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New strategies and compounds for anti-infective treatment

Editorial overview

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Robert EW (Bob) Hancock, OC, OBC, FRSC, is a professor of Microbiology & Immunology, UBC, an Associate Faculty Member of the Wellcome Trust Sanger Institute and a Canada Research Chair in Health and Genomics. His research interests include small cationic peptides as novel antimicrobials and modulators of innate immunity, the development of novel treatments for antibiotic resistant infections, the systems biology of innate immunity, inflammatory diseases and *Pseudomonas aeruginosa*, and antibiotic uptake and resistance. He has published more than 600 papers and reviews, has 44 patents awarded and is an ISI highly cited author in Microbiology with more than 45,000 citations and an *h*-index of 111 according to Google Scholar.

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Hans-Georg Sahl is a professor of Medical and Pharmaceutical Microbiology, University of Bonn. He is a speaker of the basic research unit FOR854 *Post-genomic strategies for new antibiotic drugs and targets* funded by the German Research Foundation (DFG) and coordinator of the translational unit *Novel anti-infectives* of the German Center of Infection Research (DZIF). He is interested in understanding antibiotic mechanisms on the molecular and cellular levels. Using staphylococci and chlamydia as model organisms, his focus is on the impact of antibiotics on functional organization of bacterial cell wall biosynthesis.

Resistance to antibiotics has become commonplace in pathogenic bacteria and these 'magic bullets' are losing efficacy. Current medical standards in infectious disease management, intensive care and transplantation medicine heavily rely on efficacious, classical anti-infective chemotherapeutics, and the gradual and inexorable decrease in their efficacy could reverse the successes achieved in these areas, leading to a major increase in morbidity and mortality. Despite the strong medical need, the antibacterial development pipeline is drying up and the number of innovative drugs reaching the market is rapidly decreasing. The interest of the Pharmaceutical Industry is waning on a world-wide scale since it appears increasingly difficult to discover and develop novel compounds. The reasons for this are manifold and range from immense costs for R&D programs to pharmaco-economic considerations and regulatory hurdles. However, there are also specific fundamental questions associated with the discovery, production and activities of antibiotics that need to be addressed by curiosity-driven basic research to support the anti-infective discovery and development process.

Antibiotics have always been indispensable tools for studying basic functions of bacterial cells and this in turn has helped to drive the discovery and development of new antibiotics [1]. In the last decade tremendous progress has been made in unravelling the organization of a bacterial cell. What once was considered a 'bag full of enzymes', in which anabolic and catabolic pathways are largely diffusion controlled, is now understood as a highly structured and functionally organized cell. It is now fully appreciated that all major biosyntheses are performed by highly complex synthesis machineries that require an unimagined degree of coordination over space and time to achieve, with high fidelity, vital processes such as cell division, cell wall biosynthesis, DNA replication, protein and RNA synthesis, all of which are executed in a controlled fashion. Novel microscopy techniques based on fluorescence labelling continue to provide exciting insights into the dynamics of these machineries and have entirely changed our views of the organization of prokaryotic cells. By applying these techniques we can learn more effectively about the impact of antibiotics on the dynamic function of these machineries and on the bacterial cell as a whole; that is, we can learn about antibiotic effects beyond the initial drug–target interactions. This seems likely to provide clues as to what distinguishes good antibiotic targets from poor targets, and what needs to be taken into account to improve target-based screening approaches, which in the past were rather unsuccessful. Interestingly, most of our highly successful, natural product-based antibiotics are targeting components of such machineries rather than soluble easy-to-screen cytoplasmic enzymes. Intriguingly we are becoming increasingly aware that antibiotics are often pleiotropic in their targets, with the obvious example being the targeting, by the β-lactam antibiotics, of multiple cell wall biosynthesis enzymes including penicillin

binding proteins and autolysins. This concept of multiple targets for antibiotics makes target-based screening fraught with difficulties.

