Review

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Anthrax lethal and edema toxins in anthrax pathogenesis

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The pathophysiological effects resulting from many bacterial diseases are caused by exotoxins released by the bacteria. *Bacillus anthracis*, a spore-forming bacterium, is such a pathogen, causing anthrax through a combination of bacterial infection and toxemia. *B. anthracis* causes natural infection in humans and animals and has been a top bioterrorism concern since the 2001 anthrax attacks in the USA. The exotoxins secreted by *B. anthracis* use capillary morphogenesis protein 2 (CMG2) as the major toxin receptor and play essential roles in pathogenesis during the entire course of the disease. This review focuses on the activities of anthrax toxins and their roles in initial and late stages of anthrax infection.

B. anthracis and anthrax

B. anthracis is a Gram-positive, rod-shaped, spore-forming bacterium, and is the causative agent of anthrax, an acute, rapidly progressing infectious disease that affects both animals and humans. *B. anthracis* forms spores after the death of infected hosts. The spores can remain dormant for many years in soil, and begin to grow again and secrete toxins after gaining entry into susceptible hosts. The *B. anthracis* spore, the infectious form of the pathogen, has long been considered as a potential warfare agent and has been a top bioterrorism concern since the 2001 anthrax attacks in the USA [1].

In addition to a single chromosome, *B. anthracis* contains two large extrachromosomal plasmids, pXO1 (182 kb) and pXO2 (96 kb), that are essential for its full virulence [2,3]. The pXO1 plasmid encodes the three anthrax exotoxin components: protective antigen (PA, 83 kDa), lethal factor (LF, 89 kDa), and edema factor (EF, 90 kDa). Plasmid pXO2 encodes proteins that synthesize the unique poly-D-γ-glutamic acid capsule which confers resistance to phagocytosis. There are three forms of anthrax disease defined by the route of spore entry into the body: cutaneous, gastrointestinal, and inhalational anthrax. Early studies showed that spores are phagocytosed by resident macrophages and dendritic cells, which may serve as a 'Trojan horse' to carry them from peripheral sites to local lymph nodes where they germinate to become toxin-producing vegetative bacteria [4]. Recent studies have shown a rapid localized germination event [5,6], suggesting the bacteria overcome innate immunity, resulting in systemic infection, through what has been termed a 'jailbreak' mechanism ([7] for detailed review). As major virulence factors of *B. anthracis*, the anthrax toxins play essential roles during multiple steps of the disease. In this review we discuss the roles of the anthrax toxins in initiation of anthrax infection as well as their lethal effects at the late stages of the disease.

The anthrax toxin components and receptors

The three components of the anthrax exotoxins, PA, LF, and EF, are individually non-toxic, but they pair to form the two major virulence factors of *B. anthracis*: lethal toxin (LT, composed of LF + PA) and edema toxin (ET, composed of EF + PA) [8]. PA is the cellular binding moiety, and LF and EF are the catalytic moieties of the toxins. Upon being secreted by *B. anthracis* during infection, PA binds to its cellular receptors on target host cells and is proteolytically processed by furin or furin-like proteases into the receptor-bound C-terminal fragment PA_{63} and the free N-terminal fragment PA_{20} (Figure 1). Release of PA_{20} from PA_{63} removes steric hindrance and allows PA_{63} to form an oligomeric (heptamer or octamer) structure competent for LF/EF binding [9,10]. LF/EF-binding sites are formed by residues located on adjacent PA_{63} monomers [11]. Each PA_{63} heptamer and octamer binds to three or four EF and/or LF molecules, respectively, due to steric interference between toxin molecules bound at adjacent sites [11]. The PA_{63} oligomer/LF and/or EF complex is then internalized through a receptor-mediated endocytic pathway [12]. In endosomes, acidic conditions induce conversion of the PA_{63} oligomer prepore to a protein-conducting channel through which LF and EF are translocated into the cytosol of the cells to exert their cytotoxic effects (reviewed in detail in [13]). In endosomes, the toxin complex can also be routed into intraluminal vesicles, where LF and EF are sequestered inside the vesicles ([14] for detailed review). In this case LF and EF can be released into the cytosol through back fusion of the intraluminal vesicles with endosome membranes. Because PA_{63} oligomerization triggers receptor-mediated endocytosis, only the EF/LF-binding competent PA_{63} oligomer, but not cell

