Editorial: Introduction to thematic issue ‘Molecular Effectors of Tuberculosis Pathogenesis’

The journal Pathogens and Disease (PAD) was launched in early 2013 at the instigation of its Editor-in-Chief, Dr. Patrik Bavoil of the University of Maryland Baltimore, to replace the former FEMS-IMM (Federation of European Microbiological Societies, Immunology and Medical Microbiology) journal. Its aims as defined by Editor and Members of the Editorial Board were ‘to publish outstanding primary Research Articles, Short Communications and Mini Reviews reporting on hypothesis- or discovery-driven studies relating to pathogens (eukaryotes, prokaryotes and viruses that infect humans and animals), the host pathogen interaction, the host response to infection and their molecular, cellular, microbial and environmental correlates. Commentaries and Perspectives on matters related to articles published in Pathogens and Disease or on topics of interest in infectious diseases research are also welcome’. Subsequently, thematic issues (TIs) became a specialty of PAD and some outstanding TIs have been published over the years, for instance several on microbial biofilms; coinfections due to the convergence of many classes of microorganisms; Neisseria; several on Chlamydia research; Pertussis; Rickettsia and other intracellular bacteria; pathogenesis and host response in Helicobacter infections; bacterial toxins; and a few on immunological responses to microbial infections such as ‘the Dynamic Relationship between Host and Bacterial: Resistance, Tolerance and Disease…’ (PAD, volume 75, issue 2, 1 March 2017). Throughout there has been no dedicated TI devoted to tuberculosis (TB). Indeed, PAD has not been a major venue for outstanding primary research articles on TB. In order to rectify that deficit, Dr. Bavoil first approached Dr. Patrick Brennan, a long-term Associate (handling) Editor of PAD, in 2015 with the idea of assembling the nine articles on mycobacteria published in PAD in that year into a TI. However, on consideration we could not identify a common theme among these and instead sought a plan more directly related to mycobacterial pathogenesis in accord with the central theme of the Journal.

Only in 2017 with the recruitment of Dr. Kathleen McDonough (a member of the Editorial Board of PAD) did we begin to determinedly address this challenge. Dr. McDonough’s research on the genetic and biochemical basis of TB infection and disease complements the research of Dr. Brennan on the structural basis of many of the mycobacterial cell wall/envelope effectors of TB pathogenesis. Accordingly, the chosen subject for this themed issue of PAD was ‘Molecular Effectors of Tuberculosis Pathogenesis’ reflecting our twin perspectives on the molecular nature of those mycobacterial agents responsible for the peculiar aspects of TB/mycobacterial pathogenesis and virulence, and mechanisms of how such is accomplished.

TB is the leading cause of death due to a single infectious agent, causing nearly 1.7 million deaths and 10.4 million new cases of disease in 2016 (WHO Report 2017). It has been 20 years since the first complete Mycobacterium tuberculosis (Mtb) genome sequence was published (Cole et al. 1998). This publication, along with its online companion resource Tuberculist (Lew et al. 2011), was a major turning point for the field. Research on the molecular basis of TB pathogenesis has since flourished, resulting in numerous new candidates in the drug and vaccine pipelines (Schito et al. 2015). Yet TB remains a major global health concern, the problem of drug resistance continues to grow, and new technological breakthroughs are still urgently needed to break this cycle (WHO Report 2017). Much essential groundwork has been laid on Mtb physiology, but a key question that must be asked is whether we are missing some fundamental truth(s) about Mtb biology that can be targeted to stop the insidious progression of this most successful of pathogens, and if so, what are they? This thematic issue is an attempt to stimulate such research by providing a compilation of reviews addressing current understanding of several unique aspects of Mtb biology, with a focus on critical unanswered questions and the resources that are available to investigators who are willing to tackle them.

