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Expert Review of Anti-infective Therapy

ISSN: 1478-7210 (Print) 1744-8336 (Online) Journal homepage: http://www.tandfonline.com/loi/ierz20

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To cite this article: Thomas R Laws, Adam W. Taylor, Paul Russell & Diane Williamson (2019): The treatment of melioidosis: is there a role for repurposed drugs? A proposal and review, Expert Review of Anti-infective Therapy, DOI: 10.1080/14787210.2018.1496330

To link to this article: https://doi.org/10.1080/14787210.2018.1496330

Published online: 10 Jan 2019.

EXPERT REVIEW OF ANTI-INFECTIVE THERAPY
https://doi.org/10.1080/14787210.2018.1496330

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REVIEW

The treatment of melioidosis: is there a role for repurposed drugs? A proposal and review

Thomas R Laws, Adam W. Taylor, Paul Russell and Diane Williamson

CBR Division, DSTL Porton Down, Salisbury, Wiltshire, UK

ABSTRACT

Introduction: Melioidosis is a significant health problem within endemic areas such as Southeast Asia and Northern Australia. The varied presentation of melioidosis and the intrinsic antibiotic resistance of *Burkholderia pseudomallei*, the causative organism, make melioidosis a difficult infection to manage. Often prolonged courses of antibiotic treatments are required with no guarantee of clinical success.

Areas covered: *B. pseudomallei* is able to enter phagocytic cells, affect immune function, and replicate, via manipulation of the caspase system. An examination of this mechanism, and a look at other factors in the pathogenesis of melioidosis, shows that there are multiple potential points of therapeutic intervention, some of which may be complementary. These include the directed use of antimicrobial compounds, blocking virulence mechanisms, balancing or modulating cytokine responses, and ameliorating sepsis.

Expert commentary: There may be therapeutic options derived from drugs in clinical use for unrelated conditions that may have benefit in melioidosis. Key compounds of interest primarily affect the disequilibrium of the cytokine response, and further preclinical work is needed to explore the utility of this approach and encourage the clinical research needed to bring these into beneficial use.

ARTICLE HISTORY
Received 6 December 2017
Accepted 29 June 2018

KEYWORDS
B. pseudomallei; melioidosis; repurposing; immunotherapy; anti-infectives

1. Introduction

Many pathogens are able to colonize and infect the human host by evading, exploiting, or suppressing the immune response, which leads to a variable spectrum of disease. Sepsis represents one extreme where pathogen-host interactions result in a rapid, biochemically and physiologically violent response with a fatal outcome to the host. There is little opportunity to intervene therapeutically apart from attempting to reduce the pathogen burden as quickly as possible while supporting and correcting physiological derangements. The existence of infection in both chronic and latent disease-states suggests that some form of equilibrium is reached in which the host immune system has accommodated the pathogen. If this equilibrium is not fixed, chronic infection may have profound debilitating effects on the host over time, leading to considerable morbidity and mortality. Latent infection has limited overt effects; however, latent infection potentially represents a pathogenic 'time-bomb' typically as the immune system deteriorates with age. Melioidosis, caused by *Burkholderia pseudomallei*, is an example of such and causes disease that ranges across the entire spectrum from acute to latent [1], and it has been shown that resurgent infections can come from both reinfection and relapse [2].

B. pseudomallei is an important cause of disease in South East Asia, and Northern Australia, where it is a major cause of sepsis [3]. Its geographical distribution, however, is far greater than previously considered due to lack of recognition or under-reporting in some nations [3]; thus, the global burden is unclear but has been predicted to being the cause of as many as 165,000 (95% credible interval 68,000–412,000) and 89,000 (36,000–227,000) deaths [3]. The bacterium *B. pseudomallei* is associated with soil and fresh water, especially in paddy fields, and causes infection by either direct inoculation following trauma and wound contamination with *B. pseudomallei*, or via inhalation of contaminated water or soil. Certain individuals appear more susceptible to infection and severe disease, including those with diabetes, established renal failure, and cancer [4]. Management is difficult as *B. pseudomallei* is intrinsically resistant to many antibiotics and typically treatment requires at least 2 weeks of intravenous antibiotic therapy followed by lengthy oral therapy, often with multiple antibiotics [1,5]. Despite efficacious antibiotic therapy being available, there is still significant mortality and morbidity, and there is no guarantee of clinical success, often due to severe forms of the disease [6]. Furthermore, this brings up the potential for prolonged latent disease, which represents a future problem for the individual infected. *B. pseudomallei* has also been of significant interest to the biodefence community, due to its attributes of intrinsic antibiotic resistance and its ability to cause severe infections via the respiratory route in healthy individuals [7,8].