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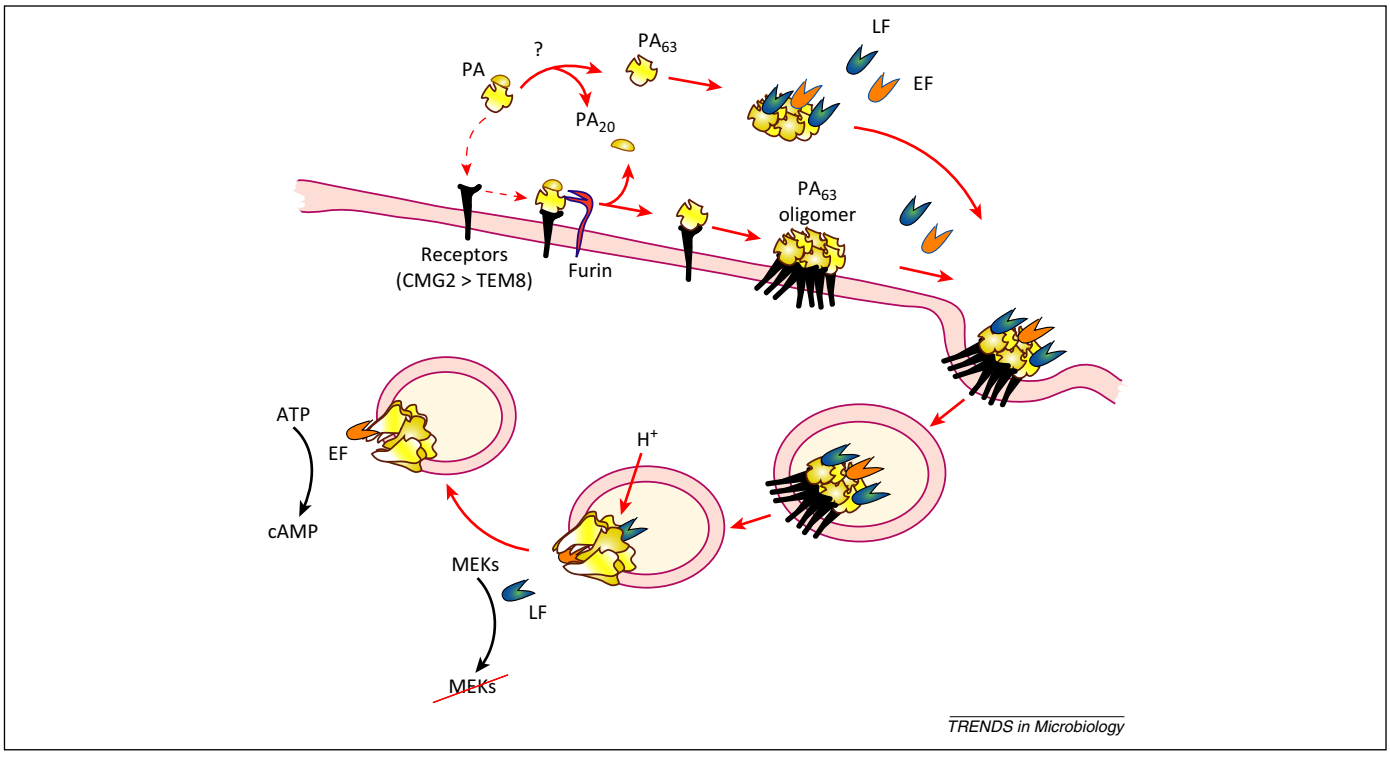

