REVIEW ARTICLE

MEDICAL PROGRESS

Melioidosis

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MELIOIDOSIS, CAUSED BY THE ENVIRONMENTAL GRAM-NEGATIVE BACIL-lus *Burkholderia pseudomallei*, is classically characterized by pneumonia and multiple abscesses, with a mortality rate of up to 40%. It is an important cause of community-acquired sepsis in Southeast Asia and northern Australia. Its known global distribution is expanding, a reflection of improvements in diagnostic microbiology and increasing numbers of cases in travelers and returning military personnel (Fig. 1).[1,2] A locally acquired case of melioidosis was recently described in the United States.[3] *B. pseudomallei* has been classified by the Centers for Disease Control and Prevention as a category B bioterrorism agent, resulting in increased research and understanding of melioidosis. This review considers recent developments in pathogenesis, diagnostics, and treatment.

THE BACTERIUM

B. pseudomallei belongs to the burkholderia genus, which contains over 40 species. Other pathogenic members include *B. mallei*, which causes glanders in horses and other solipeds and is highly virulent in humans, and *B. cenocepacia*, which is an important cause of opportunistic infection in patients with cystic fibrosis. The genus also includes *B. thailandensis*, which coexists with *B. pseudomallei* in the soil in Thailand and Australia, and *B. oklahomensis*, which is sporadically found in the midwestern United States; these two species rarely, if ever, cause disease and are much less virulent (by a factor of >100,000) than *B. pseudomallei* in hamsters and mice.[3,4]

A HIGHLY VARIABLE AND EVOLVING GENOME

The *B. pseudomallei* genome is composed of two chromosomes of 4.07 and 3.17 megabase pairs, respectively — it is one of the most complex bacterial genomes sequenced to date.[5,6] *B. pseudomallei* shares a core set of 2590 genes with other members of the burkholderia genus and is highly dynamic.[7-9] Eighty-six percent of the prototypic *B. pseudomallei* K96243 genome is common to all strains and represents the core genome, with 14% variably present across isolates. The variable region includes multiple genomic islands containing DNA acquired from other bacteria. Genomic islands are likely to be associated with virulence and the potential for infection, although specific associations with clinical outcomes have not yet been elucidated.[8] Genotyping of multiple *B. pseudomallei* colonies from several tissue sites from four patients with acute melioidosis showed substantial genetic diversity within a single patient, indicating the capacity of the organism to evolve rapidly within the host.[10]

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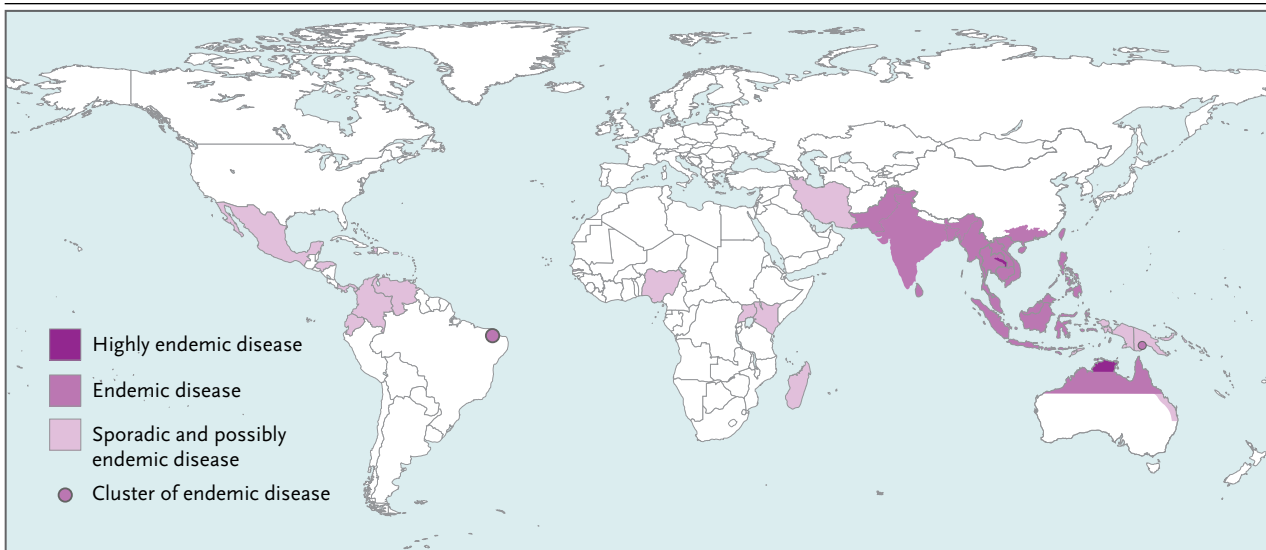
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Figure 1. Global Distribution of Melioidosis.

