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Minireview

MINIREVIEW

Tug of war between *Acinetobacter baumannii* and host immune responses

Fei-Ju Li, Lora Starrs and Gaetan Burgio*,†

Department of Immunology and infectious Diseases, John Curtin School of Medical Research, Australian National University, 131 Garran Road, Acton, ACT 2601, Australia

*Corresponding author: Tel: +61-2-6125-9428; E-mail: gaetan.burgio@anu.edu.au

One sentence summary: This review gives an overview on how Acinetobacter baumannii survives and subverts the host immune response and how in return the host innate immunity kills the bacteria.

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†Gaetan Burgio, http://orcid.org/0000-0002-7434-926X

ABSTRACT

Acinetobacter baumannii is an emerging nosocomial, opportunistic pathogen with growing clinical significance. *Acinetobacter baumannii* has an exceptional ability to rapidly develop drug resistance and to adhere to abiotic surfaces, including medical equipment, significantly promoting bacterial spread and also limiting our ability to control *A. baumannii* infections. Consequently, *A. baumannii* is frequently responsible for ventilator-associated pneumonia in clinical settings. In order to develop an effective treatment strategy, understanding host–pathogen interactions during *A. baumannii* infection is crucial. Various *A. baumannii* virulence factors have been identified as targets of host innate pattern-recognition receptors, which leads to activation of downstream inflammasomes to develop inflammatory responses, and the recruitment of innate immune effectors against *A. baumannii* infection. To counteract host immune attack, *A. baumannii* regulates its expression of different virulence factors. This review summarizes the significance of mechanisms of host–bacteria interaction, as well as different bacteria and host defense mechanisms during *A. baumannii* infection.

Keywords: inflammasome; host–pathogen interaction; innate immunity; *Acinetobacter baumannii*; toll-like receptors; outer membrane

INTRODUCTION

Bacterial infections are a leading cause of morbidity and mortality worldwide. Although the discovery of antibiotics successfully controlled bacterial infections, overuse and misuse of antimicrobials escalated the selection of difficult-to-treat multi-drug resistant (MDR) organisms. *Acinetobacter baumannii* belongs to a group of antibiotic-resistant bacteria with significant clinical prevalence, termed 'ESKAPE' pathogens. ESKAPE pathogens are comprised of *A. baumannii*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* spp (Rice 2008). The ESKAPE bacteria were classified due to their ability to effectively escape antibiotic treatments, leading to a high mortality rate in vulnerable patients (Rice 2008).

Notably, Gram-negative *A. baumannii* infections are increasing in prevalence in hospitals and combat zones worldwide (Antunes, Visca and Towner 2014). For example, during 2002 to 2004 military medical facilities reported that 83%, a total of 102 identified cases, of *A. baumannii* bloodstream infections were associated with combat zone operations, far exceeding the three cases reported during 2000 to 2002, with 80% of these *A. baumannii* isolates showing resistance to the last-line antibiotic carbapenem (Acinetobacter baumannii 2004). The increased prevalence of nosocomial *A. baumannii* infections can be largely attributed to the remarkable ability of *A. baumannii* to colonize and form biofilms on abiotic surfaces, including medical devices. This subsequently promotes bacterial contamination

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and host infection from opportunistic pathogens (Greene *et al.* 2016). Studies in nine European countries indicated a high incidence rate of *A. baumannii* in ICU patients, with *A. baumannii* present in 17.9% of patients with bacteremia and in 22.9% of trauma patients (Koulenti, Tsigou and Rello 2016). In a Turkish hospital, *A. baumannii* was the most frequent pathogen (69.5%) of 417 patients with ventilator-associated pneumonia during 2010 to 2015. Remarkably, these *A. baumannii* isolates are highly resistant to antibiotics, including broad-spectrum penicillin, cephalosporin, aminoglycoside, quinolone and carbapenem (But *et al.* 2017), which are routinely used to treat *A. baumannii* infection. More recently, during December 2016 to June 2017, in a Fiji hospital, nosocomial outbreaks of MDR *A. baumannii* affected 1.8% pediatric and 10.6% neonatal ICU patients, causing fatality of up to 68% of affected patients. It was further identified that nearly all patients (97%) received ventilator support (Zimmerman, Lyman and Andersson 2017), emphasizing the ability of *A. baumannii* to colonize abiotic medical surfaces facilitating movement of MDR bacteria between rooms and patients.

The therapeutic options for these highly resistant pathogens are very limited and as such, physicians have been forced to use the last resort antibiotics, including colistin, which may induce nephrotoxicity and select for colistin-resistant *A. baumannii* (Moffatt *et al.* 2010; Henry *et al.* 2012; Boll *et al.* 2016). To effectively treat and limit the spread of MDR *A. baumannii*, while protecting the host from further damage from the treatments, a thorough understanding of the molecular and cellular mechanisms of host–pathogen interactions is crucial.

It is well-characterized that neutrophils and macrophages are essential for host defense during *A. baumannii* infection (van Faassen *et al.* 2007; Qiu *et al.* 2012; Garcia-Patino, Garcia-Contreras and Licona-Limon 2017), with host soluble factors, including the complement cascade and antimicrobial peptides also participating in the killing of *A. baumannii* (Maisetta *et al.* 2006; Routsias *et al.* 2010; Lin *et al.* 2015; Garcia-Patino *et al.* 2017). The role of different effector cells and soluble antimicrobial factors during *A. baumannii* infection has been reviewed recently (Garcia-Patino *et al.* 2017), and will not be discussed in detail in this review. Furthermore, while a number of studies examine the mechanisms of antibiotic resistance, epidemiology and virulence factors such as biofilm formation of *A. baumannii* (Lee *et al.* 2017; Rodrigo-Troyano and Sibila 2017), there are limited number of studies focusing on how bacteria interact with the host, and subvert cellular defense mechanisms to survive and persist. This review will be discussing the *A. baumannii* components that enhance bacterial virulence, and how *A. baumannii* modulates the expression of these components to evade host immune responses, ultimately promoting bacterial survival and propagation in the host. Understanding both sides of this story may identify potential therapeutic targets to control *A. baumannii* infection in the future. Figure 1 and Table 1 depict all bacterial features described in this review, whereas all host innate immune factors discussed in this review are summarized in Figure 2.

Biology of *A. baumannii*

Acinetobacter baumannii present many unique components to assist its invasion into the host, evasion of host immune attack and persistence in the host. The most studied components of *A. baumannii* are its outer membrane proteins 'Omps'. *Acinetobacter baumannii* Omps are the most abundant surface proteins of *A. baumannii* and they form pores on the outer membrane to regulate membrane permeability. Several *A. baumannii* Omps have been identified, including OmpA (also known as Omp38), Omp33-36 and Omp22 (Lee *et al.* 2017). OmpA is a well-characterized virulence factor of *A. baumannii* (AbOmpA), responsible for a wide range of bacterial activities from promoting adhesion (Choi *et al.* 2008) to inducing host cell apoptosis (Choi *et al.* 2005). In addition to Omps, the *A. baumannii* membrane also contains membrane-bound lipopolysaccharide (LPS)—a highly immunogenic endotoxin present in all species of Gram-negative bacteria—capable of causing lethal shock (Ramachandran 2014). LPS is a well-characterized, highly immunogenic microbial molecule and evokes extensive host immune responses (Kim *et al.* 2013). Being the two predominant components of *A. baumannii* virulence, AbOmps and LPS may be potential host receptor targets to sense invading bacteria (Knapp *et al.* 2006; Kim *et al.* 2013; Kim *et al.* 2014). To counteract this, *A. baumannii* express various levels or structural modification of Omps (Sato *et al.* 2017) and/or LPS (Boll *et al.* 2016).

