is so profound that the addition of antistaphylococcal beta-lactams like nafcillin and oxacillin has been successfully deployed as adjunctive salvage therapy to successfully clear refractory MRSA bacteremia [18, 19]. Ampicillin has been used in an analogous fashion to clear persistent bacteremia due to ampicillin-resistant, vancomycin-resistant *Enterococcus faecium* (VRE) [20]. In both cases, concentrations well below the MIC rendered MRSA and VRE hypersusceptible to cationic antimicrobial peptides, a property completely missed in standard AST testing, which may serve a critical role in the resolution of severe infection. Synergy with antimicrobial peptides has also been shown for ceftaroline against *Streptococcus pneumoniae* [21] and ceftriaxone against *Salmonella enterica* [22]. The cationic defense peptide cathelicidin is a key host defense against systemic infection and bacterial meningitis. Antimicrobial synergy with cathelicidin may be an important factor driving better clinical outcomes [22].

This enhancement between antibiotics and immune response extends beyond beta-lactams. Azithromycin, a macrolide antibiotic, demonstrates synergy with host antimicrobial peptides against *Pseudomonas* and *Acinetobacter* [14]. Even certain beta-lactamase inhibitors, conceptually deployed to inhibit beta-lactam hydrolysis by beta-lactamase enzymes, can themselves act synergistically with endogenous antimicrobial peptides or peptide antibiotics like daptomycin and colistin [23, 24]. All of these properties require the use of physiological (eg, tissue culture) media to evaluate, as host antimicrobial peptides are generally not active in bacteriological media. Mechanisms for synergy between host innate immunity and antibiotics are certain to be multifactorial. This complexity may be one unrecognized pharmacodynamic faculty helping to explain the differences in clinical efficacy of different antibiotic classes irrespective of MIC in standard AST testing. For example, vancomycin is a far inferior agent to beta-lactams clinically against *S. aureus*, despite showing

potent activity in vitro [25, 26]. These effects may unfortunately contribute to the poorer outcomes of patient who are denied beta-lactam drugs due to penicillin "allergies" listed in their medical records [27].

The interaction of antibiotics and innate immunity can be mediated through additional mechanisms, including alteration of virulence factor expression, which in turn may influence host cytokine expression. For example, beta-lactams upregulate alpha-toxin and other exotoxin expression in *S. aureus* [28], which in turn stimulates a host interleukin (IL) 1–beta response [29], which is important in host recognition and clearance of bacteremia [30, 31]. Beta-lactam therapy is more effective than vancomycin in eliciting an IL1-beta response in patients with *S. aureus* bacteremia, including when used as adjunctive therapy for MRSA [32]. Conversely, bacteriostatic antibiotics inhibit this process [28]. Interestingly, the MRSA cell wall is less cross-linked in the presence of beta-lactam and induces a more potent IL1-beta response as well [33]. Reduction in peptidoglycan O-acetylation induced by beta-lactams has a similar effect [34]. Hence, the functional determination for bacteriostatic vs bactericidal antibiotics in bacteremia may diverge from their potency in AST testing in bacteriological media; it may reflect immune response factors that synergize with innate immunity or induce protective cytokine responses.

CONCLUSIONS

Looking toward the future, our separation of antimicrobial pharmacology and immunology has resulted in paradigms that limit our understanding of antimicrobial action in an era of ever-expanding resistance. The oncology field has made much greater strides recognizing the importance of host immune cooperativity in treating certain cancers, and significant advances toward cure of nearly untreatable cancers have been made through the use of immunologic and T-cell engineering therapies [35]. In infectious diseases, more objective examinations of the immune system—antibiotic cooperativity through mathematical modeling and computer simulation—have already been undertaken by some groups [36], which will hopefully help transition this field into a more formalized pharmacological discipline. Steps must be taken to reunite the understanding of innate immunity–antibiotic relationships to improve treatments, slow the development of resistance, and discover new therapeutic approaches.

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