Areas where melioidosis is highly endemic, endemic, or sporadic and possibly endemic are indicated. This reflects current knowledge that is based on limited evidence and is likely to change over time.

VIRULENCE FACTORS

Multiple potential virulence factors have been described for *B. pseudomallei*, but the relative importance of each for human disease remains largely unknown. The quorum-sensing system influences the behavior of the whole bacterial population through the extracellular secretion of *N*-acyl homoserine lactones.[7,11,12] *B. pseudomallei* contains three type III secretion system (TTSS) gene clusters that encode membrane-spanning syringes that deliver bacterial effector molecules into the host-cell cytoplasm; the TTSS3, the Inv/Mxi-Spa–type system, plays a role in intracellular survival of the bacterium by influencing host-cell processes.[7,13] TTSS3 mutations impair the intracellular survival of *B. pseudomallei* and prevent bacterial escape from endocytic vacuoles.[7] The *B. pseudomallei* genome encodes six type VI secretion systems, which are implicated in bacterial virulence, intracellular survival, and competition within bacterial communities.[14,15] Capsular polysaccharide, lipopolysaccharide, and two other surface O-polysaccharides (O-PS; types III O-PS and IV O-PS) are additional putative virulence factors.[7] Flagella may be of importance for *B. pseudomallei* motility and invasion of host cells, although their importance as a virulence factor in human disease is debated. Burkholderia lethal factor 1 is similar to *Escherichia coli* cytotoxic necrotizing factor 1 and interferes with the initiation of translation, leading to alteration of the actin cytoskeleton and ultimately to cell death.[16]

HOST DEFENSE AGAINST *B. PSEUDOMALLEI*

INTRACELLULAR ACTIVITY

B. pseudomallei can invade, survive, and replicate in a range of phagocytic and nonphagocytic cells, and its intracellular behavior is considered to be crucial for disease pathogenesis.[17,18] After cellular uptake, this bacterium can escape from the vacuole and replicate within the host-cell cytosol. *B. pseudomallei* is then capable of inducing actin tails composed of actin filaments that become polarized and enable the bacterium to move inside the cell, with the subsequent formation of cell-membrane protrusions and direct cell-to-cell bacterial spread (Fig. 2).[17] *B. pseudomallei* can also induce multinucleated giant-cell formation.[19,20]

RECOGNITION OF *B. PSEUDOMALLEI* AND THE IMMUNE RESPONSE

Pattern-recognition receptors — especially the toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) — are the first to detect host invasion by pathogens, initiate immune responses, and form the crucial link between innate and adaptive immunity.[21] Pattern-recognition receptors recognize

conserved motifs on pathogens termed "pathogen-associated molecular patterns" (PAMPs). *B. pseudomallei* expresses various PAMPs, including lipopolysaccharide, peptidoglycan, flagella, TTSS, and DNA, which are recognized by various TLRs, and related molecules such as CD14 and MD2, which can be up-regulated in patients with melioidosis.[22] TLR4-region genetic variants in humans are associated with susceptibility to melioidosis.[23] Whether the lipopolysaccharide of *B. pseudomallei* signals by means of TLR2, like the lipopolysaccharide of *Legionella pneumophila* and *Leptospira interrogans*,[22,24] or by means of TLR4,[21,25] which is regarded as the receptor for lipopolysaccharide, remains the subject of intense study. Surprisingly, CD14-deficient and TLR2-deficient mice with experimentally induced melioidosis have a markedly improved host defense, as reflected by a strong survival advantage, whereas TLR4-deficient mice are indistinguishable from wild-type mice with respect to bacterial outgrowth and survival.[22,26]