This review considers the pathogenesis of *B. pseudomallei* and considers potential strategies and targets for intervention.

CONTACT Thomas R Laws trlaws@dstl.gov.uk DSTL Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK

2. Multiple scaled points for therapeutic intervention in melioidosis

In melioidosis, there are a number of points in the disease process where therapeutics could be used to: kill the pathogen, arrest further multiplication and spread, boost, or restore the immune system to kill the pathogen, ameliorate sepsis, and restore normal physiology. There is increasing understanding of the pathogenesis of *B. pseudomallei*, but gaps in knowledge still remain given the complex nature of the pathogen and considerable variability between strains and in the host response. Some of the bacterial virulence determinants have been described by Stone et al. [9].

2.1. Killing the pathogen

Attempting to kill or cripple a pathogen in the early stages of infection is a tried and tested medical intervention that has been the bedrock of modern medicine and public health for over 200 years in the case of immunization [10] and nearly a century with chemical-based antimicrobial agents [11].

Antimicrobial therapy of melioidosis has been widely researched from *in vitro* susceptibility testing through to clinical trials with single agents or combinations [12]. However, *B. pseudomallei* is resistant to many antibiotics, and in the context of a lack of investment into new antibiotic drugs for any bacterial disease, finding a fully efficacious antibiotic is challenging, and this is beginning to have profound effects on healthcare [13].

There are rare reports of new antibiotics [14]. Attempts to develop new antibiotics include the modification of current antibiotics and consideration of antibiotic delivery systems that may offer potential strategies to extend the utility of current antibiotics. For example, dendritic cells have been used as 'mules' to take antibiotic directly into granulomas [15], and lipid encapsulation of antibiotics has shown some promise in delivering antibiotics into the lung [16].

Prophylactic immunization primes the immune system to fight off infection. Despite considerable work in animal models with putative candidates, a melioidosis vaccine for use in the clinic remains elusive for the time being [17,18].

Passive immunization introduces preformed antibody from a donor, which could be derived by purification from convalescent serum from a previously infected individual, or from an animal source that has been deliberately challenged with a pathogen or parts of a pathogen [19]. Recently, the use of monoclonal antibodies and de-speciated antibodies (to prevent hypersensitivity reactions) have provided improvements to this strategy, but again in the case of melioidosis, there has been little progress beyond proof-of-concept that such an immunotherapeutic approach can be efficacious using *in vivo* models [20–22]. In contrast, there has been commercial development of a pipeline of immuno-therapeutics for other infections [23]. Perhaps one reason why antibody-based therapies have not been considered more is that antibodies need to be able to bind to bacteria, while in the extracellular space, *B. pseudomallei* is not only capable of intracellular infection but is likely able to move between cells without entering the intracellular space (reviewed in [24]). This would likely impact on the ability for antibody-based therapies to fully clear infection without assistance from antibiotics that accumulate within cells and/or the cell mediated immune response.

2.2. Prevention of host cell subversion

B. pseudomallei is phagocytozed by granulocytes but resists intracellular killing. Within the cell, the bacteria are able to multiply and spread to adjacent cells [25]. Macrophages can kill *B. pseudomallei*; however, they need to be in an interferon-γ rich environment [26] and, as discussed later here, are subverted by other mechanisms.

The opportunities to prevent this exploitation of the host immune system start with preventing bacterial uptake by phagocytic cells, or expression or blockade of bacterial virulence factors. Small molecule inhibitors to blockade the function of virulence determinants have been, and continue to be, widely used in the development of antiviral therapies [27]. To our knowledge, to date there are no small molecule inhibitors that have been licensed against bacterial virulence determinants. However, there is significant interest in this area, and examples of broad spectrum targets of interest include type-III secretion systems [28], quorum sensing [29], and fatty acid synthesis [30]. Each of these are important in the virulence of *B. pseudomallei* [31–33]. The roles of type-III secretion systems to deliver effectors and quorum sensing to alter gene expression based on cell density are self-evident; however, the role of fatty II acid synthesis might need more explanation. Fatty acids are an essential building block of all life, and critically (for the purposes of antimicrobial drug development) the enzymes that perform this function are very different comparing mammals and bacteria [34].

The premise that neutrophils are important in the immune response to melioidosis [35] led to clinical trials using Granulocyte Colony Stimulating Factor (G-CSF, stimulator for neutrophil release) in conjunction with conventional therapies [36]. Unfortunately, G-CSF therapy did not have a significant impact on outcome. While G-CSF therapy increases the numbers of neutrophils (providing their production is not currently at maximum), it is argued that a lack of activation contributed to the apparent clinical failure. Mice deficient in osteopontin, a pleiotropic cytokine that is chemotactic for neutrophils, was shown to be more resistant to *B. pseudomallei* infection [37].