While Omps and LPS promote the virulence of *A. baumannii*, a third membrane-bound component, capsular polysaccharide (CPS)—an outer-membrane polymer with a theorized involvement in virulence—enables the bacteria to evade host immune attack during infection. The conserved gene cluster (K locus) discovered in *A. baumannii*, determines the production of CPS, with antibiotic exposure inducing CPS expression through gene regulation (Geisinger and Isberg 2015). Capsular polysaccharide has been related to biofilm formation, avoidance of both phagocytosis and complement, and antibiotic resistance of *A. baumannii* (Russo *et al.* 2010; Lees-Miller *et al.* 2013). To further enhance *A. baumannii* adherence and spread, the bacteria also presents biofilm-associated protein (Bap), and Csu pili as well as type IV pili. Unsurprisingly, *A. baumannii* demonstrates ability to regulate the expression of these adherence components. This review will briefly introduce these components. For a comprehensive review, we refer the readers to the recent review from Harding, Hennon and Feldman (2018).

Apart from membrane-bound components, *A. baumannii* also presents various transmembrane secretory systems and nutrient acquisition systems, which secrete proteins outside of the cell, to either promote bacterial virulence or assist bacteria in acquiring nutrients essential for growth. This includes the secreted outer membrane vesicles (OMVs), transmembrane type VI secretory system (T6SS) or efflux pumps, all together modulating bacterial resistance to host immune responses (Hood *et al.* 2012; Repizo *et al.* 2015), antibiotic resistance (Rumbo *et al.* 2011) or assisting intra-species competition (Carruthers *et al.* 2013; Repizo *et al.* 2015), ultimately resulting in *A. baumannii* survival. Like other structures of *A. baumannii*, the expression of these secretory components may be altered by *A. baumannii* gene regulation.

In the following section, we discuss how these *A. baumannii* components contribute to bacterial virulence, potentially through expression of different components, ultimately promotes *A. baumannii* persistence during infection.

BALANCING BACTERIAL INVASION, VIRULENCE AND SURVIVAL

Fierce *A. baumannii*—AbOmps and OMVs

Acinetobacter baumannii OmpA (AbOmpA) is critical for bacteria virulence. *In vitro*, AbOmpA promotes the adherence and invasion of *A. baumannii* into epithelial cells, elucidated from a 95% reduction in cell invasion by OmpA-deficient *A. baumannii*, compared to wild-type bacteria (Choi *et al.* 2008). Furthermore, purified OmpA triggers epithelial cell apoptotic responses

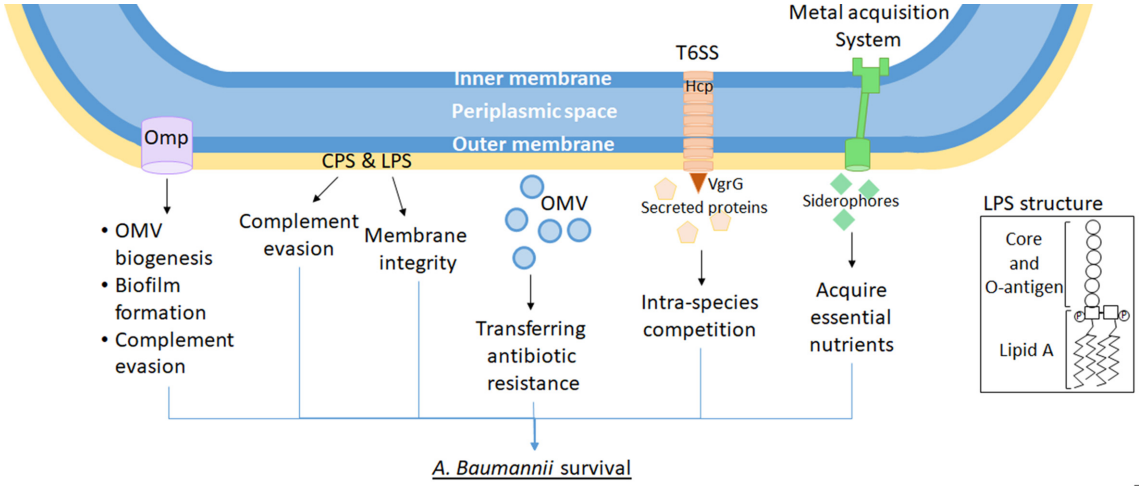

Figure 1. Components of *A. baumannii* and their mechanisms to promote bacteria survival. *A. baumannii* outer membrane proteins (Omps) form pores on the outer membrane, facilitating generation of OMVs and bacterial adherence (biofilm formation). Omps and CPS both assist *A. baumannii* to evade host immune responses such as complement attack. CPS and LPS are present on bacterial outer membranes. LPS are essential for maintaining bacterial membrane integrity, yet the lipid A component of LPS (inset) is highly immunogenic to host receptors. *Acinetobacter baumannii* can modify LPS structures to evade host immune recognition. Secretory systems of *A. baumannii* include OMV and type VI secretory system (T6SS). OMVs act as a carrier of antibiotic resistance genes between different strains of *A. baumannii*. T6SS promotes *A. baumannii* competitiveness against other bacteria species, subsequently enhancing *A. baumannii* colonization. *Acinetobacter baumannii* also presents metal acquisition systems and the related siderophores to ensure acquisition of essential nutrients for bacterial growth. *Acinetobacter baumannii* can up- or down-regulated these different components in response to antibiotic stress, yet the impacts of the regulations on *A. baumannii* fitness remains unknown.

Table 1. Summary of all *A. baumannii* components, their functions and conditions of regulation mentioned in this review.