B. pseudomallei can also activate the cytosolic inflammasome, a large, multiprotein complex formed, among others, by the NLRs NLRC4 and NLRP3, the assembly of which leads to the activation of caspase 1 and promotes the maturation of the proinflammatory cytokines interleukin-1β and interleukin-18.[21] Interleukin-18 plays a protective role during melioidosis through induction of interferon-γ, a key cytokine that contributes to protection against melioidosis.[27-29] By comparison, interleukin-1β may play a deleterious role by causing excessive neutrophil recruitment and tissue damage and by inhibiting the activation of interferon-γ production (Fig. 2).[28] *B. pseudomallei*–induced activation of caspase 1 through NLRC4, a receptor for the TTSS3 component BsaK,[30] leads to rapid macrophage cell death — a process known as pyroptosis, which serves as a host defense mechanism to restrict intracellular bacterial growth.[28,31]

The immune response initiated by pattern-recognition receptors leads to the recruitment of neutrophils, macrophages, and lymphocytes toward the site of infection. Although disproportionate neutrophil recruitment may be detrimental,[28] activated neutrophils play a critical role in early bacterial containment.[32] Murine cell-depletion studies have shown that T cells — in particular, CD4+ T cells — are important in both innate and adaptive immunity against *B. pseudomallei* infection,[29,33] although there is no association be-

tween infection with the human immunodeficiency virus (HIV) and the risk of melioidosis.[34] The proinflammatory cytokines tumor necrosis factor α and interleukin-6 — both of which are up-regulated during melioidosis — activate the coagulation system in severe melioidosis. All three of the major pathways are implicated, with the concurrent enhancement of procoagulant mechanisms and impairment of anticoagulant and fibrinolytic mechanisms.[35] The complement system, responsible for restoring host cellular homeostasis and opsonization and elimination of bacteria, becomes rapidly activated and consumed during *B. pseudomallei* infection.[7]

THE SPECTRUM OF HUMAN DISEASE

EPIDEMIOLOGY

Among the major regions where melioidosis is endemic, the Top End of the Northern Territory in Australia and northeast Thailand represent hot spots, with annual incidence rates of up to 50 cases per 100,000 people (Fig. 1).[36,37] Melioidosis is the third most common cause of death from infectious disease in northeast Thailand, exceeded only by HIV infection and tuberculosis.[36] Malaysia, Singapore, Vietnam, Cambodia, and Laos are also regions of endemic disease.[2,38] Reports have expanded the endemic zone to areas of the Indian subcontinent, southern China, Hong Kong, Taiwan, various Pacific and Indian Ocean islands, and parts of the Americas.[39] Sporadic cases have been reported in Nigeria, Gambia, Kenya, and Uganda, but the extent of the disease in Africa remains uncertain.[39-41]

