Molecular Mechanisms of Host-Pathogen Interactions and their Potential for the Discovery of New Drug Targets

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Abstract: Vaccines and chemotherapy have undeniably been the discoveries in the field of biomedical research that have exerted the biggest impact on the improvement of public health. Nevertheless, the development of bacterial resistance to antibiotics has co-evolved over time with the discovery of new drugs. This entails the necessity for continuous research on new anti-infectious agents. The current review highlights recent discoveries in the molecular mechanisms of specific host pathogen interactions and their potential for drug discovery. The focus is on facultative and obligate intracellular pathogens (Mycobacterium, Chlamydia and Legionella) and their manipulation of host cells in regard to inhibition of phagosome maturation and cell death. Furthermore, the composition and role of the SecA2 and the ESX-1 secretion pathways in bacterial virulence and manipulation of infected host cells is discussed. The central hypothesis proposed in this review is that the characterization of bacterial proteins and lipids involved in host cell manipulation (modulins) will provide an abundance of new drug targets. One advantage of targeting such bacterial modulins for drug development is that these anti-modulin drugs will not disrupt the beneficial host microflora and therefore have fewer side effects.

Key Words: Apoptosis, phagosome, mycobacterium, secretion, cell wall, antibiotics, drug resistance, infection, modulin.

INTRODUCTION

The era of chemotherapy began with the discovery of Salvarsan 606 by Dr. Sahachiro Hata in Paul Ehrlich’s laboratory in 1909 for the treatment of syphilis following the unsuccessful screening of 605 chemical compounds. The rational for this effort was based on the “Magic Bullet Theory” formulated by Ehrlich which states that if a chemical can specifically stain bacteria and not host cells, then it should also be possible to identify compounds with specific toxicity to bacteria and not host cells [1]. The subsequent discovery of antibacterial compounds of synthetic, semisynthetic and natural origins led to a great reduction in mortality and morbidity due to bacterial infectious diseases [2]. However, cases of antibiotic resistance in bacterial infections have been increasing ever since the introduction of penicillin in the 1940s, and antibiotic resistance was instantaneous for the first treatment of a tuberculosis patient with the anti-tuberculosis antibiotic streptomycin [2-4]. Presently, there are several notable human pathogens with multi-drug resistance that pose a serious threat to public health (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium tuberculosis). As a consequence there is a pressing need for the discovery of new and/or improved drugs and vaccines.

In general antibiotics inhibit essential enzymatic processes in the bacterium such as replication, transcription, translation or cell wall synthesis and either kill the bacteria (bactericidal) or inhibit bacterial replication (bacteriostatic). The current review proposes the hypothesis that a new class of anti-bacterial compounds (anti-modulins) that target the bacterial proteins/lipids that modulate the host immune response will be a significant asset for treatment of multidrug-resistant bacteria. By attacking specific virulence mechanisms of pathogens, these compounds have the benefit of not harming the normal human microflora which should result in reduced side effects for the patient. In addition, this approach may prove to be especially advantageous for the defense against facultative and obligate intracellular pathogens that rely on the manipulation of the host cell for their intracellular survival and long-term persistence in the host. Several host-pathogen interactions with the potential for new drug targets will be discussed. The focus is on the intracellular pathogen Mycobacterium tuberculosis but examples of other human pathogens that employ similar strategies to manipulate the host cell are given throughout the review.

BACTERIAL TARGETS THAT MODULATE SPECIFIC HOST CELL DEFENSE PATHWAYS

1) Inhibition of Phagosome-Lysosome Fusion (“Phagosome Maturation”)

The first line of defense of the cellular innate immune response are the phagocytes that roam the body in search of invaders to ingest and kill. After initial phagocytosis the phagosome gradually changes its composition by fusing sequentially with early endosomes, late endosomes and finally lysosomes; a process defined as “phagosome maturation” [5-7]. The end result is a phagolysosome that has a low pH and a high concentration of activated proteases, lipases and DNAses ready to degrade the phagosome content [8, 9].

This is an extremely efficient process that will kill most ingested bacteria. However, many facultative and obligate in-
intracellular pathogens have developed strategies to avoid being in a phagolysosome.