As already mentioned, *B. pseudomallei* is able to resist intracellular killing. The process known as xenophagy, effectively an autophagic process, is subverted by many pathogens, and autophagy itself is believed to be important in the formation of tumors and chronic inflammatory conditions. There is considerable research interest in manipulating autophagy pathways as a tumor therapy, and stimulation of autophagy is potentially a strategy that could be employed for melioidosis [38].

2.3. Prevent/mitigate manipulation of caspase systems

A key enzyme in the cell death pathway is caspase-1, which when activated causes cell death and cleaves the pro-inflammatory cytokines IL-1β and IL-18. The role of caspase-1 in HIV has attracted considerable interest. The pyroptotic cell death pathway was found to be subverted in HIV infection,

promoting CD4 T-cell destruction, and hence the promotion of inflammation through inhibition of caspase-1 (increasing apoptosis and reducing pyroptosis) was found to be beneficial [39]. In the case of melioidosis, it is known that ablation of caspase-1 will render mice highly susceptible, and this is believed to be because critical interferon-γ production is reduced without the cleaving of IL-18 [40,41]. This would suggest that pyroptosis is important in raising inflammation against *B. pseudomallei*. The main outputs of pyroptosis (IL-1β and IL-18) were investigated for their utility in protecting against *B. pseudomallei* infection [42]. The role and importance of IL-18 in inducing IFN-γ was confirmed; however, IL-1β was found to deleterious. Activation of pyroptosis was found to be dependent upon Nod-Like Receptor (NLR) C4 [42,43]. It was also found that NLRP3 contributed to IL-1β production but in a pyroptosis independent manner [42]. This seemingly is the cause of the imbalance where IL-1β is detrimental. Type III secretion is important in the interaction between *B. pseudomallei* and NLRC4 [44]. Recently, capsase-6 has also been shown to be important in response to *B. pseudomallei*, where capsase-6 deficient macrophages were shown to be less bactericidal and capsase-6 mice exhibited higher sensitivity alongside raise IL-1β and IL-10 production [45].

2.4. Rebalancing of the cytokine network

An effective immune response to infectious disease must be carefully counterbalanced to effectively kill and eradicate the invading pathogen, yet it must be 'reined in' to prevent collateral damage to the host. Immune pathways permitted to continue unopposed by appropriate feedback is the basis of the so-called 'cytokine storm' leading to the sepsis pathway, which is associated with high mortality. In the pathogenesis of melioidosis, IL-18 is activated via caspase-1 along with IL-1β; IL-18 then stimulates IFN-γ, which restricts bacterial multiplication. IL-1β can lead to excessive neutrophil recruitment, which in the case of melioidosis provides advantage to the bacteria. Murine studies have shown the benefit of IL-18 production (via IFN-γ) in contrast to worse outcomes associated with overproduction of IL-1β [42]. Further evidence of the detrimental effects of IL-1β is the apparent survival advantage of a small sub-population of diabetic patients with melioidosis that receive the antidiabetic drug glyburide [46]. Study of the patients' transcriptome showed reduction of many inflammatory markers (including IL-1), correlating with the effects of the drug's prevention of activation of cryopyrin inflammasome [47]. The benefits of glyburide were also seen in a murine model of infection with reduced bacterial load and inflammation (notably IL-1β) [48]. Another study, however, found that treatment with glyburide worsened disease associated with reducing inflammation [49]. The latter study found that glyburide had an immune-tempering effect that was driven via an IRAK-M specific route, reducing inflammation including IL-1 levels. Further work has shown that glyburide acts upon neutrophilic interaction with *B. pseudomallei* [50] where again it was demonstrated to reduce IL-1 release. As previously mentioned, IL-1β inhibits IFN-γ release, and IFN-γ is likely to be very beneficial. Murine studies have shown that supplementation with exogenous IFN-γ has a synergistic effect with ceftazidime and improves survival [51], further establishing that IL-1β can be deleterious in melioidosis. Collectively, this evidence suggests that reduction in IL-1β with enhancement of IFN-γ signaling could be of benefit therapeutically.

2.5. Prevention of immunopathology

One retrospective study in Thailand suggested that approximately 9% of individuals with melioidosis will die from sepsis [6]. Given individual variations in responses to pathogens and the complexity of the host response, there may be multiple mechanisms, as yet not all understood, which lead to sepsis. Immuno-therapy to prevent severe sepsis against a variety of pathogens has universally failed to meet expectations [52], and the recognition of the diverse nature of sepsis requires stratification not only of the causative microbe but aspects of the host [53] so that therapy may have to be tailored to the individual [54]. Nonetheless, the science resulting from the sepsis field may still present new therapeutic options for severe melioidosis.