The M. tuberculosis H37Rv DNA sequence (Cole et al. 1998) provided a critical platform for addressing some of the technical difficulties associated with the slow growth of Mtb and rudimentary tools for genetic manipulation. Suddenly genes and their promoters could be predicted prior to cloning, and transposon mutations could be mapped rapidly by DNA sequencing. Such tools have now been widely exploited to identify genes of importance for Mtb grown in different conditions by using population-based mutational analyses (Sassetti, Boyd and Rubin 2001; Dejesus et al. 2017; Rock et al. 2017). Likewise, advances in DNA sequencing provided a tremendous boon for molecular typing and TB epidemiology, and the number of sequenced Mtb genomes now exceeds 5900 (https://www.ncbi.nlm.nih.gov/genome/?term=mycobacterium:tuberculosis). While the resulting datasets provide a rich source of information, the mutant libraries, tools for genetic manipulation of Mtb and clinical strains from diverse sources used in different studies also provide powerful physical resources for investigators in the field. Numerous repositories have been developed to distribute these materials, and Hazbón et al. (2018) in this thematic issue address the challenge of finding them by providing an extensive review...
of resources available to the TB research community. The availability of many attenuated mycobacterial strains also removes the requirement for containment facilities in the course of genetic manipulation of some Mtb, further expanding the range of investigators with diverse expertise who can contribute to the development of creative solutions for TB control. Another powerful breakthrough made possible by this molecular genetic revolution is the elegant use of imaging with fluorescence-based reporters to follow the interactions between Mtb and its host in real time. MacGilvary and Tan (2018) provides an elegant and practical guide to the use of fluorescent reporter technology for such research on TB.

The Mtb genome sequence has allowed comparison of the overall genetic blueprint of Mtb with those of other better studied bacteria, such as Escherichia coli and Bacillus subtilis. One thing we have learned is that Mtb often follows its own rules with respect to conserved nucleotide signaling strategies. For example, genome analyses first showed that Mtb encodes up to 16 distinct adenylyl cyclases (ACs), compared to only one in other model organisms, including E. coli. The absence of catabolite repression in Mtb is another point of departure between cAMP signaling in Mtb versus the bacterial paradigm established in E. coli. Rather, the cAMP produced by Mtb ACs contributes to virulence by modulating host macrophage responses when secreted by Mtb, and by extensively regulating Mtb gene expression and physiology when retained within the bacterium, as reviewed by Johnson and McDonough (2018). Similarly, Mtb uses (pp)pGpp to respond to starvation through a conserved stringent response, and (pp)pGpp is a critical regulator of virulence in Mtb. The stringent response in Mtb also follows a path distinct from the paradigms established for either E. coli or B. subtilis. Prusa et al. (2018) provide a comprehensive review of this novel stringent response in Mtb, including its potential as a target for treatment.

Small regulatory RNAs (sRNAs) and toxin–antitoxin (TA) systems are relatively newly discovered regulatory elements in many bacteria, and Mtb has considerable novelty in both types of molecules. TA systems include a toxin that inhibits bacterial growth and a cognate antitoxin that regulates the effects of the toxin. Genome sequence analyses have identified an excessive number of Type II TA loci in TB complex bacteria compared to just a handful in non-pathogenic mycobacteria. Sladen et al. (2018) discuss the potential importance of these abundant VapBC, MazEF and ParDE/RelBE family regulators for Mtb virulence and persistence. The discovery of numerous regulatory RNAs in Mtb required next generation sequencing methods such as RNAseq, because the presence of non-coding RNAs cannot be easily deduced from only the genome sequences. RNA sequences such as riboswitches or antisense RNAs act on contiguous or overlapping genes, while trans-acting small RNAs (sRNAs) regulate expression of distal genes. Schwenk and Arnvig (2018) provide a valuable primer on the different types of regulatory RNAs found in Mtb, whose roles in pathogenesis are only just beginning to be explored.