Bacterial cell biology may also provide a better understanding of the cellular and environmental context in which antibiotics are produced. Bioinformatics tools are now available to easily identify, in individual genomes and the meta-genomes of microbial consortia, interesting gene clusters encoding natural compounds. Moreover the methods of Synthetic Biology are offering excellent ideas on how to redesign gene clusters to enable them to express entirely new compounds, although in most cases we are still struggling with problems of heterologous expression of gene clusters in suitable host organisms or even with the production of modified clusters in the natural host. Obviously, there is still a long way to go to translate the immense amount of available genetic information into antibiotic products that can be analyzed and developed. There remains a lot more to learn about how and when antibiotics are produced in a cell, about auxiliary functions, self-protection mechanisms and about regulation and spatial organization of production machineries. We also need to better know how nature applies antibiotics and somehow ensures, in spite of all of the resistance mechanisms present in the environment, that they are still effective after millions of years of application. For research on medical applications, this could mean that we need to focus more, for example, on analyzing concentration windows that avoid resistance development and on providing better rational for combination therapies if we are to preserve antibiotic efficacy for sustained usage rather than consuming them within a short period of time.

There is yet another set of strategies to employ that fall under the umbrella of adjuvant strategies. Therapeutic adjuvants are agent designed to increase the efficacy of primary treatments, or antibiotics in the case of bacterial infections. They may or may not have direct anti-infective activity but are likely to be applied together with conventional agents when they are used clinically and many have already been shown to have this type of activity in animal models. The most profound example of success in this regard is the β-lactamase inhibitors that are included in combination with β-lactams to defeat resistance mechanisms. In addition to such anti-resistance adjuvants that directly attack the mechanisms by which bacteria become resistant to antibiotics, there are also anti-virulence agents that attack bacterial virulence determinants and/or the lifestyle of an organism in the human body, and host directed therapies, particularly immunomodulators, which improve the ability of the host to mount an effective attack on invading microbes.

In this issue of Current Opinion in Microbiology, Sass and Brötz-Oesterhelt, Pasquina *et al.* and Roemer *et al.* describe new targets and potential drug candidates in the area of cell envelope biosynthesis and cell division. The various pathways for synthesis of cell wall polymers, such as peptidoglycan, wall teichoic acids and capsules, appear to function in a highly interdependent and integrated fashion. Not only do they share certain substrates such as UDP-activated sugars and the central carrier bactoprenol phosphate, but there also seems to be at least transient physical interactions between some of their components during the synthesis of building blocks and the coordinated assembly of the individual polymer blocks into the final cell envelope structure. This concerted action of many players can be perturbed at multiple sites, which not only offers new target sites for antibiotic intervention, but also provides opportunities to overcome existing widespread resistance mechanisms and, for example, restore methicillin susceptibility in *Staphylococcus aureus* (Roemer *et al.*). Methicillin resistance in Staphylococci (e.g. MRSA) is based on the acquisition of an additional penicillin binding protein (PBP 2a) that can take over the transpeptidase function of PBP 2 when this is blocked by methicillin. However, PBP 2a is not capable of catalyzing the essential transglycosylation reaction and thus it has to cooperate with the PBP2 transglycosylation domain to enable the production of functional peptidoglycan cell wall. The physical integration of an additional protein into the highly dynamic, multi-protein machinery seems to require perfect functioning of all other components such that inhibitors of other reactions within the synthesis and decoration pathways, with little or no antibiotic activity on their own, may well synergize to restore the methicillin sensitivity phenotype.

An impressive example within this scenario is the inhibition of wall teichoic acid (WTA) as described by Pasquina *et al.* WTA is a multifunctional cell envelope polymer that gets attached to peptidoglycan outside the cell. WTA is not essential for growth *per se* and in certain genetic backgrounds can be knocked-out, however, the inhibition of, for example, the WTA export process in wild type cells is lethal and also re-sensitizes bacterial cells towards methicillin. Cell division is another central process in the bacterial life cycle that has not been specifically targeted by antibiotics currently in use. It requires a well characterized protein machinery, the so-called divisome, that needs to be assembled at mid cell and that also requires de novo synthesis of peptidoglycan in a coordinated fashion at the same site. Interestingly, the divisome and the peptidoglycan synthesis machineries are partially overlapping by sharing some essential components at the molecular level, and certain inhibitors that primarily interfere with the division process, impair cell envelope pathways as a secondary effect and restore, for example, methicillin sensitivity. FtsZ is a pacemaker in the divisome assembly process and a prime target for targeting by antibiotics. Sass and Brötz-Oesterhelt summarize recent developments on inhibitors of FtsZ and other cell division components and also describe a new

class of antibiotics, the acyldepsipeptides (ADEPs), which impact on cell division in a most remarkable, indirect way. The primary target of ADEPs is the intracellular protease ClpP which becomes dysregulated after ADEP binding such that FtsZ becomes a target for degradation through uncontrolled ClpP activity.