Virulence factor	Functions	Found in	Modulation
OmpA	Induce cell apoptosis (Choi *et al.* 2005), complement resistance (de Leseleuc *et al.* 2014), biofilm formation, cell invasion (Choi *et al.* 2008), OMV biogenesis (Moon *et al.* 2012)	Most *A. baumannii* strains (Beveridge 1999)	Unknown
CPS	Complement resistance (Geisinger and Isberg 2015), biofilm formation (Russo *et al.* 2010)	Most *A. baumannii* strains (Lees-Miller *et al.* 2013)	Up-regulated upon antibiotic or ROS exposure (Geisinger and Isberg 2015; Chin *et al.* 2018)
OMVs	Transferring OmpA (Kwon *et al.* 2009), toxin delivery (Jha *et al.* 2017),	Most *A. baumannii* strains (Beveridge 1999)	Up-regulated upon antibiotic exposure (Koning *et al.* 2013)
LPS	Membrane integrity (Boll *et al.* 2016), induce cell apoptosis (Beceiro *et al.* 2014), antibiotic resistance (Moffatt *et al.* 2010)	Most *A. baumannii* strains (Beveridge 1999)	Loss during colistin resistance development (Boll *et al.* 2016)
T6SS	Interspecies competition (Carruthers *et al.* 2013; Weber *et al.* 2013)	Most *A. baumannii* strains (Weber *et al.* 2013)	Activate upon contact with competing bacteria (Weber *et al.* 2015)
Micronutrient acquisition systems	Nutrient acquisition (Wang *et al.* 2014; Gebhardt *et al.* 2015)	Most *A. baumannii* strains (Mortensen and Skaar 2013)	Up-regulated under nutrient-deprived conditions (Zimbler *et al.* 2009; Kroger *et al.* 2016)
Type IV pili	Twitching motility (Harding *et al.* 2013)	Most *A. baumannii* strains (Piepenbrink *et al.* 2016)	Up-regulated during growth in human serum (Jacobs *et al.* 2012)
Bap	Biofilm formation (Brossard and Campagnari 2012)	Most sequenced *A. baumannii* strains (Goh *et al.* 2013)	Up-regulated while growing under low iron conditions (Azizi *et al.* 2016)
Csu Pili	Biofilm formation (abiotic surface only) (Tomaras *et al.* 2008)	Most *A. baumannii* strains (Moriel *et al.* 2013)	Antibiotic exposure (K. H. Moon *et al.* 2017; Farshadzadeh *et al.* 2018)

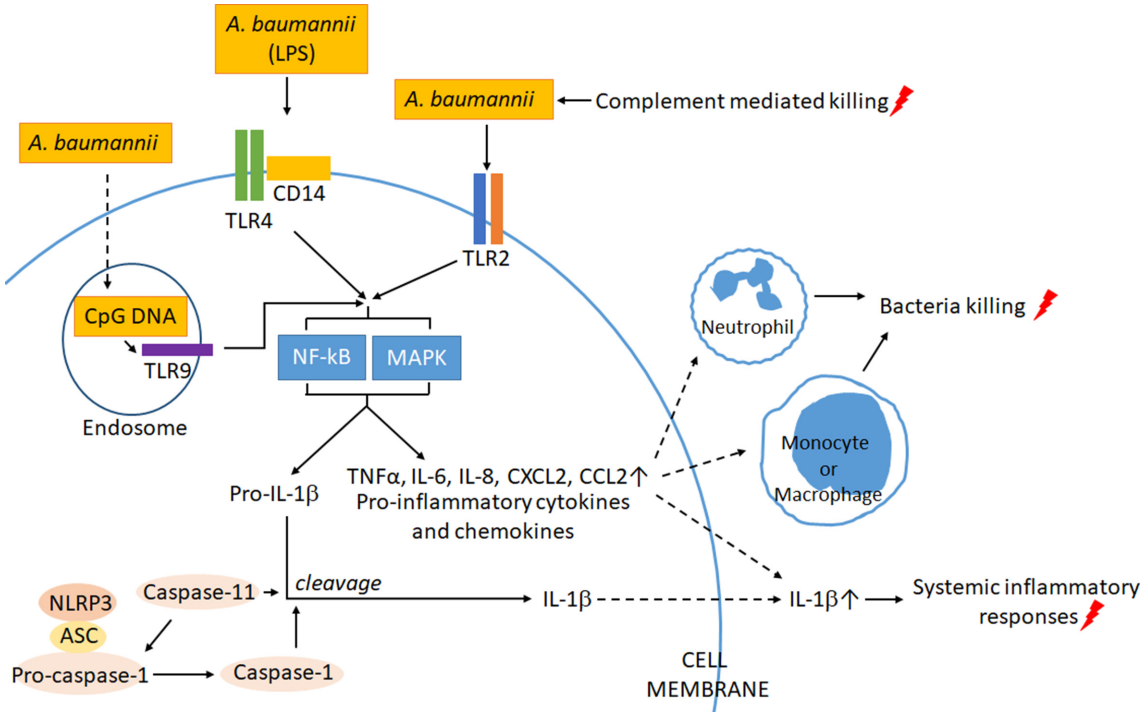

Figure 2. Host innate immune responses to *A. baumannii* infection. The initial encounter of host cells and *A. baumannii* triggers signaling through recognition by TLR4/CD14, TLR2; or TLR9 in the endosome, activating downstream mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB), leads to pro-inflammatory cytokines and chemokines secretion. Pro-IL-1β is cleaved by activated caspase-1 or caspase-11 into mature IL-1β for release and promote systemic inflammation. Recruited neutrophils, monocytes or macrophages contribute to bacterial clearance through ROS production and phagocytosis. IL: interleukin; TNFα: tumor necrosis factor α. Red lightning bolt highlights *A. baumannii*-killing effectors. Figure not to scale.

similar to wild-type *A. baumannii* strain ATCC19606 (Choi *et al.* 2005), highlighting the crucial role of AbOmpA in host cell-invasion by bacteria and triggering host cell death. Similarly, for *in vivo* murine models, intra-tracheal inoculation of an isogenic AbOmpA⁻ mutant induces lower neutrophil infiltration, less severe lung lesions and lower bacteremia compared with *A. baumannii* ATCC19606 strain (Choi *et al.* 2008). The lower bacteremia triggered by AbOmpA⁻ mutant suggests that OmpA is responsible for *A. baumannii* dissemination into the bloodstream. Overall, the lower immune response and less severe pathology elicited by AbOmpA⁻ mutant emphasizes the role of AbOmpA in bacterial virulence *in vivo*.

Interestingly, AbOmps are crucial for the bacteria's survival by evading the host immune response, thus promoting bacterial virulence. *Acinetobacter baumannii* outer membrane fractions along with other virulence factors including CPS, T6SS, plasminogen-binding protein (CipA) and heme consumption are all implicated in assisting *A. baumannii* in bacteriolytic complement evasion (de Leseleuc *et al.* 2014; Geisinger and Isberg 2015; Repizo *et al.* 2015; Koenigs *et al.* 2016). One way they achieve this is by subverting the host immune system. For example, outer membrane fractions of *A. baumannii* isolate ATCC19606, specifically the Omps with size 38 (i.e. AbOmpA), 32 and 24 kDa, have been identified as ligands for factor H, a soluble regulator which patrols the bloodstream and binds to self-antigens, preventing activation of the complement pathway (Ferreira, Pangburn and Cortés 2010). When *A. baumannii* is pre-treated with trypsin to cleave-off surface proteins, the survival of the bacteria in normal human serum significantly decreases (Kim *et al.* 2009), suggesting that the complement evasion of *A. baumannii* is dependent on the interaction between bacterial membrane proteins and host soluble factors (i.e. factor H) found in serum. However, King and colleagues (King *et al.* 2009) examined the relationship between their clinical *A. baumannii* isolates and the alternative complement pathway, and elucidated that their clinical complement-resistant *A. baumannii* isolate (LK41) avoids deposition of complement factor C3, yet it does not bind to factor H. To date, the exact role of factor H and AbOmpA in *A. baumannii* serum resistance remains elusive.