Figure 1. Mode of action of anthrax toxins. Following secretion by *Bacillus anthracis*, the anthrax toxin components distribute quickly into various tissues. Upon binding to cell surface receptors, protective antigen (PA) is cleaved by furin or furin-like proteases, yielding the C-terminal fragment PA_{63} (dashed arrows), which then spontaneously forms oligomers that gain the ability to bind to lethal factor (LF) and edema factor (EF). PA can also be processed in circulation by unidentified proteases, and the resulting PA_{63} can oligomerize and bind to LF and EF before binding to the toxin receptors capillary morphogenesis protein 2 (CMG2) and tumor endothelium marker 8 (TEM8) to form the PA_{63} oligomer–receptor complex. CMG2 is the major toxin receptor *in vivo*, whereas TEM8 plays only a minor role in anthrax toxin pathogenesis. The toxin complex is then internalized through the receptor-mediated endocytosis. Once inside endosomes, the toxin complex encounters an acidic environment, which induces the formation of the PA_{63} oligomer channel in the membrane, allowing LF and EF to enter the cytosol to exert their cytotoxic effects.

surface-bound PA monomer, is internalized into cells [12,15].

Anthrax toxin receptors

Two cell surface mammalian proteins were identified as receptors that mediate anthrax toxin entry into host cells: tumor endothelium marker 8 (TEM8), also known as anthrax toxin receptor 1, and CMG2, also named anthrax toxin receptor 2 [16,17]. TEM8 and CMG2 are homologous cell surface proteins containing an extracellular von Willebrand factor A (vWA) domain responsible for binding to PA as well as to their natural ligands. CMG2 has been shown to bind collagen IV, laminin, and fibronectin, whereas TEM8 was shown to interact with collagen I, gelatin, and collagen VI [18]. The physiological functions of TEM8 and CMG2 are unknown. TEM8 was first described as a tumor endothelial marker due to its upregulation in human colorectal cancer endothelium, making it a potential target for cancer therapy [19]. CMG2 was identified as one of the genes upregulated in human endothelial cells undergoing tubule formation in a 3D collagen matrix assay, suggesting a role in angiogenesis [20]. Mutations in human CMG2 cause two human autosomal recessive conditions, juvenile hyaline fibromatosis and infantile systemic hyalinosis, now described with the single term hyaline fibromatosis syndrome (HFS) [21]. These conditions are characterized by multiple subcutaneous skin nodules and gingival hypertrophy with excess extracellular protein deposition. Mutations in human TEM8 cause a human autosomal recessive condition called GAPO syndrome [22], characterized by growth retardation, alopecia (hair loss), pseudoanodontia (failure of tooth eruption), and progressive visual impairment. Histology reveals moderate excess of extracellular matrix protein deposition in many tissues from GAPO patients. Because TEM8 and CMG2 mediate receptor-driven toxin internalization, it is tempting to speculate that they may have a parallel function as physiologic receptors for cellular uptake and degradation of particular extracellular proteins, an essential process in maintaining extracellular matrix homeostasis.

CMG2- and TEM8-null mice and double-null mice have been generated to characterize the roles of the two receptors in anthrax pathogenesis. Interestingly, these mice recapitulate some of the clinical signs seen in human HFS and GAPO patients. TEM8-null mice display dental dysplasia and moderate excess extracellular matrix deposition [23,24]. CMG2-null mice have severe collagen deposition in their uteri, disabling their parturition ability [25,26]. However, both receptor-null mice have less severe phenotypes than human HFS and GAPO patients, perhaps reflecting species differences in extracellular matrix turnover physiology.

Analyzing CMG2- and TEM8-null mice revealed that CMG2 is the physiologically relevant anthrax toxin receptor *in vivo* [24,27]. CMG2-null mice are not only resistant to LT and ET challenge but also to *B. anthracis* infection. By contrast, TEM8-null mice remain susceptible to both the toxins and *B. anthracis* infection. CMG2 has a 10-fold