The magnitude of melioidosis in the Americas remains to be elucidated. Two cases reported in the United States were thought to have been acquired in Honduras.[42] Severe melioidosis in Puerto Rico has been described in a patient with chronic granulomatous disease and in a person with diabetes, both of whom became ill during the rainy season.[43,44] Sporadic cases of melioidosis have been reported in Ecuador, Guadeloupe, and Aruba, and the emergence of melioidosis in Brazil is an example of increasing recognition in areas where the disease has become manifest as a result of enhanced awareness and diagnostic tests.[45] Aruba was the location of an outbreak in sheep, goats, and pigs in the 1950s[46] and may have been the location for an infection acquired by a child with cystic fibrosis who recently presented
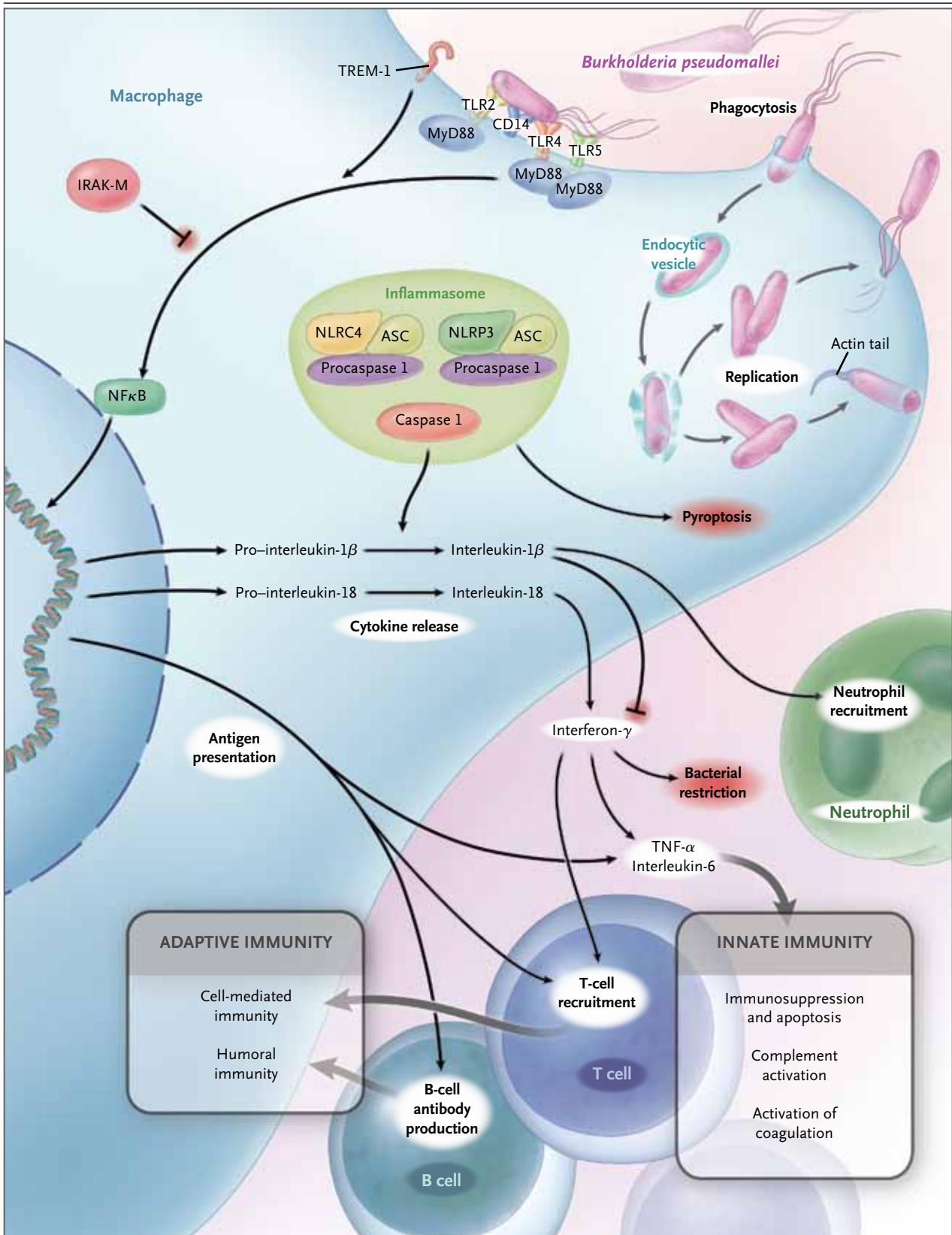
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Figure 2 (facing page). Immunopathogenesis of Melioidosis.

Burkholderia pseudomallei can invade macrophages and may then survive and replicate for prolonged periods. Although some bacteria are destroyed after phagocytosis, a proportion of the organisms escape endocytic vacuoles and either break out directly to the extracellular space or infect other cells through actin-based membrane protrusions. Toll-like receptors (TLRs) expressed on host cells are the first to detect invading *B. pseudomallei*, leading to nuclear factor-κB (NF-κB) induced activation of the immune response through the release of proinflammatory cytokines. The TLR response is tightly regulated: triggering receptor expressed on myeloid cells 1 (TREM-1) amplifies TLR-induced signals, and interleukin 1 receptor–associated kinase (IRAK)–like molecule (IRAK-M) dampens the immune response during melioidosis. Intracellular inflammasome receptors — most notably, the nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) NLRC4 and NLRP3 — recognize bacterial virulence factors and endogenous danger signals, leading to caspase 1–mediated release of interleukin-1β and interleukin-18, in addition to pyroptosis (caspase-dependent cell death that restricts intracellular bacterial growth). Interleukin-18 further induces protective interferon-γ production. Neutrophils are recruited toward the site of infection, and the complement and coagulation cascade becomes activated. As infection progresses, the adaptive immune response leads to T-cell recruitment in response to interferon-γ production, which gives rise to a cell-mediated immune response and the production of antibodies by B cells. Most of the data in this model are derived from in vitro studies and animal models of melioidosis. The apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) serves as an NLR adaptor molecule, whereas myeloid differentiation factor 88 (MyD88) serves as the central TLR adaptor molecule.