To achieve this goal, one strategy is to inhibit the maturation of the phagosome. Thus it was demonstrated originally in the 1970’s that Mycobacterium tuberculosis (Mt) containing phagosomes do not acquire the characteristics of a phagolysosome [10]. During the following three decades, the characteristic composition of the Mt-phagosome was studied intensively and a wealth of information was gathered (for review [11-13]). In essence, the Mt-phagosome resembles an early phagosome which is reflected by its neutral pH due to exclusion of the host cell vacuolar proton pump, its accessibility to recycling cell surface molecules such as the transferrin receptor (important for supply of iron) and exclusion of lysosomal markers such as CD63. The Mt-phagosome contains small Rab GTPases characteristic of early endosomes (Rab4, Rab5, Rab14 [14]) but not for late endosomes and lysosomes (Rab7, Rab9). In addition, the recruitment of host cell coronin-1 to the Mt-phagosome is an important step since Mt cannot inhibit phagosome-lysosome fusion in coronin-1 deficient mice [15]. Furthermore, lipids also play an important role since the Mt-phagosome has reduced levels of phosphatidylinositol 3-phosphate (PI3P), which consequently inhibits the recruitment of other FYVE-domain containing proteins that are important for the fusion with late endosomes [5, 7, 11-13, 16].

How do Mt bacteria achieve the inhibition of phagosome maturation? The secreted serine/threonine protein kinase G (PknG) of Mt was characterized as an important mediator of phagosome maturation inhibition [17]. Interestingly, an inhibitor of PknG (AX20017) added to macrophages during infection resulted in the maturation of the Mt-phagosome and intracellular killing of the bacteria [17]. The PknG kinase was identified as one of eleven potential serine/threonine kinases in the genome of Mt due to its homology with eukaryotic kinases [18]. Therefore, it was not unreasonable to assume that the PknG inhibitor, AX20017, might cross-react with kinases of the host cell. This hypothesis was tested by screening the effect of AX20017 on 28 eukaryotic kinases, which revealed no inhibitory activity of the compound on the tested eukaryotic kinases [19]. Furthermore, the resolution of the structure of the kinase-inhibitor complex revealed that the inhibitor is buried deep within the adenosine-binding site of the kinase and the AX20017-binding pocket is shaped by a unique set of amino acid side chains that are not found in any human kinase [19]. The authors conclude that these results not only explain the specific mode of action of AX20017 but also demonstrate that virulence factors which are highly homologous to host molecules can be successfully targeted to block replication of pathogens [19]. This important conclusion implies that bacterial targets for drug development should not be excluded a priori due to high homology to eukaryotic proteins.

In addition, at least four independent studies used genetic screens to isolate mutants of Mt that are defective in inhibition of phagosome maturation, thus providing an array of new potential drug targets [20-23]. One of these genetic screens identified four mutations in genes of the ESX-1 secretion system [22]. This system is encoded by the RD1-locus which is not present in the genome of the tuberculosis vaccine strain M. bovis BCG and is the main cause of attenuation in BCG [24, 25]. The results by MacGurn et al. thus confirmed a previous report that the ESX-1 system is important for the capacity of the bacteria to inhibit phagosome maturation [26]. Nevertheless, the secreted effector protein(s) seems to be encoded outside the ESX-1 complex [22]. The importance of the ESX-1 system for inhibition of phagosome maturation was further corroborated by the identification of a novel ESX-1-dependent secreted protein (espB) which is encoded by Rv3881c or Mh3881c in M. tuberculosis or M. marinum, respectively [27, 28]. The deletion of espB results in acidification of the bacterial phagosome [28]. It is therefore likely that any drug that inhibits this secretion system will also inhibit the capacity of the bacteria to modulate the phagosome maturation process.