This dissimilarity in susceptibility to complement, as observed by King *et al.* (2009), is likely related to the different expression of OmpA in the clinical isolates studied. Numerous studies have suggested that *A. baumannii* shows different levels of Omp expression across strains. Sato *et al.* (2017) compared the level of Omp between five MDR clinical isolates from Teikyo University hospital during an outbreak around 2010 to the standard *A. baumannii* ATCC19606 strain. It was observed that two of the clinical isolates showed higher OmpA mRNA levels when compared to the standard ATCC19606 strain. In addition to OmpA, reduced mRNA levels of Omp33-36 in two clinical strains compared to ATCC19606 isolate were also reported. This differential expression across strains was further supported by Dupont and colleagues (2005) who showed that the expression of *A. baumannii* Omp at 43 and 29 kDa were reduced in one clinical strain (Ab1), compared to other strains (clinical Ab2, Ab3 and ATCC19606) tested. Since Omps form porins and mediate the permeability of the bacterial outer membrane, it is reasonable to speculate that Omp expression can affect virulence, and it follows that changes in Omp expression may be related to the diverse antibiotic resistance of *A. baumannii* across strains.

Importantly, OmpA is involved in additional bacterial virulence including biogenesis of OMVs, complement resistance and biofilm formation (Gaddy, Tomaras and Actis 2009; Kim *et al.*

2009; Moon et al. 2012). OMVs are a type of secretion system utilized by Gram-negative bacteria to transfer microbial products or bacterial toxins to induce host cell apoptosis. *Acinetobacter baumannii* OMVs are associated with abundant expression of OmpA and other virulence factors (Kwon et al. 2009). Unsurprisingly, since AbOmpA is involved in OMV biogenesis, the OMVs from AbOmpA mutant and wild-type strain (ATCC19606) show different protein constituents (Moon et al. 2012). This suggests the virulence of *A. baumannii* is dependent not only on each separate factor, but also to their interactions with each other and with the host. Intra-tracheal administration to mice of *A. baumannii* OMVs induced secretion of the cytokines IL-1β, IL-6 and the chemokine ligand 2 (CCL2) (Jun et al. 2013). *In vitro*, *A. baumannii* OMVs show phospholipase C, hemolytic and leukotoxic (ability to induce shape change in granulocyte) activities, which, when compared to ATCC19606 control strain, the OMVs derived from clinical MDR-strains display enhanced hemolytic and leukotoxic activities (Jha et al. 2017). The interaction between *A. baumannii* OMVs and host cells, therefore induces both host immune responses and host cell cytotoxicity.

Acinetobacter baumannii (ATCC19606) OMV morphology differs between the stages of bacterial growth (Koning et al. 2013). During late log-phase of growth, *A. baumannii* OMVs are larger compared to the OMVs generated during early log-phase, yet the number of OMVs produced peaks during the stationary phase of bacterial growth. Furthermore, a sub-lethal concentration of ceftazidime induces OMV formation in *A. baumannii* (Koning et al. 2013). It was not identified whether these secreted OMVs contained the antimicrobial substance, nevertheless, this could be a potential bacteria defense mechanism, where it secretes antibiotic-containing OMVs, removing the antibiotic and thus, enhancing its resistance. On the other hand, increased secretion of OMVs may be an indirect consequence of the increased formation of porins (i.e. Omps) by *A. baumannii*, resulting in increased OMV biogenesis. It is critical for further studies to be conducted to understand the correlation between *A. baumannii* OMV morphology, composition, bacterial virulence and interaction with the host, since these are clearly important in bacterial pathology. Taken together, while these studies illustrate the importance of Omps and OMV in the pathogenesis of *A. baumannii*, it remains unclear how the bacteria achieve the trade-off between decreasing expression of different Omps and increasing bacterial virulence

Lipopolysaccharide—promoting virulence while maintaining membrane integrity

The other major component of the *A. baumannii* cell wall is LPS in addition to Ab Omps. LPS is composed of a hydrophobic lipid A region, attached to a carbohydrate chain, and is believed to play a role in *A. baumannii* virulence. It has previously been shown that four LPS mutants of *A. baumannii*, derived from ATCC19606, induce significantly lower cell death in the human lung epithelial cell line (A549) *in vitro* compared to the wild-type ATCC19606 (Beceiro et al. 2014). Similarly, Lin et al. (2012) showed that inhibition of LPS synthesis in hyper-virulent *A. baumannii* (HUMC1, MDR) decreases mouse susceptibility to systemic infection, and reduced overall bacterial burden *in vivo*, providing further support that expression of LPS enhances *A. baumannii* virulence and pathology. Interestingly, no correlation was found between *A. baumannii* strain virulence *in vivo* and its LPS content per bacterium *in vitro* (Lin et al. 2012), suggesting the inflammatory response to *A. baumannii* is not solely dependent on the quantity of LPS.

It is well known that *A. baumannii* alters either LPS structure or LPS expression to evade host receptor recognition and antibiotic colistin binding (Boll et al. 2016). Unsurprisingly, since LPS is highly immunogenic, the LPS-lacking *A. baumannii* examined by Lin et al. (2012) elicits less pro-inflammatory cytokines, including tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) *in vivo*. Likewise, Moffatt and colleagues showed that LPS-deficient *A. baumannii* evoked lower transcription factor NF-κB activation through toll-like receptor 4 (TLR4) signaling, and therefore less TNFα secretion *in vitro* (Moffatt et al. 2013). This means the bacteria are able to evade the host immune response since the host no longer develops inflammatory responses through these pathways, and hence, these LPS-lacking *A. baumannii* are able to survive.

However, loss of *A. baumannii* LPS may result in a concomitant change of surface molecule composition. Transcriptomic analysis of LPS-deficient *A. baumannii* (ATCC19606R) compared to the wild-type strain (ATCC19606), showed that the loss of LPS was compensated via upregulation of transport lipoproteins (Henry et al. 2012; Boll et al. 2016), which may induce permeability changes such as those shown by Moffatt et al. (2010), often reflecting associated changes in virulence and antibiotic resistance. As previously mentioned, LPS-deficient *A. baumannii* induces lower epithelial cell (A549) death *in vitro* (Beceiro et al. 2014) than wild-type strains, and while the loss of LPS in some strains of *A. baumannii* allows them to evade the immune system and increase their antibacterial resistance, increased permeability must be considered a 'cost' to the bacteria, and it follows that there must be a balance between the loss of LPS and the ability of the bacteria to survive. Overall, these intricate relationships between LPS expression, membrane permeability and bacterial virulence in *A. baumannii* remain unclear.

While reports regarding *A. baumannii* LPS structural/expression variation and bacterial host immune evasion are still lacking, another nosocomial gram-negative bacterium, *K. pneumoniae*, avoids phagocytosis by both mouse alveolar macrophages (MH-S) and amoebae *Dictyostelium discoideum* via LPS lipid A palmitoylation (March et al. 2013). Since some highly virulent *A. baumannii* isolates can also evade phagocytosis (Bruhn et al. 2015), future research may validate, or refute, a relationship between *A. baumannii* LPS modification and bacterial evasion of phagocytosis.