with melioidosis in Massachusetts.[47] The specific ecologic niches of *B. pseudomallei* appear to vary among locations where melioidosis is endemic, but the recent finding that *B. pseudomallei* is colonizing and thriving in the rhizosphere and aerial parts of native and imported grasses in northern Australia provides insights into global epidemiology and potential dispersal.[48]

CLINICAL MANIFESTATIONS

Melioidosis primarily affects persons who are in regular contact with soil and water. Infection results from percutaneous inoculation (e.g., by means of a penetrating injury or open wound), inhalation (e.g., during severe weather or as a result of deliberate release), or ingestion (e.g., through contaminated food or water) (Fig. 3). Melioidosis is predominantly seasonal; 75 to 81% of cases occur during the rainy season.[2,49] Incidence peaks between 40 and 60 years of age, but melioidosis is well recognized in children.[50] Melioidosis has been transmitted to infants through breast milk from mothers with mastitis.[2]

Since up to 80% of patients with melioidosis have one or more risk factors for the disease, it has been suggested that melioidosis should be considered an opportunistic infection that is unlikely to have a fatal outcome in a previously healthy person, provided that the infection is diagnosed early and appropriate antibiotic agents and intensive care resources are available.[37] Risk factors for melioidosis include diabetes (present in 23 to 60% of patients), heavy alcohol use (in 12 to 39%), chronic pulmonary disease (in 12 to 27%), chronic renal disease (in 10 to 27%), thalassemia (in 7%), glucocorticoid therapy (in <5%), and cancer (in <5%).[36,37]

The incubation period for melioidosis has been evaluated in a single published study, in which 25% of patients who recalled a specific event such as an injury had clinical manifestations 1 to 21 days (mean, 9 days) later.[51] The inoculating dose, strain virulence, mode of infection, and risk factors in the host are all likely contributors to the incubation period, clinical presentation, and outcome. An incubation period of a day or less was documented after aspiration of *B. pseudomallei* in a near-drowning event,[52] whereas the longest recorded apparent incubation period was 62 years.[53]