Mycobacteria are not the only pathogens that manipulate phagosome maturation and, as discussed for Mt, the identification of bacterial effector proteins should provide good targets for drug development against these pathogens. The intracellular pathogen Legionella pneumophila, causative agent of Legionnaires’ pneumonia, avoids phagosome maturation by recruiting early secretory vesicles from the host cell endoplasmic reticulum (ER) and thus transforms its phagosome into a ribosome-studded compartment that resembles the host cell rough ER [29-32]. The protein family of small GTPases are important regulators of intracellular vesicle trafficking and among them is the group of Rab proteins that has more than 60 members in mammalian cells [33, 34]. The small GTPase Arf1, for example, is involved in the retrograde transport of vesicles from the Golgi to the ER [35]. Legionella targets and activates host cell Arf1 via secretion of a guanine nucleotide exchange factor (RalF) by the Dot/Icm secretion system [36, 37]. The activation and recruitment of host cell Arf1 will presumably establish vesicular trafficking of the host cell Golgi to the Legionella phagosome with components that usually are transported to the host cell ER and thus support the creation of the ER-like characteristics of the phagosome. The second important intracellular target of Legionella is another small GTPase, Rab1, that is essential for anterograde ER to Golgi vesicle trafficking [33, 34]. Two recent publications demonstrated that the Dot/Icm secreted protein, independently named either DrsA or SidM, is a Rab1 specific, guanine nucleotide-exchange factor that increases the activity of intracellular Rab1 [38, 39]. In addition, LidA of Legionella binds to Rab1 and synergizes with DrsA/SidM for the recruitment of activated Rab1 to the Legionella phagosome [39]. Although its function is less well defined, it seems as if SidJ is another protein important for maintaining the Legionella phagosome [40]. In summary, the manipulation of host cell small GTPases involved in ER to Golgi anterograde and retrograde trafficking by secreted Legionella proteins is clearly important in establishing the unique characteristics of the Legionella phagosome.

Finally, members of the obligate intracellular bacteria of the genus Chlamydia are a leading cause of sexually transmitted disease cases in the USA and for infection-induced blindness world wide [41, 42]. The bacteria replicate within a membrane-bound vacuole that avoids fusion with the endosomal/lysosomal pathway and instead fuses with vesicles derived from the Golgi-apparatus [43-45]. The Chlamydia vacuole recruits intracellular Rab6 proteins [46, 47]. Chla-
mydia remains genetically intractable and therefore it is a challenge to identify bacterial proteins that manipulate the host cell. In an elegant study Cortes et al. used a combination of biochemical and genetic approaches to identify several proteins of Chlamydia that are contained in the vacuolar membrane and interact with host cell Rab1, Rab10 and Rab11 [46]. This study was an important step in laying the groundwork for a molecular analysis of the Chlamydia-mediated host cell manipulation, and in identifying a series of interesting drug targets.

2) Inhibition of Host Cell Death

Programmed cell death (PCD) or apoptosis plays an important role in the innate immune response (IR) against pathogens, a defense strategy that is evolutionarily conserved and extends even into the plant world [48-50]. It is therefore essential for persisting, intracellular pathogens to have strong anti-apoptosis mechanisms. Viral inhibition of host cell apoptosis has been extensively studied and there are numerous examples of viral proteins directly interfering with host cell apoptosis signaling [51-53]. Furthermore, an increasing number of protozoal pathogens have been shown to manipulate the PCD of the infected host cell [54, 55]. This review examines the recent advances in the understanding of the Mtb-mediated host cell apoptosis inhibition but also introduces some important anti-apoptotic mechanisms of other bacterial pathogens that may serve as potential drug targets (also reviewed in [56-59]).

Virulent but not avirulent strains of mycobacteria inhibit apoptosis of primary human alveolar macrophages and therefore the capacity of Mtb to inhibit apoptosis was proposed to be a virulence factor [60]. The importance of apoptosis in the host’s innate immune response was underlined by a report that apoptotic cell death reduced mycobacterial viability, whereas necrotic cell death had no effect on bacterial viability [61-63]. In line with these findings is a report demonstrating that the susceptibility of different mouse strains to mycobacterial infections could be linked to the capacity of infected macrophages to either undergo necrotic or apoptotic cell death upon infection, with the former imparting a susceptible phenotype and the latter a resistant phenotype [64]. The importance of apoptosis for the acquired immune response against Mtb was suggested by the demonstration that phagocytosis of apoptotic bodies containing mycobacteria by DCs could lead to the presentation of mycobacterial lipid and peptide antigens and subsequent activation of specific T-cells [65]. The phagocytosis of apoptotic bodies seems to be an important mechanism by which DCs gain access of extracellular antigens to MHC I molecules for priming of cytolytic T-cells, a process defined as “crosspriming” [66]. Remarkably, apoptotic bodies containing mycobacteria have the capacity to protect mice from challenge by virulent Mtb [67]. All of these results support the importance of the inhibition of apoptosis for the virulence of M. tuberculosis.