3. Potential therapeutic opportunities

3.1. Therapeutics to kill/inhibit the pathogen

An interesting endeavor might be to select candidates from the pharmacopeia, looking for effects on *B. pseudomallei* growth and viability as has been attempted for other infections. Kruszewska and colleagues investigated greater than 160 pharmaceuticals for antimicrobial properties against strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans* [55]. Later, Younis and colleagues test 727 pharmaceuticals for activity against strains of *Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa*, and *Enterobacter cloacae* [56]. Two drugs were shown to have efficacy in an *in vivo* mouse model of *S. aureus* infection [56]. Between these two studies, numerous in-use pharmaceuticals have been identified to have direct antimicrobial effects (Table 1) and may be worthy of investigation against *B. pseudomallei*.

One strategy is to have *B. pseudomallei* targeted for killing by the host using of antibodies. There are potential limitations of using antibody therapies to treat an infection that seems to have an intracellular life style. Despite this, monoclonal therapies can have beneficial effects even after pulmonary bacterial challenge [20]. However, such a therapy would need clinical development from first principals, negating the advantage of many of the other therapies discussed in this article.

3.2. Therapeutics to prevent host cell subversion

Given the importance of IL-18 and IFN-γ, and their apparent effectiveness *in vivo* when given with ceftazidime, their potential use is obvious. Recombinant IFN-γ-1b (Immukin®) is licensed for use in chronic granulomatous disease and severe malignant osteopetrosis and use off-licence in mild to severe atopic dermatitis and has also been trialed for the treatment of Friedreich's ataxia. Treatment with rIL-18 may play a role in the pathogenesis

Table 1. In-use pharmaceuticals found to have some direct antimicrobial effects in studies [55,56].

Drug	Class	Clinical Use
Alendronate Clodronate	Bisphosphonate	Inhibit recruitment and promote apoptosis of osteoclasts reducing resorption and turnover of boneUsed in:Paget's DiseaseHypercalcaemia secondary to malignancy Prevention/therapy of post-menopausal osteoporosis Prevention/therapy of glucocorticoid-induced osteoporosis Therapy of cancer metastases in bone
Aciclovir	DNA Polymerase Inhibitor	Antiviral for *Herpes viridae* Used in: Prophylaxis/therapy of *Herpes simplex* 1 and 2 and *Varicella zoster* infection
Alverine		Antispasmodic Used in: Gastro-intestinal disorders characterized by smooth muscle spasm Dysmenorrhea
Butorphanol Tramadol	μ-opioid receptor partial agonist and antagonist	Opioid analgesia
Diclofenac Etodolac Naproxen Oxaprozin Tolfenamic acid	Non-steroidal anti-inflammatory (NSAID)	COX-1/COX-2 inhibitors Anti-inflammatory, analgesia, antipyrexial
Emastadine Levocabastine	H1 receptor antagonist	Topical antihistamine for allergic conjunctivitis
Fluvastatin	Statin	HMG-CoA reductase inhibitor Reduction of cholesterol
Ketamine	Anesthetic	Anesthetic
Losartan Telmisartan	Angiotensin II receptor antagonist	Anti-hypertensive Also used in cardiac failure
Matipranolol	Non-selective beta blocker	Topical for glaucoma
Mesalazine	Aminosalicylate	Ulcerative colitis
Oxymethazoline	α1 adrenergic receptor agonist and α2 adrenergic receptor partial agonist	Nasal decongestant
Proxymetacaine	Antagonist on voltage-gated sodium channels	Topical ophthalmic local anesthetic
Ribavarin	Adenosine/Guanosine analog	AntiviralUsed in:Respiratory Syncytial Virus Hepatitis C Arenavirus infection (e.g. Lassa Fever) Bunyavirus infection (e.g. Congo-Crimean Hemorrhagic Fever)
Rutin	Dietary Supplement	Vascular protection
Sulodexide	Anticoagulant	Prophylaxis/therapy of vascular thrombo-embolic disease
Tegaserod	5-HT4 agonist	Gut motility agent
Temozolomide	Alkylating agent	Chemotherapy for brain tumors
Ticlopidine	Adenosine diphosphate (ADP) receptor inhibitor	Prophylaxis/therapy of vascular thrombo-embolic disease
Tropicamide	Anticholinergic	Short acting mydriatic
Ebselen	Organoselenium compound mimicking glutathione peroxidase	Potential as a therapy for reperfusion injury, stroke, hearing loss and tinnitus, and bipolar disorder Activity against *Clostridium difficile*
5-fluoro-2′-deoxyuridine Raltitrexed	Thymidylate Synthase inhibitor	Chemotherapy for colorectal tumors
Epirubicin Daunorubicin Idarubicin	Anthracycline	Chemotherapy for a variety of malignancies
Toremifene[1] Tamoxifen[2] Clomifene[3]	Selective estrogen receptor modulator	Chemotherapy for some prostate malignanciesPrevention/therapy of breast malignancies Infertility treatment
Isoprenaline	Non-selective β adreno-receptor agonist	Treatment for bradycardia and heart block
Oxiconazole Bifonazole Clotrimazole Econazole Miconazole	Azole	Antifungal
Triclabendazole	Benzimidazole	Antihelminthic
Carmofur 5-Fluorouracil Ftorafur	Pyrimidine analog	Chemotherapy for a variety of malignancies
MK-886	Leukotriene antagonist	5-lipoxygenase-activating protein inhibitor and COX-1 inhibitor Anti-inflammatory
5-Nonloxytryptamine	Serotonin receptor agonist	Variety of different types of agonist used as antidepressants or migraine therapy
Dactinomycin	Actinomycine	Chemotherapy for a variety of malignancies
Acetazolamide	Carbonic anhydrase inhibitor	Used in:GlaucomaEpilepsyPeriodic paralysisCentral sleep apnoealdiopathic intracranial hypertensionAltitude sicknessDural ectasiaCystinuria
Furosemide	Loop diuretic	Used in:Peripheral edema due to cardiac or hepatic failurePulmonary edemaCerebral edemaHypertension