B. pseudomallei infection has protean clinical manifestations, and severity varies from an acute fulminant septic illness to a chronic infection (the presence of symptoms for >2 months, accounting for 11% of all cases) that may mimic cancer or tuberculosis (see Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[1,49] In a descriptive study involving 540 patients in tropical Australia over a 20-year period, the primary presenting feature was pneumonia (in 51% of patients), followed by genitourinary infection (in 14%), skin infection (in 13%), bacteremia without evident focus (in 11%), septic arthritis or osteomyelitis (in 4%), and neurologic involvement (in 3%)[37] (Fig. 3). The remaining 4% of patients had no evident focus of infection. Over half of patients have bacteremia on presentation, and septic shock develops in approximately one fifth.[37] Internal-organ abscesses and secondary foci in the lungs, joints, or both are common.
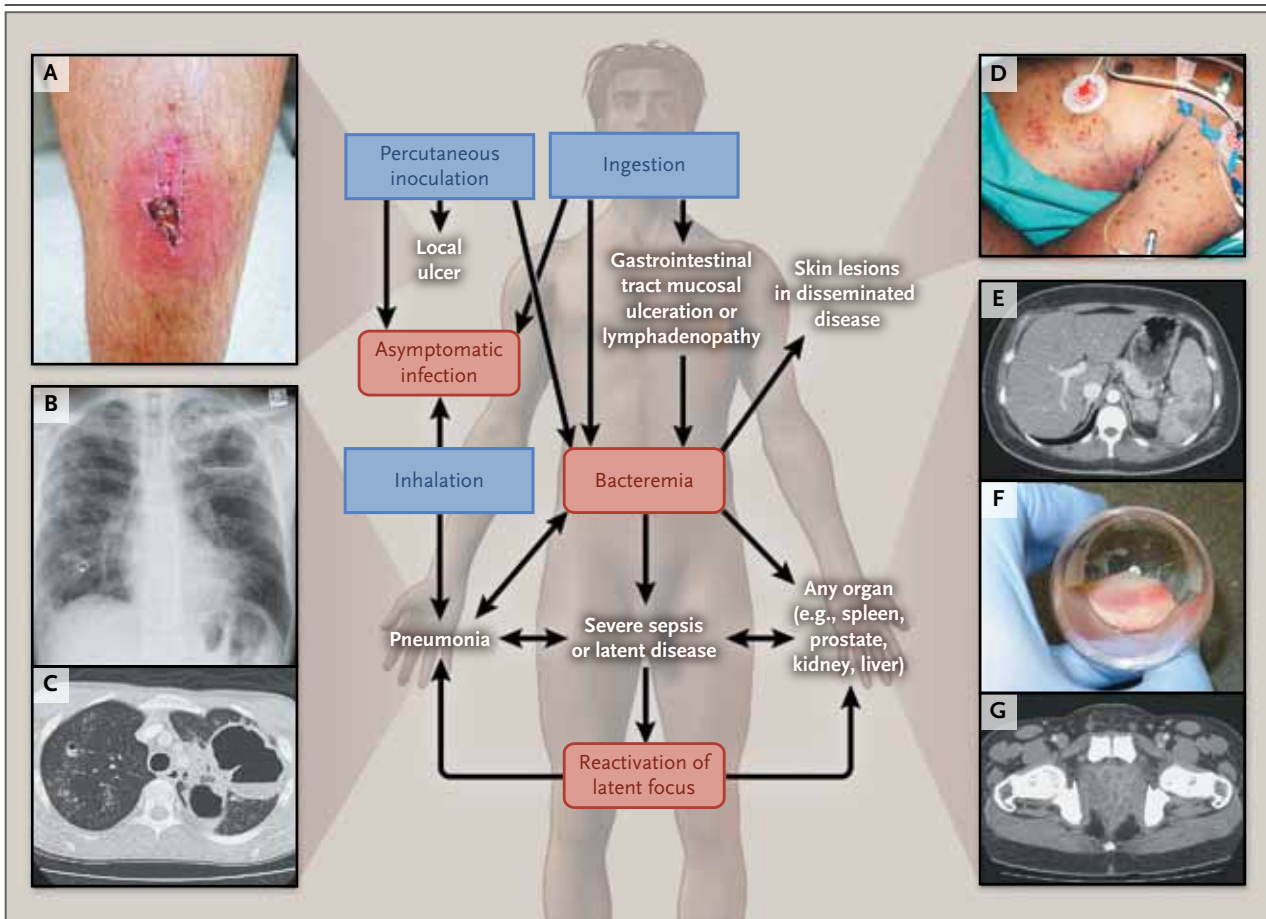
Figure 3. Clinical Events after Infection with *B. pseudomallei*.

Melioidosis may have a wide range of clinical manifestations, and severity varies from an acute fulminant septic illness to a chronic infection. Shown are the routes of infection (blue boxes: percutaneous inoculation, inhalation, and ingestion), the natural history of infection (red boxes: asymptomatic infection, bacteremia, or reactivation of latent focus), and the diverse disease manifestations (white text). Panel A shows cutaneous melioidosis in a healthy host. Panel B shows lung abscesses on the chest radiograph of a patient with acute melioidosis pneumonia, and Panel C shows the corresponding computed tomographic (CT) scan. Panel D shows the skin manifestations in a fatal case of disseminated melioidosis. Panel E shows splenic abscesses on an abdominal CT scan. Panel F shows aspirated pus in a patient with prostatic and periprostatic abscesses, and Panel G shows the abscesses on a CT scan from the patient.