In general, apoptosis can be induced via two pathways: the extrinsic pathway, which involves death receptors like CD95 that activate Caspase-8/10 upon ligation, and the intrinsic pathway, which is triggered upon intracellular stress sensed by the mitochondria and initiated by activation of Caspase-9. Both pathways converge at the level of the Caspase-3/6/7 activation which then triggers the subsequent events associated with apoptosis, e.g. fragmentation of genomic DNA (for a detailed review see [68, 69]). Some evidence suggests that Mtb inhibits the intrinsic pathway of apoptosis induction since Mtb induces upregulation of anti-apoptosis genes mcl-1 and A1 which both encode for Bcl-2-like proteins localized in the mitochondria. In addition, these results were corroborated by functional data using either anti-sense oligonucleotides to knock-down mcl-1 expression [70] or A1 knock-out mice to demonstrate the importance of these genes for Mtb mediated host cell apoptosis inhibition [71, 72]. On the other hand, virulent Mtb strains inhibit FasL-induced apoptosis in Fas (CD95) expressing cells [73], which suggests an Mtb-mediated inhibition of the extrinsic apoptosis pathway. The same group reported recently that Mtb lipoglycan stimulates the activation of NF-κB via TLR-2 and that the subsequent upregulation of cellular anti-apoptotic protein FLIP leads to inhibition of FasL-mediated apoptosis [74]. Furthermore, it was suggested that Mtb stimulates the secretion of soluble TNF-R2, which leads to the reduction of bioactive TNF-α in the medium and therefore less stimulation of the TNF-R1 [75]. Altogether, virulent Mtb appear able to inhibit induction of host cell apoptosis via multiple pathways; clearly the extrinsic apoptosis pathway via death receptors (FasL, TNF-R) is affected but also the intrinsic pathway, through the increase in anti-apoptotic mitochondrial proteins like mcl-1, is modulated by Mtb.

The molecular mechanisms of the Mtb-mediated apoptosis inhibition are poorly understood largely due to a lack of defined apoptosis mutants. In order to identify genes of Mtb that are important for the inhibition of apoptosis, we recently performed a “gain-of-function” genetic screen. The apoptosis-inducing, nonpathogenic M. smegmatis (Msmeg) was complemented with genomic DNA fragments of virulent M. tuberculosis and 312 individual Msmeg-clones were screened for a reduction in infection-induced apoptosis. This led to the identification of three independent regions in the genome of Mtb that could mediate inhibition of apoptosis in a human macrophage-like cell line [76] and V.Briken unpublished data). Surprisingly, the anti-apoptotic gene of one of these regions (nuoG) encodes for a subunit of the NADH-dehydrogenase, NDH-1. The deletion of nuoG in Mtb lead to an increase of apoptosis in macrophages after infection and an attenuation of the mutant in infected mice [76]. How the NDH-1 mediates apoptosis inhibition is not clear, but what is certain is that the enzymatic activity of NDH-1 is lost by the deletion of nuoG (C. Vilcheze, W.R. Jacobs Jr. [HHMI] and V.Briken, unpublished results). Therefore it seems likely that the enzymatic activity of NDH-1 plays an essential role in host cell apoptosis inhibition. If that conclusion is valid, then any drug that specifically inhibits this enzyme, but not its eukaryotic homolog located in the respiratory chain of mitochondria, will be of interest for therapeutic use. The anti-apoptotic genes in the other genomic regions remain to be identified but should also provide new drug targets for interference with the capacity of Mtb to inhibit host cell apoptosis.

Superoxide dismutase A (SodA) of Mtb has been demonstrated to be involved in apoptosis inhibition of host cells.
The study by Hinchey et al. also very convincingly shows the importance of host cell apoptosis for the induction of a cytolytic T-cell response by comparing antigen specific CD8+ T-cells in mice infected with wild-type and SodA deficient Mtb. The mice infected with the SodA mutant produced an increased cytolytic T-cell response when compared to mice infected with wt-Mtb which underlines the importance of host cell apoptosis for an efficient induction of the acquired host immune response [78]. Superoxide dismutases of pathogens have been implicated in the bacterial defense against phagosomal reactive oxygen species (ROS)[8]. However, their role in inhibition of host cell apoptosis has not been appreciated, although interestingly it is well documented that eukaryotic superoxide dismutase is very important in protecting cells against apoptosis induction via protection from mitochondrial ROS [79, 80]. SodA is a major secreted protein of Mtb and is thus an appealing drug target. The major challenge will be to identify a drug that does not interfere with the host cell’s superoxide dismutases.