EVADING HOST ATTACK AND PERSISTENCE OF *A. BAUMANNII* IN THE HOST

The armor of *A. baumannii*—CPS

Many *A. baumannii* clinical isolates express inducible CPS polymer encasing the bacterium to shield *A. baumannii* from host responses including complement attack (Geisinger and Isberg 2015). The production of CPS is not only up-regulated by antibiotic exposure (Geisinger and Isberg 2015) but may also be influenced by host defenses. *Acinetobacter baumannii* isolated from lungs post-infection in mice lacking functional reactive oxygen species (ROS) and lysozyme shows reduced capsule expression, compared to *A. baumannii* isolated from wild-type lung (Chin et al. 2018). This suggests that *A. baumannii* use CPS up-regulation to protect themselves against ROS and lysozyme degradation, and provides indirect evidence that these host factors play a role in defense against *A. baumannii*.

Previous studies have shown that CPS expression is associated with *A. baumannii* virulence. When infected with *A. baumannii* with enhanced capsule production, inbred C57BL/6 mice show decreased survival by up to 40%, as well as increased bacterial burden in the blood and spleen when compared to mice infected with normal CPS-expressing *A. baumannii* (Geisinger and Isberg 2015). This discovery is supported by Chin *et al.*, who showed that capsule-enriched *A. baumannii* (AB5075, clinical MDR strain) exhibits greater dissemination *in vivo* and induces higher lethality in mice (Chin *et al.* 2018). Furthermore, in rat soft tissue, Russo and colleagues (2010) suggested that capsule-positive *A. baumannii* show enhanced bacterial survival, implicating a strong link between the CPS and bacterial virulence, therefore improving bacterial persistence *in vivo*. CPS also facilitates *A. baumannii* colonization and biofilm formation by promoting bacterial adherence to various surfaces (Russo *et al.* 2010), which is fundamental for *A. baumannii* to spread, and limits our ability to eliminate *Acinetobacter* species.

It is also evident that different strains of *A. baumannii* not only express CPS at different levels but also express different CPS monosaccharide composition and/or acetylation pattern (Shashkov *et al.* 2015; Wright *et al.* 2017), which provides further intrigue into exactly what in CPS influences virulence, with this research still being undertaken. It is also unknown if alternating CPS expression or modification induces any fitness or virulence costs to the bacteria, and is thus, a vital piece of information in understanding their persistence and how we may harness this for therapeutics.

Making *A. baumannii* fit—the secretory systems

Pathogenic bacteria express dedicated secretion systems to deliver pathogenic factors into host cells, promoting bacterial virulence through improved bacterial attachment or increased ability to forage resources from the environment. Many other mechanisms for delivery of pathogenic factors also exist. In particular, *A. baumannii* possess type II, V, VI secretion systems and chaperon-usher pathways which are responsible for *A. baumannii* survival on abiotic surfaces and *in vivo* (Lee *et al.* 2017). Of these, the *A. baumannii* type VI secretory system (T6SS) is the most characterized. Components of T6SS include secreted hemolysin-co-regulated protein (Hcp) and valine-glycine repeat protein G (VgrG); these two components assemble to form a tube (Hcp) with spiky head (VgrG) that punctures the membrane and facilitates delivery of bacterial effector proteins to target cells (Lee *et al.* 2017). Additionally, *A. baumannii* T6SS also contains a peptidoglycan hydrolase 'TagX' and other structural proteins, which cooperate to facilitate T6SS biogenesis and functionality (Weber *et al.* 2016). Genomic analyses identified conserved T6SS loci and expression of Hcp among *A. baumannii* strains (ATCC19606, 17978, SDF, AYE and three clinical isolates) (Weber *et al.* 2013), implicating an essential role of T6SS for *A. baumannii* infection.

Active T6SS contributes to bacterial virulence *in vivo*. For instance, survival of *Galleria mellonella* larvae is significantly increased if infected with *A. baumannii* with non-functional T6SS (T6SS structural protein TssM mutant strain) compared to the wild-type *A. baumannii* DSM30011 isolate (Repizo *et al.* 2015). Moreover, *A. baumannii* shows T6SS-dependent ability to kill *Escherichia. coli* in a poly-microbial environment to facilitate bacteria colonization and enhance the fitness of the pathogen, subsequently promoting bacterial spread. The ability of *A. baumannii* to target and kill *E. coli* is dependent on the strain's secretion of Hcp; detection of Hcp protein in culture supernatants of *A. baumannii* culture (ATCC17978, non-clinical DSM30011 isolate) indicates functional T6SS and is related to a strain's ability to compete against *E. coli* (Carruthers *et al.* 2013; Repizo *et al.* 2015). Further study of the *A. baumannii* strain DSM30011 showed that *A. baumannii* can also outcompete *K. pneumoniae* and *P. aeruginosa* in a T6SS-dependent manner (Repizo *et al.* 2015). In addition to promoting *A. baumannii* intra-species competition, the activity of T6SS is highly versatile in enhancing bacterial fitness and/or virulence, through improving biofilm formation and survival in serum (i.e. complement resistance) *in vitro*, as observed in clinical T6SS-positive *A. baumannii* isolates (strain unidentified) (Kim *et al.* 2017). Altogether, the expression of T6SS by *A. baumannii* substantially improves bacterial survival in the host.

With regard to the important role of T6SS in augmenting bacterial fitness through virulence, it is highly possible that *A. baumannii* T6SS can counteract host attack mechanisms. A recent study indicated that another gram-negative bacteria, *Edwardsiella tarda*, is able to inhibit the NLRP3 inflammasome in a 'T6SS effector *E. tarda* virulent protein P (EvpP)' dependent manner (Chen *et al.* 2017). However, even with evidence suggesting that there are tetR-like repressors in *A. baumannii* plasmids that regulate expression of T6SS (Weber *et al.* 2015), we have limited understanding of the ability of T6SS to protect *A. baumannii* from host attack. Interestingly, the activation of T6SS expression was correlated to loss of antibiotic resistance genes including β-lactams, aminoglycosides and tetracycline resistance of *A. baumannii*. Nevertheless, it remains unclear how *A. baumannii* acquire this T6SS-repressive plasmid (Weber *et al.* 2015).

Key to a healthy diet—nutrition acquisition systems

In order to acquire essential nutrients to proliferate within host cells, *A. baumannii* employs various strategies, from high-affinity siderophores that passively attracts the nutrient needed, to more sophisticated acquisition systems, which actively harvest the nutrients in the environment. The major siderophore found in most *A. baumannii* strains is acinetobactin, which mediates iron uptake (Gaddy *et al.* 2012). In *A. baumannii* ATCC19606 strain, acinetobactin biosynthesis and transport are required for the bacteria to grow under iron-restricted conditions (Zimbler *et al.* 2009).