A notable difference in presentation between patients in tropical Australia and those in Southeast Asia is suppurative parotitis, which accounts for up to 40% of cases of melioidosis in children in Thailand and Cambodia but is extremely rare in Australia.[50] In Australia, prostatic melioidosis is present in approximately 20% of male patients, and neurologic melioidosis is manifested as brainstem encephalitis, often with cranial-nerve palsies (especially cranial nerve VII), or as myelitis with peripheral motor weakness.[37] Recurrent melioidosis occurs in approximately 1 in 16 patients, often in the first year after the initial presentation.[37,54] Roughly a quarter of recurrences are due to reinfection, with the remainder due to relapse from a persistent focus of infection.[54] Mortality rates for melioidosis are approximately 40% in northeast Thailand (35% in children)[36] and 14% in Australia.[37]

DIAGNOSIS AND THERAPY

A delay in diagnosis can be fatal, since empirical antibiotic regimens used for suspected bacterial sepsis often do not provide adequate coverage for *B. pseudomallei*. Guidelines for empirical treatment of community-acquired pneumonia in endemic regions recommend the administration of antibi-

otic agents with activity against *B. pseudomallei* in patients with risk factors for melioidosis. A culture of *B. pseudomallei* from any clinical sample is the sine qua non for the diagnosis of melioidosis. Laboratory procedures for maximizing the culture and identification of *B. pseudomallei* have been developed, but a delay in the identification of *B. pseudomallei* or a misidentification as another species is not uncommon in laboratories that are unfamiliar with this organism.[55] A direct polymerase-chain-reaction assay of a clinical sample may provide a more rapid test result than culture, but the assay is less sensitive, especially when performed on blood.[56,57] Serologic testing alone is inadequate for confirming the diagnosis, especially in endemic regions where the background seropositivity rate can be more than 50%.[58] If empirical therapy for melioidosis is begun and *B. pseudomallei* is not subsequently detected in adequate cultures of specimens obtained before therapy, completion of a full course of antimicrobial therapy is generally not recommended.

Melioidosis has a notoriously protracted course; cure is difficult without a prolonged course of appropriate antibiotics. *B. pseudomallei* is inherently resistant to penicillin, ampicillin, first-generation and second-generation cephalosporins, gentamicin, tobramycin, streptomycin, and polymyxin. Of the newer antibiotics, ertapenem, tigecycline, and moxifloxacin have limited in vitro activity against clinical isolates of *B. pseudomallei*, and the minimum inhibitory concentration for doripenem is similar to that for meropenem.[59] Various mechanisms of acquired antibiotic resistance have been identified, including efflux pumps, enzymatic inactivation, bacterial-cell-membrane impermeability, alterations in the antibiotic target site, and amino acid changes in *penA*, the gene encoding the highly conserved class A β-lactamase.[60,61]

The treatment of melioidosis consists of an intensive phase of at least 10 to 14 days of ceftazidime, meropenem, or imipenem administered intravenously, followed by oral eradication therapy, usually with trimethoprim–sulfamethoxazole (TMP-SMX) for 3 to 6 months (Table 1).[55,62] Carbapenems, such as meropenem and imipenem, have lower minimum inhibitory concentrations and superior results in in vitro time-kill studies than ceftazidime, but a randomized comparative study in Thailand did not show a survival advantage of imipenem over ceftazidime.[63] The current recommendation[62] for the oral phase of therapy

is TMP-SMX, which replaces the previous recommendation[64] to give this medication in conjunction with doxycycline. A careful search for internal-organ abscesses is recommended, such as with the use of computed tomography or ultrasonography of the abdomen and pelvis. Adjunctive therapy for abscesses includes drainage of collections and aspiration and washout of septic joints.

The rate of resistance to TMP-SMX, as assessed with the use of Etest (AB Biodisk), is reported to be approximately 13% for Thai isolates but much lower for Australian isolates (0 to 2.5%).[2,65] An alternative agent for eradication therapy is amoxicillin–clavulanate; although it is inferior to TMP-SMX because it is associated with a higher rate of