Finally, a third anti-apoptotic protein of Mtb has recently been identified that is part of the family of serine-threonine protein kinases. As described above PknG is important for the inhibition of Mtb phagosome maturation. The promoter of PknE has been shown to be induced by nitric oxide (NO) stress conditions [81]. The deletion of PknE resulted in a mutant that was more susceptible to NO exposure and also capable of inducing a higher level of apoptosis in human macrophages compared to wt [82].

An increasing number of pathogenic bacteria are being identified that mediate host cell apoptosis inhibition, and among them the best studied to date is Chlamydia spp. (for detailed review see [59]). The first description of the anti-apoptotic capacity of Chlamydia demonstrated that infected cells are resistant to apoptosis induction via a wide array of external pro-apoptotic ligands [83]. Subsequent reports demonstrated that infection with Chlamydia mediated the specific degradation of pro-apoptotic proteins which lead to the inhibition of mitochondrial and CD95 induced apoptosis [84-87]. Unfortunately, Chlamydia bacteria are still genetically intractable and thus genetic screens or other genetic approaches to identify bacterial mediators of apoptosis inhibition are not possible. This drawback is compounded by the biology of Chlamydia which, as an obligate intracellular pathogen, does not grow in vitro and therefore the biochemical approaches to identifying important mediators are difficult. Combining genomics and bioinformatics in silico has allowed the prediction of target proteins. One such candidate, the CPAF (Chlamydia protease-like activity factor) was recently shown to be important for the degradation of pro-apoptotic host cell proteins [88-91]. In addition, this protease is involved in the degradation of host cell transcription factors important for initiation of MHC class I and II gene transcription, thus reducing the levels of these two major classes of antigen presenting molecules in the infected cells [92]. Consequently, CPAF is a prominent drug target for inhibiting the capacity of Chlamydia to modulate the infected host cell. Further bioinformatic analysis revealed a list of 100 hypothetical proteins in the Chlamydia genome that are predicted to be in the membrane of the inclusion body and could thus modulate the host cell response. This information can now be used to generate antibodies against the proteins, confirm their localization and subsequently help to further our understanding of this complex host-pathogen interaction [88, 93-96].

In a twist on the current theme of clear-cut bacteria mediated host cell apoptosis inhibition, Abu-Zant et al. report that Legionella pneumophila, despite Caspase-3 activation early during infection in human macrophages, inhibits the subsequent stages in the apoptosis pathway by upregulation of at least 12 anti-apoptosis genes of the host cells [97, 98, 99]. A separate study implicated Dot/Icm-dependent upregulation of anti-apoptotic genes in the prevention of host cell death after low-dose Legionella infection, presumably for injection of the yet to be determined bacterial effector proteins into the host cell cytosol [100]. This hypothesis was recently confirmed by identifying the Dot/Icm substrate, SdhA, as important for Legionella mediated apoptosis inhibition [101]. The Legionella SdhA mutant is deficient in intracellular growth and induces caspase activation, mitochondrial membrane damage and apoptosis [101]. Legionella SidF is able to directly bind and inactivate two pro-apoptotic members of the Bcl2 family of mitochondrial proteins which is important for its capacity to inhibit host cell apoptosis [102]. These two recent reports thus provide potential targets for drug development. Interestingly, during the later stages of infection in mice, Legionella will induce apoptosis, a step that is potentially important for the virulence of the bacteria since in the nonpermissive BALB/c mouse strain no caspase-3 activation and no host cell apoptosis induction is observed [103]. In summary, Legionella may have evolved to inhibit host cell death pathways during its early infection phase, which is required for intracellular replication; once a critical mass of bacteria is in the host cell, the induction of cell death is advantageous for the spread of the bacteria to uninfected cells.

Pseudomonas aeruginosa is an important human pathogen that inhibits TNF-α induced macrophage cell death [104] and infection-induced cell death in corneal epithelial cells [105]. In the related plant pathogen P. syringae secreted protein AvrPtoB has been demonstrated to have E3 ubiquitin ligase activity and that this activity is necessary to inhibit host cell apoptosis [106, 107]. It remains to be seen if an AvrPtoB homolog in P. aeruginosa has the same activity and could thus serve as new drug target. This is especially important for future treatment of P. aeruginosa because of the high incidences of isolates with multiple antibiotic resistances [108]. Only one more bacterial effector protein has been identified to date which is involved in apoptosis inhibition; the BePA protein of Bartonella henselae and B. quintana was shown to be translocated into the cytosol of infected endothelial cells via a type IV secretion system and to localize to the host cell plasma membrane. The intracellular levels of cyclic adenosine monophosphate (cAMP) and subsequently cAMP-responsive gene expression were increased after infection. These events protected infected cells from apoptosis induction by cytotoxic T lymphocytes [109, 110].