The vast majority of antibiotics in current clinical use were derived from natural products that are still considered as the most promising source for new antibiotic compounds, substantially because of their striking structural diversity and novelty. The search for new natural compounds has been intensified in recent years and microbial isolates from previously under-investigated habitats such as marine ecosystems are being increasingly characterized. In this issue, Bills and coworkers have focussed on a specialized group of fungi that colonize mammalian dung, a particularly rich and competitive habitat that is poorly studied to date. The available literature suggests that coprophilic fungi could indeed be a source for new natural products although unsurprisingly well-known classes of natural products are also found.

Another strategy for identifying new classes of compound is to identify those targets that are most critical to the functioning of cells. In particular in this issue Zoraghi and Reiner discuss protein:protein interactions (PINs) and hubs as sources of new antibiotics. Cells can be considered as dynamic networks of interacting components (largely proteins and nucleic acids) that interact physically, biochemically or to mediate transcription and translation. In particular certain proteins are called hubs because they participate in PINs with many other partners. By definition such hub proteins are thought to be exceptionally important since they directly communicate within cells receiving and transmitting signals to multiple partners, and thus are considered optimal targets for antibiotic targeting. Zoraghi and Reiner provide concrete examples of the approach to determining promising novel targets in *S. aureus* (pyruvate kinase) and *M. tuberculosis* (PknK) that might otherwise not have been identified.

As new compounds are developed there is an increasing need to move rapidly towards more sophisticated views of how they will work and there is no doubt that the success of a compound is determined in part by its pharmacokinetics and pharmacodynamics (PK/PD). Jian Li and colleagues provide an update on the use of *in vitro* PK/PD models that show excellent performance and have strong predictive value in determining how to deliver compounds to minimize antibiotic resistance.

In addition to attempts to develop new bactericidal drugs, the threatening rise in antibiotic resistance clearly warrants the evaluation of alternative anti-infective strategies, particularly the adjuvant strategies mentioned above. Compounds that modulate microbial virulence or special growth states that exist in the body or support host defence functions seem to be well suited to decreasing severity of infections, boosting the effectiveness of conventional antibiotics and lowering the pressure for microbes to develop resistance. In addition to the inhibition of toxin secretion, interference with bacterial quorum sensing and biofilm production appears to represent promising intervention strategies. Attacking biofilms, which are surface associated consortia of organisms that adopt a distinctive growth state, is particularly important since biofilms cause more than 65% of all infections and are highly adaptively resistant to multiple classes of antibiotics. de la Fuente-Núñez *et al.* provide an update on recent advances in understanding the physiology of biofilms and particularly on the development of new agents that specifically attack bacteria in the biofilm state, hindering development or actually causing dispersal of biofilms.

One particularly intriguing area that has been minimally employed for bacteria but is a mainstay of anti-cancer and anti-viral therapy is immunomodulation, basically increasing the potency of natural host defences against infections. Nijnik provides a state-of-the-art review on the development and clinical applications and trials of immunomodulatory approaches for prevention and treatment of infectious diseases. An alternative strategy is to use bacteriotherapy, which employs a selected consortium of beneficial bacteria from the gut microbiota to selectively impact on immune defences while providing competition for pathogenic microbes. Adamu and Lawley provide an exciting application of this concept for the treatment of intestinal dysbiosis caused by *Clostridium difficile* infection.

The alarming and steady rise in antibiotic resistance and very limited new antibiotics entering the clinic or under development has caused enormous concern amongst health officials in our society. It is thus exciting to see that permeating up from academia are some new exciting ideas about how to move towards innovative treatments that will work in the antibiotic resistance era.

References

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