Table 1. Treatment of Melioidosis.*	
Antimicrobial Drug	**Dose**
Initial intensive therapy†	
Ceftazidime	50 mg/kg of body weight (up to 2 g), every 6–8 hr
Meropenem	25 mg/kg (up to 1 g), every 8 hr
Imipenem	25 mg/kg (up to 1 g), every 6 hr
Oral eradication therapy‡	
TMP-SMX	
Body weight	
>60 kg	2×160 mg of TMP–800 mg of SMX (960 mg), every 12 hr
40–60 kg	3×80 mg of TMP–400 mg of SMX (480 mg), every 12 hr
<40 kg, adult	1×160 mg of TMP–800 mg of SMX (960 mg) or 2×80 mg of TMP–400 mg of SMX (480 mg), every 12 hr
<40 kg, child	8 mg of TMP/kg–40 mg of SMX/kg, every 12 hr

* Dose information is from Peacock et al.[55] and Chetchotisakd et al.[62]
† Intensive therapy is defined as intravenous administration of one of the listed medications for a period of 10 to 14 days. Four or more weeks of parenteral therapy may be necessary in patients with severe disease (e.g., those with ongoing septic shock, deep-seated or organ abscesses, extensive lung disease, septic arthritis, osteomyelitis, or neurologic melioidosis). The addition of trimethoprim–sulfamethoxazole (TMP-SMX), which is available in a fixed drug ratio of one part TMP to five parts SMX, at a dose of 8 mg of TMP and 40 mg of SMX per kilogram of body weight (up to 320 mg of TMP and 1600 mg of SMX) every 12 hours should be considered for patients with neurologic, prostatic, bone, or joint melioidosis. A switch to meropenem is indicated if the clinical condition worsens with the administration of ceftazidime (e.g., organ failure develops), if a new focus of infection develops during treatment, or if repeated blood cultures at 7 days remain positive.
‡ Oral therapy is typically required for 3 to 6 months. If the organism is resistant to TMP-SMX or the patient has unacceptable adverse events in response to the medication, the second-line choices are amoxicillin–clavulanate and doxycycline. Amoxicillin–clavulanate is recommended at a dose of 20 mg of amoxicillin and 5 mg of clavulanate per kilogram of body weight given orally, three times daily.

relapse, amoxicillin–clavulanate is used in some locations in children and pregnant women.[66] Resistance of *B. pseudomallei* to carbapenems has yet to be documented, and primary resistance to ceftazidime is extremely uncommon.[65] In very rare cases, acquired resistance to ceftazidime develops during therapy; the mechanisms for acquired resistance include point mutations and gene deletion.[67]

PREVENTION AND VACCINE DEVELOPMENT

Melioidosis is potentially preventable, but there is no evidence base for the development of guidelines for prevention. Although it has been recommended that people with cystic fibrosis be warned about traveling to areas where melioidosis is endemic, no advice is given to tourists in general, despite the steadily increasing number of cases in returning travelers, many of whom have diabetes. It is recommended that people with risk factors such as diabetes or immunosuppressive therapy stay indoors during periods of heavy wind and rain, when aerosolization of *B. pseudomallei* is possible.[68] There is no evidence to support direct human-to-human transmission through respiratory spread. A human vaccine is currently not available for melioidosis, but this is an active area of research in animal models involving the use of live attenuated, subunit, plasmid-based DNA, and killed whole-cell vaccine candidates. No vaccine candidates have been associated with sterilizing immunity.[69,70] Recommendations for postexposure prophylaxis after inadvertent laboratory exposure to *B. pseudomallei* or in the event of accidental release of *B. pseudomallei* are provided in Table S1 in the Supplementary Appendix.[55] Melioidosis has been reported after renal transplantation[71] and is increasingly being recognized in patients receiving immunosuppressive therapy, especially high-dose glucocorticoids.[37] The approach to the treatment of patients who are immunosuppressed or are about to begin immunosuppressive therapy and are asymptomatic but have serologic evidence of exposure is provided in Table S2 in the Supplementary Appendix.

SUMMARY

The identification and reporting of melioidosis cases are increasing worldwide, given improved diagnostic microbiology and general awareness of the disease among health care workers. The higher mortality in Thailand, as compared with Australia, suggests that efforts to reduce mortality should be directed toward resource-restricted settings, with an emphasis on the rapid administration of antimicrobial drugs, early recognition of sepsis, and adequate fluid resuscitation. Further reductions in mortality in areas with state-of-the-art medical facilities may be difficult to achieve, and improvement may depend on new therapeutic strategies resulting from fundamental research on bacterial pathogenesis and host–pathogen interactions.

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