Finally, several other pathogens are able to inhibit host cell apoptosis but their interaction with the host cell is poorly understood. The obligate intracellular pathogen Rickettsia rickettsii, the etiologic agent of Rocky Mountain spotted
fever, has been shown to inhibit apoptosis of infected endothelial cells in an NFκB dependent manner [111, 112]. Porphyromonas gingivalis, is a major cause of periodontal diseases and, as an opportunistic pathogen, is present even in the absence of obvious pathology. It is estimated that about 50% of adults in developed countries have some form of chronic periodontitis [113]. In gingival epithelial cells, Porphyromonas induces major changes in the host cell proteome[114]. One way the bacteria mediate host cell apoptosis inhibition is through manipulation of the JAK/STAT pathway that controls mitochondria mediated cell death pathways [114]. However, a conflicting report demonstrates an induction of apoptosis in a caspase-3 independent pathway by the same pathogen [115]. These differences may be due to variations in host cells and/or bacterial isolates used for the studies. Finally, Coxiella burnetii , the causative agent of Q fever, inhibits apoptosis in human and monkey alveolar macrophages [116]. This inhibition seems to involve the prevention of cytochrome C release from mitochondria [117] but can be overcome by stimulating the macrophages with IFN-γ [118].

BACTERIAL TARGETS THAT REGULATE VIRULENCE FACTOR EXPRESSION AND/OR SECRETION

1) Cell Wall Synthesis

Inhibition of cell wall synthesis is a primary antibiotic target for Gram-positive and Gram-negative bacteria and will continue to provide new targets for drug development [119]. Mycobacteria are Gram-positive and have a very complex cell wall, that not only protects the bacteria from toxic compounds within the macrophages (e.g. reactive oxygen species), but also contains components with important immunomodulatory function [11, 120-123]. Understanding the biosynthetic pathways of these immunomodulators will generate new targets for drug development. There have been several recent reviews on this topic (124-128), and here the discussion will be limited to one component of the complex cell wall and its implication in pathogenicity of Mtb.

Lipoarabinomannan (LAM) is an important lipoglycan that has been implicated in the inhibition of phagosome maturation, host cell apoptosis, IFN-γ signalling and host cell IL-12 cytokine secretion [11, 122, 123, 129]. For example, the coupling of Mtb-LAM to latex beads inhibited their fusion with lysosomes [130, 131]. Interestingly, LAM isolated from mycobacterial species that are able to inhibit phagosome maturation is through manipulation of the JAK/STAT pathway that controls mitochondria mediated cell death pathways [114]. However, a conflicting report demonstrates an induction of apoptosis in a caspase-3 independent pathway by the same pathogen [115]. These differences may be due to variations in host cells and/or bacterial isolates used for the studies. Finally, Coxiella burnetii , the causative agent of Q fever, inhibits apoptosis in human and monkey alveolar macrophages [116]. This inhibition seems to involve the prevention of cytochrome C release from mitochondria [117] but can be overcome by stimulating the macrophages with IFN-γ [118].

Another group of potential drug targets are the enzymes that add the arabinose residues to its biosynthetic cell wall precursor lipomannan (LM), since purified LM can induce host cell apoptosis and secretion of IL-8, IL-12 and TNF-α [134-136]. It is therefore likely that an inhibitor of LAM biosynthesis at this level will induce an increase of LM in the cell wall of mycobacteria. This should render the bacterium less virulent since it would now induce host cell apoptosis and cytokine secretion instead of inhibiting these responses, which would stimulate the innate and subsequent acquired immune responses [122]. The embC gene of the nonpathogenic M. smegmatis has been shown to be critical in modifying the mannan core since its deletion resulted in the absence of full-length PILAM but instead only LM with a couple of arabinose residues could be isolated [137-139]. Nevertheless, to date it has not been possible to create an embC mutant in the background of a virulent strain of mycobacteria, pointing to the possibility that embC has another essential function in these strains. Eukaryotic cells do not express LAM and therefore enzymes involved in its biosynthesis should be sufficiently different from enzymes in eukaryotic cells to serve as drug targets.