These *A. baumannii* nutrient acquisition systems are essential for bacterial survival *in vivo*, proven by both Gebhardt *et al.* (2015) and Wang *et al.* (2014) using genome-wide transposon mutagenesis. More details of the regulations of these micronutrient acquisition systems employed by *A. baumannii* were reviewed by Harding, Hennon and Feldman (2018). Interestingly, these nutrient acquisition systems not only ensure bacterial survival but also promote pathogen virulence. For example, *A. baumannii* acinetobactin expression can induce epithelial cell (A549) apoptosis, as cell apoptosis decreases when infected with an acinetobactin-deficient mutant, compared to the parental control strain (ATCC19606) (Gaddy *et al.* 2012). In addition, both murine models and *G. mellonella* larvae are less susceptible to *A. baumannii* acinetobactin-deficient mutant compared to relevant controls (Gaddy *et al.* 2012). Remarkably, it was suggested that *A. baumannii* strain ATCC17978 expresses an acinetobactin-independent gene cluster involved in iron acquisition under iron-chelation conditions (Zimbler *et al.* 2009), highlighting the importance of nutrient availability.

In addition to iron, zinc and manganese are also essential for *A. baumannii* growth (Kroger *et al.* 2016). Therefore, *A. baumannii* also expresses a zinc acquisition system (znuABC) and

a manganese transporter. When wild-type mice were infected with an equal mixture (1:1) of *A. baumannii* (ATCC17978) and the zinc-acquisition system mutant strain (ΔznuB), the parental *A. baumannii* outcompetes the ΔznuB mutant strain for lung colonization (Hood *et al.* 2012). Similarly, when wild-type mice are intra-nasally infected with an equal mixture (1:1) of *A. baumannii* (ATCC17978) and the manganese transporter (ΔmumT) mutant strain, parental *A. baumannii* colonies in lung and liver are significantly higher than the mutant (Hood *et al.* 2012). Therefore, the various metal acquisition systems of *A. baumannii* ensures the availability of different nutrients to facilitate bacteria growth in the host.

The host is able to restrict the growth of bacterial infections by depriving of essential nutrients, including transition metals and amino acids. One mechanism of host nutrient restriction is calprotectin, a neutrophil-derived protein that chelates zinc and manganese. During *A. baumannii* infection, calprotectin effectively limits *A. baumannii* (ATCC17978) dissemination and protects the host from *A. baumannii*-induced lethality, evidenced by Hood and colleagues' study using calprotectin-deficient mice (myeloid-related protein 14 deficient mice (*MRP-14*$^{-/-}$), also known as *S100A9*$^{-/-}$) (Hood *et al.* 2012). Unfortunately, *A. baumannii* had developed a way to counteract the action of calprotectin. Under low zinc conditions, *A. baumannii* upregulates different zinc-uptake mechanisms including znuABC or Zn metallochaperone ZigA, to enhance zinc acquisition (Kroger *et al.* 2016; Mortensen *et al.* 2014; Nairn *et al.* 2016). More details of these zinc acquisition mechanisms were summarized by Harding and colleagues (Ferreira, Pangburn and Cortés 2010).

Moreover, it was also demonstrated that zinc deprivation not only targets *A. baumannii* growth, but also affects the zinc-dependent hydrolyzation activity of carbapenemase, affecting carbapenem resistance of *A. baumannii*. As a result of zinc chelation, carbapenem-resistant *A. baumannii* (AB0057) shows decreased minimal inhibitory concentration of imipenem, under zinc-selective chelator (TPEN) treatment (Hood *et al.* 2012). The decreased carbapenem resistance due to zinc chelation is reversible with the addition of excessive zinc (Hood *et al.* 2012), showing that the bioavailability of zinc is the key factor. It is therefore reasonable to conclude that different *A. baumannii* nutrient acquisition mechanisms also contribute to bacterial virulence, as the expression/regulation of these mechanisms assists the bacteria in overcoming host nutrient limitation, ensures bacterial survival and, subsequently allows the bacteria to induce damage and cell lethality within the host.

The sticky *A. baumannii*

Apart from the afore-mentioned AbOmp, CPS and T6SS, *A. baumannii* presents many additional components including biofilm-associated protein (Bap), Csu pili and type IV pili to maintain its biofilm formation, promoting bacterial persistence (Brossard and Campagnari 2012; Harding *et al.* 2013; Tomaras *et al.* 2008). While the Csu pili are thought to be responsible for *A. baumannii* adherence on abiotic surfaces to facilitate its spread only (Tomaras *et al.* 2008), the Bap is responsible for *A. baumannii* to adhere not only to eukaryotic cells during infection, but also enhancing bacterial adherence to abiotic surfaces (Brossard and Campagnari 2012). Like other components of *A. baumannii*, both Bap and Csu pili levels vary under different stimuli. For example, Azizi and colleagues (2016) demonstrated the up-regulation of Bap in *A. baumannii* growing under low iron conditions. Moreover, *A. baumannii* utilize a two-component regulatory system to alter the expression of Csu pili (Tomaras *et al.* 2008), in response to environmental factors such as antibiotic exposure (Moon, Weber and Feldman 2017; Farshadzadeh *et al.* 2018).

On the other hand, type IV pili are known to play a role in a range of processes from cell adhesion to bacterial motility. Though most studies recognized the role of type IV pili in bacterial adherence and movement (Harding *et al.* 2013), Jacobs *et al.* reported up-regulation of genes responsible for type IV pili biogenesis during growth in human serum, indirectly suggesting that type IV pili may be important in promoting the complement resistance or facilitating escape of *A. baumannii* (Jacobs *et al.* 2012). In other gram-negative bacteria such as *Pseudomonas aeruginosa*, type IV pili adhesion triggers a downstream signaling cascade, subsequently enhancing bacterial virulence (Persat *et al.* 2015). Additionally, in *E. coli*, Bieber and colleagues suggested that presence of type IV pili bundle-forming pilus (bfp) directly related to bacterial virulence, as inactivating bfp biogenesis reduces pathogenicity (Bieber *et al.* 1998). However, a direct link between type IV pili-dependent twitching motility and the virulence of *A. baumannii* is still lacking.

Innate host responses to *A. baumannii*

While different *A. baumannii* virulence factors are relatively well-described, there is limited knowledge on how the host responds to the various bacterial components of *A. baumannii*. The first line of host defense involves innate immune pattern-recognition receptors (PRRs) sensing conserved structures of microbial organisms, called pathogen-associated molecular patterns (PAMPs). Host cells also release damage-associated molecular patterns (DAMPs), similar to PAMPs, that are also sensed by PRRs. The most studied family of PRRs is toll-like receptors (TLRs), with TLR2 and TLR4 being the predominant cell surface sensors of bacterial infections (Ramstead *et al.* 2016). In this section, we will describe the current knowledge of the major players in the interaction of the host and the pathogen.

Recognition of different *A. baumannii* components by TLRs

As described previously in this review, OmpA is an important component of *A. baumannii* virulence. As a consequence, recombinant AbOmpA induces host TLR2 mRNA up-regulation and cell surface expression in the human respiratory epithelial cell line (HEp-2) (Kim *et al.* 2008). Additionally, in murine dendritic cells, blocking the TLR2 signaling pathway reduces AbOmpA-stimulated IL-12 production (Lee *et al.* 2007). Together, this data implies a potential role of TLR2 in the recognition of AbOmpA, contributing to host immune defense against *A. baumannii* infection.

