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Editorial

Anthrax as a Biothreat and our Current Understanding of this Disease

Rolf König*

Department of Microbiology & Immunology and the Sealy Center for Molecular Medicine, The University of Texas Medical Branch, Galveston, TX 77555-1019, USA

***Corresponding author**

Rolf König, Department of Microbiology and Immunology, The University of Texas Medical Branch, Medical Research Building, Room 4.142A, Mail Route 1019, Galveston, TX 77555-1019, USA, Tel: 409-747-0395; Fax: 409-772-5065; E-mail: rokonig@utmb.edu

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Bacillus anthracis, the causative agent of anthrax infections, is a gram-positive, rod-shaped, spore-forming bacterium that survives as a spore in alkaline soils for decades [1]. Spores rarely germinate in soil but can germinate in the rhizosphere of certain grass species at high ambient temperatures, the optimal germination temperature being 39°C [2]. In mixtures of warm, stagnant water and soil, spores can germinate and multiply in the phagocytic amoeba *Acanthamoeba castellanii* [3]. Vegetative cells are fragile, compete poorly with other soil microflora, and require high amounts of nutrients for propagation and survival [1]. Grazing animals can ingest the spores, which enter the animal's blood stream and germinate in macrophages. In healthy animals, the infection will be eliminated by circulating macrophages (lethal oral dose 1.5-5 x 10^8 spores), however, in stressed animals, especially during a period of hot, dry weather with poor access to food sources, animals can succumb to infection following ingestion of much smaller numbers of spores [1]. Flies also contribute to the spread of disease: necrophilic blow flies and their larvae feed on the decaying carcasses, and then disperse bacilli and spores to nearby vegetation, whereas hemophagic flies spread anthrax directly from diseased to healthy animals via cutaneous infection [1].

Human infections with *B. anthracis* have been infrequent, and are usually restricted to rural areas with susceptible livestock in underdeveloped countries or occur through inhalation of spores released from contaminated animal hides. However, biological attacks since 2001 show the growing potential of anthrax as a bioweapon. Symptoms and mortality rates following anthrax infections differ depending on the entry site with inhalational anthrax due to the exposure to airborne spores having the most severe sequelae [1]. Mortality due to inhalational anthrax can be as high as 92%, but treatment with broad-spectrum antibiotics (e. g. , ciprofloxacin or doxycycline) or anti-toxin antibodies during the early onset of symptoms can reduce mortality by 50% [4,5]. However, the initial clinical manifestation is nonspecific and may resemble an influenza infection. This initial phase lasts two to four days and is followed by a fulminant phase of respiratory distress, cyanosis, and diaphoresis [4,6]. Once patients enter the fulminant phase, mortality rises to 97% regardless of treatment [4]. Gastrointestinal anthrax resulting from the consumption of contaminated meat is rare in industrialized countries, but mortality rates can be as high as 25-60% [7]. The most common form is cutaneous anthrax resulting from spores introduced through skin lesions. Most cases occur in Africa, Asia, and Eastern Europe due to limited vaccination of farm animals and workers [8]. The mortality rate for untreated cases is 5-20%, but drops to less than 1% with antibiotic treatment [7,9]. Recently, a new form of infection with anthrax spores, injectional anthrax, was recognized in the UK and Germany among persons who inject drugs [7,10]. The source was contaminated heroin injected into the skin or muscle, causing severe soft tissue infection and necrosis, followed by an increased risk of septic shock, and a mortality rate of 34% despite antibiotic treatment compared to 1% in patients with cutaneous anthrax and treated with antibiotics [7].

Virulence genes of *B. anthracis* are located on two plasmids, pXO1 and pXO2. Plasmid pXO2 encodes genes for the synthesis of a poly-γ-D-glutamic acid capsule, which protects the bacilli from destruction by complement and phagocytes, and from phagocytosis. The protective role of the capsule is most important during the initial phase of infection. Toxin genes encoded on plasmid pXO1 are important during the terminal phase of the infection. These genes, *pag*, *lef*, and *cya*, encode protective antigen (PA), lethal factor (LF), and edema factor (EF), respectively [11,12]. The three protein products from two toxins, namely edema toxin composed of PA and EF, and lethal toxin composed of PA and LF. PA mediates toxin entry into cells by binding to the ubiquitous anthrax toxin receptor, a type I membrane protein [13,14]. Binding of PA to its receptor exposes the N-terminal region of PA to a host cell surface protease [13]. The 63-kDa proteolytic cleavage product of PA heptamerizes and forms a ring structure with competitive binding sites for three molecules of LF and/or EF [15,16]. The anthrax toxin receptor binds to two PA protein domains and ensures accurate and timely insertion into the target cell membrane [17]. Subsequently, the toxin complex is taken up by receptor-mediated endocytosis [18,19]. Anthrax toxins inhibit several signal transduction pathways: the calmodulin-dependent EF acts as an adenylate cyclase and forms cyclic AMP (cAMP) from ATP [20], whereas LF is a zinc metalloprotease that targets mitogen-activated protein

kinase (MAPK) kinases (MKKs) [21-25]. Due to their ability to enter leukocytes and affect multiple signaling pathways, anthrax toxins inhibit innate and adaptive immune responses against *B. anthracis* infection [26-33].

Understanding the molecular mechanisms by which *B. anthracis* evades immune responses resulting in bacteraemia is a major focus of research in several laboratories worldwide. Important is also the ability to recognize infection at a very early stage in order to interfere with disease progression. These combined efforts can eventually lead to effective measures against this dangerous biothreat.

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