The Mtb genome also provided new insights into Mtb’s physiological idiosyncrasies. For example, the unusually high number of genes annotated with putative roles in production and consumption of fatty acids provided some of the first evidence of Mtb’s near obsession with fatty acid oxidation and its importance to the biology of Mtb during infection; the role of phthiocerol dimycocerosate (PDIM) as a ‘virulence factor’ of Mtb—the former ‘attenuation factor’ of Mayer B. Goren—is a perfect example (Cambier et al. 2014). Wilburn et al. (2018) describe the mechanisms by which Mtb acquires and metabolizes fatty acids and cholesterol from its mammalian host to drive its pathogenesis, suggesting new targets for therapeutic intervention. Mtb is also exceptional in using a combination of mycothiol and ergothioneine rather than glutathione to maintain its intracellular redox balance during infection, and this complex topic is deftly navigated by Facl et al. (2018). The conserved family of redox-sensitive WhiB transcription factors first identified by genome analyses is now known to have multiple diverse roles in Mtb virulence, linking redox sensing with lipid production and manipulation of host macrophage responses. The intimate relationship between Mtb and its host macrophages has been an intense focus of interest for decades. However, fundamental new insights on the molecular effectors of these interactions continue to advance understanding of precisely how Mtb subverts the host immune system, with the promise for improved interventions, as discussed in a comprehensive yet accessible review by Upadhyay et al. (2018).

While the molecular genetic revolution has tremendously advanced our fight against TB, its greatest value is as a means rather than an endpoint, and our need to understand the underlying fundamentals of Mtb physiology has not changed. In this regard, features of the unusual cell envelope of Mtb have been a seminal area of study for many decades. Most of this work predated genome sequencing, relying on the evolution of mass spectrometer (MS) and nuclear magnetic resonance (NMR) to their present advanced state. However, genome definition and ensuing genetic manipulations (e.g. transposon and knock-out mutagenesis and (over)expression of genes of interest under the control of the appropriate promoters in replicative or integrative plasmids) combined with classical enzymology has allowed complete definition of the anabolic pathways responsible for the synthesis of the full range of exotic complex lipids that underlie the entire pathogenesis of TB and the cell wall ‘core’ that ensure their proper cellular location (Jackson, McNeil and Brennan 2013; Angala et al. 2014). PDIM is one example of such potent ‘virulence factors’; LAM in its variable structural oddities across various Mycobacterium spp. is another, but its role in the immunopathogenesis of TB is much more variable, of a yin-yang nature. Turner and Torrelles (2018) review how understanding of the multifaceted roles of mannose-capped lipoarabinomannan (ManLAM) in mediating interactions with host cells provides compelling evidence for a key role of this potent effector in dictating the immune response to Mtb. Recent studies showing that mycobacterial cell envelope biosynthesis and function are spatially coordinated and controlled from within laterally distinct microdomains in the plasma membrane are described by Puffal et al. (2018). The relationship of these microdomains to the development of mycobacterial extracellular vesicles (MEVs) that are derived from the Mtb cell envelope awaits further investigation. Nonetheless, MEVs are thought to be involved in iron acquisition and modulate the immune response when secreted by intracellular Mtb within macrophages due to their carriage of lipo-glycans such as lipoarabinomannan (LAM) (Gupta and Rodriguez 2018). This review by Rodriguez and Gupta is highly significant in that it provides a comprehensive understanding of the mycobacterial content of a variety of MEV derived under varying conditions; there is the dominant presence of polar lipids, such as the PIMs (phosphatidyl inositol mannosides) and other more conventional phospholipids, but, depending on the mycobacterial species and conditions of MEV production, also acyl-trehaloses (cord factor?), acyl-glycerols, lipoproteins, etc., all of which have been previously implicated in the immunopathogenesis of TB.
As the field of mycobacterial physiology and genetics has matured over the last two decades, it has increasingly enhanced a wide range of efforts to understand the molecular bases of Mtb biology in the context of physiologically relevant environments, as evidenced by the articles in this thematic issue. However, in some ways this grand journey has taken us back to our starting place, but with a much larger and more diverse tool kit. Yet, it is more essential than ever that we understand the molecular effectors that determine Mtb’s destiny at any given time. In this regard, the newly emergent fields of systems biology, transcriptomics, lipidomics and metabolomics are important additions to longstanding biochemical, structural and microbiological approaches. The current emphasis on a more holistic focus on the fundamentals of Mtb physiology, with the benefit of molecular genetic tools that were not previously available, bodes well for the next decade of TB research. This powerful arsenal of new insights and tools for discovery of how Mtb eats, sleeps and interacts with its mammalian hosts at the molecular level is also available to an ever wider range of creative investigators. Together we are poised to make exceptional strides in understanding of this complex pathogen with the common goal of eliminating TB.

Conflict of interest. None declared

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