2) Secretion Systems

The importance of secretion systems not only for bacterial viability but also for pathogenicity is well established. Highlighted here are two secretion systems that, albeit being first described and best studied in mycobacteria, are not unique to this genus and have a great potential as a target for development of new drugs against Gram-positive bacteria.

The early secretory antigenic target 6 (ESAT-6) system 1 (ESX-1) secretion apparatus is present and functional in virulent and nonpathogenic species of mycobacteria and shows some functional homology with type IV secretion systems of Gram-negative bacteria such as the Dot/Icm system of L. pneumophila [140]. ESX-1 is absolutely required for pathogenicity of virulent mycobacteria and therefore it is likely that although the secretory machinery is conserved among species, virulent mycobacteria have a unique subset of secreted effector proteins [140]. Genetic screens identified Rv3868, Rv3870, Rv3871 and Rv3877-79 as being essential for the secretion of ESAT-6 and CFP-10 [141-143]. Other substrates for ESX-1 mediated secretion are encoded by Rv3116c/EspA [144], Rv3483c and Rv3881c/EspB, although the ESX-1-dependent secretion of the latter two has only been demonstrated in the fish pathogen M. marinum so far [27, 28]. The importance of the ESX-1 system for necrosis of epithelial cells [24], and the inhibition of phagosome maturation [26, 28] was demonstrated. In addition, a recent report demonstrated a direct interaction of secreted ESAT-6 with Toll-like Receptor (TLR)2 to inhibit TLR signaling on macrophages [145]. It is likely that the ESX-1 system is also involved in other host-pathogen interactions but evidence for this hypothesis remains to be discovered. The development of drugs against the core proteins of this secretion system are therefore likely to disrupt the interaction of virulent M. tuber-
culosis with host cells at several levels and thus increase the efficacy of these drugs. ESX-1 secretion systems do not seem to be unique to mycobacteria, since the human pathogen Staphylococcus aureus secretes ESAT-6-like proteins which are important for virulence of the bacteria [146].

Another secretion system important for pathogenicity of bacteria is the alternate SecA system, SecA2. Whereas SecA is found in many bacteria and is an essential secretion system, the alternate SecA2 system is found in at least nine species of pathogenic Gram-positive bacteria and is not essential for the in vitro growth of the bacteria [147]. Among the bacteria that encode alternate SecA systems are M. tuberculosis [148], Listeria monocytogenes [147] and Streptococcus agalactiae [149] and Streptococcus gordonii [150, 151]. The deletion of secA2 in M. tuberculosis attenuated the bacteria for growth in mice [152]. Subsequent proteomic analysis of secreted proteins in wild-type and SecA2 deficient bacteria demonstrated that an important subset of secreted proteins depends on the SecA2 system [152]. Nevertheless, a significant subset of SecA2-dependent secreted proteins most likely remains to be discovered since their transcription/translation may only be induced after ingestion of the bacteria by the macrophage.

One SecA2-dependent secreted protein is the superoxide dismutase A (SodA) of Mtb. This protein is important to inhibit infection-induced apoptosis in human and murine macrophages [78]. A yet to be identified SecA2-dependent effector is important to mediate the inhibition of IFN-γ signaling in Mtb-infected macrophages [153]. It is likely that SecA2 is implicated in other host-pathogen interactions and it is a broad involvement that explains the attenuation of the SecA2-Mtb mutant and makes the SecA2 system a prominent target for drug development.

CONCLUSION

The increase in antibiotic resistance in a multitude of important human pathogens highlights the need for continued drug development. A class of drug targets that has not been previously exploited is bacterial proteins and lipids that are important for bacterial virulence because they manipulate the infected cells. The targeting of these pathogenicity factors, instead of general housekeeping proteins and lipids, might have the advantage of identifying drugs that specifically target pathogenic bacteria without disrupting the normal microflora. Nevertheless, it remains to be seen if inhibition of the virulence mechanisms of the bacteria alone is sufficient for effective treatment. Alternatively, these antimodulin drugs could be used in conjunction with traditional antibiotics to increase their efficiency and reduce development of drug resistance. Only a detailed understanding of human pathogens will allow for generation of new drug classes for treatment of resistant bacteria. This basic research will always be in demand since the acquisition of drug-resistance by pathogens will never end.

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