Table 2. Inhibition of IL-1β can be achieved by a number of drugs.

Drug	Class	Clinical Use
Anakinra	Recombinant interleukin 1 receptor antagonist	Rheumatoid Arthritis
Canakinumab	Monoclonal Antibody	Cryopyrin-associated periodic syndromes (CAPS). Gout
Chloroquine	4-aminoquinoline	Antimalarial
Rilonacept	Dimeric fusion protein consisting of IL-1 receptor component and IL-1 receptor accessory protein	Cryopyrin-associated periodic syndromes (CAPS)
Sitagliptin	Dipeptidyl peptidase-4 (DPP-4) inhibitor	Diabetes

of adenomyosis, Hashimoto's thyroiditis and upregulates amyloid-beta production in neurons. It has, however, shown potential in the therapy for age-related wet macular degeneration [57].

Neutrophil (and other phagocytic cell) activation may be a potential pathway as a therapeutic target. In keeping with all bacterial infections, immune reactions to melioidosis are provoked by the interaction of pathogen-associated molecular patterns (PAMPs) and host cell pattern recognition receptors (PRRs), and the resulting signaling cascade. There has been considerable interest in this interaction in the development of vaccine adjuvants, particularly those biasing Th1 responses, and also as adjuvants in cancer therapy [58]. The idea is not new: Coley used a bacterial lysate consisting of killed *Streptococcus pyogenes* and *Serratia marcescens* causing regression in some tumors at the end of the 19th century [59]. Continued trials with 'Coley's toxin' have been controversial, but they continue albeit with far more refined and defined moieties such as the CpG oligo-deoxy-nucleotides [60,61]. CpG ODN 1826 was found to be protective in murine models of melioidosis [62]. This finding was confirmed later where an intranasal deliver of class C CpG 2 days prior to an intranasal challenge of *B. pseudomallei* provided measurable benefits to the animals [63]. There are a number of other adjuvants that are in clinical use such as MF59 [64] that might have benefited in treating an entrenched *B. pseudomallei* infection by encouraging IFN-γ release and cellular killing.

B. pseudomallei has a variety of virulence factors that provide potential targets for vaccines and chemotherapeutic agents. Vaccine options have been considered extensively [17]. Chemotherapeutic targets include the type-III secretion system [28,65]; quorum sensing [29], and the type II fatty acid biosynthesis pathway [30].