However, the contribution of TLR2 recognition in host defense during *A. baumannii* infection *in vivo* remains elusive. *Tlr2* knockout mice show decreased clearance of non-MDR *A. baumannii* (ATCC15150) in the lung at early stages of infection, despite having similar weight loss and lung pathology as wild-type mice (Kim *et al.* 2014). In contrast, *Tlr2*$^{-/-}$ knockout mice are protected against the carbapenem-resistant strain *A. baumannii* RUH2037; exhibiting higher C-X-C motif chemokine ligand 2 (CXCL2) and CCL2 release, earlier immune effector cell (e.g. granulocyte) recruitment to the lung and lower lung bacterial load post *A. baumannii* RUH2037 infection compared with wild-type mice (Knapp *et al.* 2006). This suggests that TLR2 is somehow involved in the recognition and clearance of non-MDR, as well as being responsible for the recruitment of some effector

cells during MDR infection. Future work will clarify the interaction between AbOmpA and TLR2 as well as the contribution of TLR2 to host defence during *A. bamannii* infection with different strains, and which other bacterial factors are invovled in these interactions.

Unlike AbOmp, a large body of evidence indicates that LPS is highly stimulatory to host TLR4 receptors and its co-receptor CD14. The activation of TLR4 on macrophages by *A. baumannii* induces nitric oxide synthase (iNOS) to produce bactericidal nitric oxide (NO) (Kim *et al.* 2013). Isolated *A. baumannii* LPS elicits TLR4/CD14-dependent TNFα and IL-6 secretion as well as immune effector cell recruitment *in vivo* from wild-type mice (Knapp *et al.* 2006). *Tlr4*$^{-/-}$ mice show higher lung bacterial load and a higher rate of bacterial dissemination compared to wild-type C57BL/6 mice, post intra-nasal inoculation of carbapenem-resistant *A. baumannii* (RUH2037) (Knapp *et al.* 2006). Thus, the recognition of *A. baumannii* LPS by host TLR4 is indispensable for host defense. As discussed previously in this review, the inflammatory response triggered by *A. baumannii* is not directly proportional to LPS quantity, but may be dependent on LPS composition. It is suggested LPS lipid A modifications, through pmrC gene up-regulation, is related to TLR4 sensing of LPS, and the subsequent inflammatory responses elicited (Lin *et al.* 2012).

In contrast, only a few reports investigated other PRRs involved in triggering host responses during *A. baumannii* infection. For instance, TLR9 receptors, found intracellularly in endosomes, are known to recognize both bacterial and viral CpG DNA. In *Tlr9*$^{-/-}$ knockout mice, more severe lung lesions and greater bacterial dissemination to liver and spleen was reported compared to wild-type mice following intranasal inoculation of *A. baumannii* (ATCC17978, non-MDR). In infected *Tlr9*$^{-/-}$ mouse lungs, lower levels of TNFα and interferon-γ (IFNγ) were also observed (Noto *et al.* 2015). *In vitro* experiments showed that both live and chemically-killed *A. baumannii* leads to NF-κB activation in a TLR9-dependent manner (Noto *et al.* 2015). This implies that TLR9 may participate in immune defense against *A. baumannii*, and that activation of TLR9 is not dependent on bacterial activity, such as the release of virulence factors through its secretion system. Furthermore, Noto and colleagues suggested purified *A. baumannii* DNA does not result in TLR9-dependent NF-κB activation (Noto *et al.* 2015). However, it is the first report to indicate that *A. baumannii* are able to invade human kidney epithelial cells (HEK293) and induce TLR9 signaling. Together, *A. baumannii* elicits protective TLR9 signaling in the host, yet the bacterial component recognized by TLR9 remains to be identified.

Overall, TLRs plays an important role in recognizing different *A. baumannii* components post bacterial invasion, triggering subsequent protective inflammatory response. While the involvement of TLR2 and TLR4 were undeniable, the participating intracellular host receptors remains to be characterized in more depth.

Host inflammasome activation and regulation of inflammatory responses

In addition to the well-recognized roles of neutrophils and macrophages, as well as soluble antimicrobial factors previously reviewed by Garcia-Patino *et al.* (2017), recent studies propose a role for downstream inflammasomes during *A. baumannii* infection. The PRR signaling cascades triggered upon bacterial recognition lead to TNFα and pro-IL-1β secretion, amplifying the inflammatory response. IL-1β is an important pro-inflammatory cytokine, which tightly regulates levels of inflammation in response to infection. The maturation of pro-IL-1β into the secretory form of 'IL-1β' depends on the proteolytic activity of caspase-1, which is activated by a multi-protein complex called 'inflammasome'. The families of inflammasomes include NLRP3 (NLR family pyrin domain containing 3), NLRC4 (NLR family CARD domain-containing protein 4), AIM2 (absence in melanoma 2) and more (Man and Kanneganti 2015). The role of NLRP3 has been characterized comprehensively in various diseases, including microbial infections. Many stimuli are known to activate the NLRP3 inflammasome, including bacterial, viral or fungal factors, ROS, extracellular ATP, potassium (K^+) efflux or lysosomal damage as examples (Man and Kanneganti 2015). The assembly of the NLRP3 inflammasome following PRR signaling and pathogen-associated molecular pattern (PAMP)—including LPS or damage-associated molecular pattern (DAMP) sensing- is termed the 'canonical inflammasome pathway'. In addition to caspase-1, caspase-11 is involved in non-canonical NLRP3 inflammasome activation. Caspase-11 is activated upon binding to cytosolic LPS; activated caspase-11 leads to both caspase-1-dependent and -independent release of IL-1β (Py *et al.* 2014; Man and Kanneganti 2015).

Patients in surgical and respiratory intensive care units with *A. baumannii* pneumonia (strain unidentified) show high IL-1β levels from bronchoalveolar lavage fluid (BALF) (Wu *et al.* 2003), suggesting an involvement of inflammasome pathways. Additionally, *Nlrp3*$^{-/-}$ mice infected intra-nasally with clinical MDR strain (AB8879) showed higher bacterial burden and impaired neutrophil recruitment to the lungs compared to wild-type mice (Dikshit *et al.* 2017). However, the difference in bacterial burden was not observed if wild-type and *Nlrp3*$^{-/-}$ mice were infected with non-MDR *A. baumannii* (ATCC19606 or ATCC15150) (Dikshit *et al.* 2017; Kang *et al.*, 2017). Dikshit and colleagues (2017) identify that the clinical MDR isolate (AB8879) is more virulent compared to the antibiotic-sensitive strain (ATCC19606), as the MDR isolate induces less early lung neutrophil influx and higher BALF IL-1β levels. The increased IL-1β levels suggests further activation of NLRP3 by MDR *A. baumannii* which evades first-line of host attack. This phenomenon is supported by the clinical report of Wu *et al.*, which indicates that patients with a high bacterial burden show significantly higher BALF IL-1β (Wu *et al.* 2003). This heightened inflammasome activation contributes to additional neutrophil recruitment and bacteria clearance during late stage of infection (Dikshit *et al.* 2017). Additionally, caspase-11 also plays a role in the secretion of IL-1β in early infection, and during *A. baumannii* clearance *in vivo*. Compared with wild-type mice, *Casp-11*$^{-/-}$ mice exhibit higher susceptibility and more severe pathological lesions in response to MDR *A. baumannii* (ATCC BAA1605) (Wang *et al.* 2017). The activation of both caspase-11 and NLRP3 has been identify to be dependent on type I interferon (IFN) signaling *in vitro* (Li *et al.* 2018). Recent report by Li *et al.* indicate that clinical MDR *A. baumannii* (CN40) induces TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent type I IFN signaling and downstream histone modification (H3K27ac), which subsequently increases caspase-11 promoters (Li *et al.* 2018).