A multitude of small compounds have been considered as putative inhibitors of the Type III secretion system, and these are summarized below from two review articles [28,65]. However, to our knowledge, none of these compounds are in clinical use and would therefore require significant development prior to use in melioidosis patients. The only inhibitor of type III secretion that has been used in clinical trials is an antibody fragment (KB001-A) found to interact with the type III secretion system of *P. aeruginosa* [66]. Releases in the public media, however, revealed reported commercial concerns with regard to success criteria, and it has since been shelved. Variants of thiazolidinones (a class of small molecule that has been very useful in drug discovery [67]) have been used clinically for unrelated purposes (rosiglitazone and pioglitazone, which are both insulin sensitizers); however, it is clear that the structure of these molecules can to be optimized for effect in inhibiting type III secretion systems in both bacteria from the genus *Salmonella* and *Yersinia* [68]. With regard to compounds that can inhibit type II fatty acid synthesis, Anthranilic acid has been used clinically previously [69] and has chemistry associated with other clinically used products. Indole-3-carbinol, which is similar (SB418001), has also been used clinically [70]. Also dithiolethiones have been studied in some detail, more so in the form of Oltipraz, which was tolerated in humans with some adverse effects [71]. 4-aminopyridime (or famridine) bears similarity to the aminopyridine that targets bacteria [72] and has been shown to be tolerated in a clinical trial [73]. The easiest fatty acid synthesis inhibitor to repurpose might be Isoniazid, which is already in clinical use for the treatment of *Mycobacterium* infection. Another drug that is worthy of investigation is fosmidomycin. This drug is currently used as an antimalarial and targets DXP reductoisomerase, a critical enzyme in the nonmevalonate pathway. This drug also targets this pathway in at least some Gram negative bacteria [74].

B. pseudomallei bacteria are able to escape phagosomes via the type III secretory system and spread intracellularly; avoiding cell mediated killing processes that require compartmentalization of the bacteria. Autophagy is a collective term for the targeted degradation and recycling of cellular components. Autophagy inducing compounds offer an alternative approach as a host-directed therapy to promote killing of intracellular bacteria (xenophagy) by targeting the host autophagic pathway. Examples of autophagy promoting drugs being investigated as host-directed therapies include vitamin D [75], metformin [76,77], statins [78], verapamil [79–85], carbamezapine [86,87], and valproic acid [86,87]. Verapamil hydrochloride is a calcium channel antagonist that shows potential as a host-directed autophagy therapy to reduce intracellular *Mycobacterium tuberculosis* [79–85]. Norverapamil is the metabolite of verapamil and shows a similar ability to reduce intracellular bacteria *in vitro* [79,80]. Verapamil is being investigated as an inhalable therapy for inhalational *M. tuberculosis* with the verapamil-antibiotic combination demonstrating an enhanced antibacterial effect [88]. Carbamazepine and valproic acid have both demonstrated enhanced *M. tuberculosis* clearance from RAW 264.7 macrophages *in vitro* [86]. The diabetic drug metformin is an autophagy inducer and also shows potential as a host-directed autophagy therapy for *M. tuberculosis* [77]. Given the similarities in intracellular infection characteristics between *M. tuberculosis* and *B. pseudomallei*, the autophagy compounds currently being investigated for *M. tuberculosis* could be an approach for use as a host-directed melioidosis therapy. We have seen antimicrobial effects of verapamil and norverapamil against intracellular *Burkholderia thailandensis* within an *in vitro* macrophage assay (A. Taylor, unpublished data).

The receptor COX-2 is a potential target for treatment of melioidosis [89] as the downstream effector from COX-2, prostaglandin E2 (PGE2), aided intracellular survival of *B. pseudomallei* by biasing macrophage activation toward the alternative pathway. COX-2 inhibitors protected some mice in a murine model of respiratory melioidosis, and it is

presumed that this protection was driven by increased classical activation of macrophages [89]. It is also possible that some of the protection afforded by COX-2 inhibition was driven by a reduction in IL-1 as COX-2 can reduce the release of IL-1β via the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome [90]. Currently, celecoxib is the only specific inhibitor of COX-2 that is licensed for use; however, non-steroidal anti-inflammatory drugs (NSIADs) such as diclofenac, aspirin, acetorphan, and ibuprofen all affect COX-2, although these are less specific in their action. It is of note that some members of this class of drugs have antibacterial activity (Table 1) and may interfere with the type II fatty acid biosynthesis pathway.

3.3. Therapeutics to rebalance the cytokine network

The potential therapeutic use of IL-18 and IFN-γ has already been discussed. At present, compounds that act on the inflammasome are restricted to pharmacological tools to elucidate the pathway. As understanding increases, the opportunity for therapeutic intervention may become apparent.

The antidiabetic glyburide [91] clearly has potential in treating melioidosis given the findings from patients who are on the drug, and it has shown (in combination with glucantime) to have some activity against *Leishmania major*, another intracellular pathogen where IFN-γ production is critical for host defense [91,92].

Thalidomide and its derivatives pomalidomide and lenalidomide enhance $CD8^+$ T cell activity [93], NK cell activity, and the release of IFN-γ [94]. Moreover, these drugs inhibit the release of IL-1, TNF-α, and IL-6 [95]. These effects are all potentially positive for the host during melioidosis, and these cytokines are prognostic markers for melioidosis disease severity [96]. $CD8^+$ T cells and NK cells are thought to contribute to host defense by rapid release of IFN-γ [97]. Moreover, the cytotoxic functions of NK cells and $CD8^+$ T cells are likely to contribute to the control of *B. pseudomallei* infection in its intracellular phase. In experimental settings, thalidomide has been considered for the treatment of AIDS [98,99] and tuberculosis [100]. In mice, thalidomide has been demonstrated to mitigate *K. pneumoniae* disease by reducing inflammation [101]. Also, in a hepatitis patient cohort, thalidomide successfully reduced signs of liver damage [102]. In the clinical setting, thalidomide is mainly used to treat leprosy [103]. Thalidomide therapy is not without disadvantages; it has had a long and troubled past with regard to its chiral form's teratogenetic affect [104] and is a known contraindication of a drug of interest to this manuscript (anakinra).

Another approach that has recently shown promise is the use of the anticancer therapeutic hexamethylene bisacetamide (HMBA) [105]. This therapeutic was found to increase the production of IL-12 and IFN-γ but not TNF-α, IL-6, and IL-8 in human peripheral blood mononuclear cells infected with *B. pseudomallei*.

Anakinra is a recombinant form of human IL-1 receptor antagonist (RA), a protein that competes with IL1 for binding to the IL-1R, thereby blocking signal transduction. IL-1RA has been previously considered for the tempering of severe sepsis, and, despite interesting preliminary data, the clinical trial was halted. A non-statistically significant 9% enhancement of survival was observed in the IL-1RA treated sepsis patients [106]. Anakinra has been shown to have efficacy in a murine model of respiratory melioidosis [42,107]. Other drugs exist that perform the same function (table 2).

Chloroquine, which has a long history of use in the treatment of malaria, also inhibits IL-1β release [108] as well as conferring other immune suppressive functions [109] that are being considered in trials in autoimmune disease. It also increases the pH on the phagolysosome to the detriment of intracellular pathogens, and hence it is used in chronic Query (Q)-fever. Chloroquine also has been demonstrated to show similar suppressive effects on the virulence of *B. pseudomallei* through mediation of pH in the phagolysosome [110].

Recent studies have indicated that the Damage Associated Molecular Pattern (DAMP) HMGB1 may also contribute to immune dysregulation, and two studies have demonstrated that antibody ablation of this target can be beneficial to a murine host under conditions of *B. pseudomallei* infection [111,112]. As yet there are no known drugs that are in clinical use that target HMGB1 or the pathways immediately below HMGB1.

3.4. Therapeutics to prevent, ameliorate, or treat immunopathology

The key focus in preventing sepsis is by attempting to quantify predictive derangements in physiology in a timely manner. Unfortunately, the presenting signs and symptoms vary considerably with age and are often non-specific, mimicked by many other medical and surgical problems. A lack of a consistent and specific and sensitive diagnostic system exacerbates the problem. Preventing the immune system spiraling into a catastrophic positive feedback loop has been an area of sepsis research for many years, with many established and novel therapeutics being investigated and trialed and in the main failing [53]. Meta-analysis of immunoglobin therapy suggests benefit in sepsis, but as for antibiotic administration, immunotherapy has to be given in a timely manner, and further research is needed [113]. Recombinant activated protein C (also known as autoprothrombin IIA and blood coagulation factor XIV) treatment was initially promising; however, meta-analysis has shown this to be ineffectual [114]. Recently, beta-blockers have been considered [115].

At present, antibiotics, supportive therapy to maintain blood pressure, end-stage organ oxygenation and perfusion are the mainstay in the care of the sepsis patient [116]. Hypoxic injury to the brain, kidneys, liver, or heart has obvious deleterious consequences. Furthermore, injury to the intestine may exacerbate an already desperate situation, where there may be a sudden release of huge amounts of PAMPs into the blood from translocation of the gut flora. Moreover, as the integrity of the gut is lost due to hypoxic damage, release of proteolytic enzymes from the gut can contribute to pathology [117].

Ultimately, what can be critical in the therapeutic success of treating melioidosis is the quality of the supportive care offered. This can include a range of healthcare, such as the

supportive therapy discussed above, to ensuring antibiotic therapy is taken. The effectiveness of supportive care is demonstrated by a comparison of melioidosis case fatality rates between countries with different healthcare systems []. While better therapeutics might lead to faster recovery for all melioidosis patients, they are unlikely to translate to improved survival in areas with good healthcare (where most individuals survive) but may reduce the period of treatment and thereby reduce the likelihood of complications and acquired resistance in the microbiota driven by prolonged exposure to antibiotics.