This bacterial strain-specific role of inflammasome activation is less observed for *in vitro* studies. Upon *A. baumannii* (ATCC15150) stimulation, wild-type murine macrophages show activated caspase-1 and IL-1β secretion (Kang *et al.* 2017). In *Nlrp3*$^{-/-}$ mouse macrophages, active caspase-1 is not detected and IL-1β secretion is reduced (Dikshit *et al.* 2017; Kang *et al.* 2017; Li *et al.* 2018). In *Casp-11*$^{-/-}$ or *Casp-1/11*$^{-/-}$ macrophages, low levels of active caspase-1, and limited to no IL-1β secretion, is reported for different strains of *A. baumannii* infection across different studies (Dikshit *et al.* 2017; Kang *et al.* 2017;

Wang et al. 2017). The roles of additional mediators of NLRP3 during A. baumannii infection are also described. IL-1β secretion in vitro is reduced when wild-type macrophages are pre-treated with ROS inhibitor (NAC or APDC), K^+ efflux inhibitor (KCl) or cathepsin B inhibitor (CA074Me) (Dikshit et al. 2017; Kang et al. 2017). It is therefore concluded that A. baumannii induces IL-1β secretion in both caspase-11 and NLRP3/caspase-1-dependent manner. Upon A. baumannii infection, macrophages also release additional mediators that contribute to the activation of NLRP3.

After inflammasome activation, it is important to uncover the mechanisms of IL-1β regulation during A. baumannii infection and whether IL-1β signaling contributes to bacterial clearance. The role of IL-1β signaling in vivo has been evaluate by Kang and colleagues using IL-1 receptor-deficient mice ($IL\text{-}1R^{-/-}$) (Kang et al. 2017). It is indicated that IL-1β signaling does not play a role in in vivo clearance of the bacteria, but it does propagate lung injury during non-MDR A. baumannii (ATCC15150) infection (Kang et al. 2017). Although the report suggested a redundant role of IL-1β signaling, the importance of IL-1β during A. baumannii infection remains to be fully characterized.

To avoid uncontrolled inflammatory responses and excessive lung injury, signaling of IL-1 may be inhibited by the expression of IL-1 receptor antagonist (IL-1Ra), which competes with IL-1 in binding to IL-1R, yet it does not generate downstream signaling (Arend et al. 1998). The equilibrium between IL-1 and IL-1Ra levels mediates the initiation and termination of the pro-inflammatory response, and therefore important for the prognosis of diseases such as lethal E. coli septic shock, human immunodeficiency virus (HIV), arthritis, inflammatory bowel disease and more (Arend 2002). Holub and colleagues identify an elevated IL-1Ra level in patients with community-acquired bacterial infection (Holub et al. 2013). Additional study by Hsu and colleagues show that patients with MDR A. baumannii pneumonia has higher IL-1Ra allele 1 frequency (i.e. allelic polymorphism) (Hsu et al. 2012), implying a regulation of IL-1 signaling by IL-1Ra during A. baumannii infection. While in vivo animal studies on the IL-1Ra regulation during A. baumannii or other bacterial infection is lacking, Borghi and colleagues examine the mechanism of IL-1/IL-1Ra balance during Candida albicans infection. In vaginal Candidiasis, the activation of NLRP3 and downstream IL-1β production is counteracted by IL-22-triggered NLRC4-dependent elevation of IL-1Ra (Borghi et al. 2015). Furthermore, two studies suggest the use of IL-1Ra as treatment to S. aureus infection might be deleterious, as the treatment with IL-1Ra leads to decreased mice survival to staphylococcal sepsis (Ali et al. 2015) and increased lung bacterial load in rabbits infected with Panton–Valentine leukocidin $(PVL)^+$ S. aureus (Labrousse et al. 2014). Collectively, much work is needed to determine whether activation of the inflammasome complexes is dispensable, as well as the regulation of downstream IL-1/IL-1Ra signaling during A. baumannii infection.

CONCLUSION AND FUTURE DIRECTION

Infection with difficult-to-treat MDR A. baumannii presents a worldwide threat. Understanding the molecular mechanisms involved in A. baumannii infection enables the development of novel therapeutic targets to potentially direct modifications that induce bacterial fitness costs, or alter unwanted host inflammation responses. Most of these factors are studied in vivo but while the use of murine models greatly improves our understanding of host pathogenesis during A. baumannii infection, it does have its limitations. Since the virulence, antibiotic resistance and bacterial surface composition of A. baumannii varies between strains, the host response is highly dependent on the bacterial strain studied. The availability of various clinical isolates and commercial strains can often lead to inconsistent outcomes, particularly if the infection data is analyzed in isolation of genomic or proteomic data. In view of the arising genome-editing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) screening, Transposon Directed Insertion Sequencing (TraDIS), and High Throughput Screening (HTS), editing either bacterial or host genomes will promote our understanding of host–pathogen interactions during different clinical A. baumannii infections. These innovative tools may help in identifying a set of genes required for A. baumannii infection or persistence across different clinical strains, and thus facilitate the development of novel cross-protective vaccines or inhibitors.

In parallel, the link between A. baumannii expression of bacterial components and bacterial virulence remains unclear. Likewise, the fractions of A. baumannii responsible for the host–pathogen interaction in various signaling pathways needs to be further examined. Therefore, characterizing the extent of inflammatory responses elicited by different clinical A. baumannii strains, in conjunction with the bacterial genomic sequence data, may provide more understanding of the extent of different bacterial fraction contributing to triggering host immune responses. Understanding how A. baumannii regulates its gene expression in response to host immune attack is crucial as this may be the primary cause of strain differences, but it may also provide future drug targets utilizing bacterial defense mechanisms to limit bacterial infection. Furthermore, as inflammasomes are crucial in developing host inflammation responses during A. baumannii infection, therapies targeting the inflammasome pathway to either promote bacterial clearance through agonist injection, or reduce excessive inflammation with inhibitors may also be a promising option. In view of the rising number of highly virulent MDR strains, it is important to understand the mechanisms of host–pathogen interactions so that novel therapies may be invented and implemented. In this respect, we noted the current limited knowledge of the host–pathogen interactions during A. baumannii infection and proposed future research directions using innovative technologies.

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