4. Conclusion

In conclusion, the pathogenesis of melioidosis (and indeed, other bacterial infections) provides a number of targets and processes that may be amenable to therapeutic intervention. Traditional strategies have been discussed briefly with the premise of a number of experimental pharmacological agents exploiting new avenues for intervention such as the type III secretion pathway, fatty acid synthesis and quorum sensing, some of which may have close analogs currently in clinical use. It is clear from this review that a number of drugs, particularly from certain classes, notably NSAIDs, cancer chemotherapeutics, and disease-modifying drugs and various others such as rapamycin, thalidomide (and derivatives), chloroquine, and glyburide, have the potential to be repurposed for melioidosis therapy. Further work is needed to evaluate these candidates. However, these drugs have a wide spectrum of biological effects, not only those they were designed to fulfill but also some side-effects. For this reason, consideration needs to be given to the overall pharmacological effect. For example, anesthetic drugs that are bactericidal may also cause numerous extraneous effects.

With the constant threat of antimicrobial resistance, and its potential to render treatable diseases untreatable, the melioidosis community has an opportunity to be the forerunners of new anti-infective therapies that might have a benefit across the whole gambit of infectious diseases. Repurposing drugs that are in clinical use to correct/re-direct the immune response represents a real opportunity that is potentially low in risk and might deliver rapidly. Moreover, this approach has the potential to be impervious to standard microbial resistance mechanisms, as the whole spectrum of the immune response will be brought to bear on the invading *B. pseudomallei*. We therefore urge that these anti-infective strategies be tested further in preclinical studies to build the case for use in the treatment of sufferers of this infection.

5. Expert commentary

The question of profitability continues to be an albatross around the neck of people trying to devise any new anti-infective strategies. Melioidosis is a fascinating example of this because it impacts two groups of people.

The first group are those people living in endemic areas who suffer from this disease, who are often from low-income groups working on the land and thus exposed to the bacteria. If we were to compare melioidosis to tuberculosis (which does have a significant amount of investment), the former is disadvantaged for funding, for a number of reasons. Firstly, the number of at-risk people is simply lower. Secondly, there is no evidence for horizontal transfer, indicating that only those performing high-risk activities are susceptible and those in more affluent areas are not. This means that any novel therapeutic is unlikely to be either profitable for pharma or affordable for those who suffer from this disease.

The other groups interested in melioidosis are the biodefence and military medicine communities, who have the capability to repurpose or support the licensure of some anti-infective therapies.

These communities are working together to accelerate development of therapeutics that is affordable for sufferers/at-risk people and suitable for biodefence. So far, no novel therapeutic has transitioned beyond the 'bench.' However, a significant benefit is that there is now a substantial body of scientific research that informs on the biology of melioidosis and possible targets to treat it.

The issue with leveraging this information to create novel ways to treat endemic infection and provide biodefence is the lack of investment in the translation of laboratory science into clinically useable therapeutics. This brings us to the second part of the review that addresses whether much of the cost of translation might be bypassed by repurposing drugs used to treat unrelated infections. We draw the reader's attention to a great variety of in-use pharmaceutics that have significant potential in the treatment of melioidosis. Drug repurposing would have the advantage of using drugs for which patents have expired and that can be produced cheaply as 'generics.' However, this route will almost surely require public funding; there is little profit to be made from expanding the use of a 'generic' pharmaceutical.

In conclusion, this review addresses how a treatment for melioidosis might be arrived at within a short time frame, in the hope that investment might be found for this strategy.

6. Five-year review

While melioidosis continues to kill people, it is incumbent on the scientific community to transition therapeutic concepts into real benefits. We hope to see either new antimicrobial drugs (perhaps the fruits of labors to counter the rise of antibiotic resistance) or a return to repurposing therapies from other areas of medicine as a real option to treat this disease.

Key issues

- Melioidosis is a tropical disease caused by infection with the Gram negative bacteria *B. pseudomallei*. These infections are problematic due to the intrinsic resistance of *B. pseudomallei* to many antibiotics.
- Melioidosis research suffers from the disease not affecting affluent groups, but some additional funding is leveraged because it is considered a biothreat agent.
- Repurposing therapies used elsewhere in medicine might provide a cost-effective way to transition recent findings regarding pathogenesis into the clinic.
- Disease is a result of biological interactions that can be scaled from the bacteria's capacity to grow in the body

and how it causes immune dysregulation and ultimately sepsis and potentially death.
- There are varying amounts of evidence that a variety of 'off-the-shelf' medicines can target these biological processes with potentially beneficial effects.
- More work in this area is needed.

Funding

The manuscript was funded by the UK Ministry of Defence.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosure

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

ORCID

Thomas R Laws http://orcid.org/0000-0002-0142-9162

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