INSTITUT PASTEUR

Microbes and Infection 13 (2011) 1146–1155

www.elsevier.com/locate/micinf

Review

Key aspects of the molecular and cellular basis of inhalational anthrax

Christopher K. Cote*, Susan L. Welkos, Joel Bozue

Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA

Received 15 June 2011; accepted 7 July 2011
Available online 21 July 2011

Abstract

Bacillus anthracis is the etiologic agent of the disease inhalational anthrax, an acute systemic infection initiated by inhaling spores, which if not rapidly detected and treated, results in death. Decades of research have elucidated novel aspects of anthrax pathogenesis but there are many issues left unresolved.
Published by Elsevier Masson SAS on behalf of Institut Pasteur.

Keywords: Bacillus anthracis; Spores; Inhalational anthrax; Germination; Phagocytes

In this review, we endeavored to summarize key cellular and molecular aspects of *Bacillus anthracis* pathogenesis by describing the process from inhalation of *B. anthracis* spores, dissemination, intoxication, and ultimately death of the infected host. Due to space limitations, this review is not meant to be exhaustive, and we regret any unintended omission of research conducted on this topic. The conclusions described within this review article are based upon data collected through in vitro experimentation, numerous animal models, and human post-mortem observations. The detailed technical aspects of the research which are not discussed in this review should be taken into consideration when analyzing individual data sets. These factors include but are not limited to the strain of *B. anthracis*, specific in vitro methodology, and particular animal model employed.

1. Anthrax

Anthrax is an acute infection caused by the gram-positive, spore-forming bacterium *B. anthracis*. In nature, the predominant route of infection leading to anthrax is through ingestion of *B. anthracis* spores by animals [1]. Typically, humans are considered incidental hosts, and when put into perspective with other bacterial diseases, human anthrax cases are rare. The vast majority (greater than 95% by some accounts) of reported human cases world-wide are cutaneous infections. While cutaneous anthrax can resolve without antibiotic intervention, as much as 20% of untreated cases may be fatal. The hallmark of cutaneous anthrax is a painless lesion referred to as an eschar [1]. The second most common form of anthrax observed in humans is oropharyngeal/gastrointestinal anthrax caused by consuming contaminated meat that has not been thoroughly cooked. Oropharyngeal anthrax is often characterized by lesions in the oral cavity (i.e., tongue, tonsils, or pharynx). These lesions, if left untreated, can result in massive swelling and eventual airway blockage. Gastrointestinal anthrax may result in lesions within the stomach or other areas of the intestinal tract. These lesions can result in obstruction, perforation, or hemorrhage. Anthrax initiated by ingestion of bacteria is often difficult to diagnose due to the non-specific symptoms (i.e., sore throat or abdominal pain) [1] and may result in 25–60% fatality rates if left untreated [2]. A relatively new form of injectional/septacemic anthrax has been reported among intravenous drug users in Western Europe. Injectional anthrax was first described in 2000 [3]; however, recent cases have resurrected this proposed fourth form of anthrax. The source of the *B. anthracis* spores in the latest outbreak was hypothesized to be contaminated heroin thought to originate from Pakistan, Afghanistan, or Iran.

* Corresponding author. Tel.: +1 301 619 4936.
E-mail address: christopher.cote2@us.army.mil (C.K. Cote).

1286-4579/$ - see front matter Published by Elsevier Masson SAS on behalf of Institut Pasteur.
doi:10.1016/j.micinf.2011.07.005

Inhalation of *B. anthracis* spores results in the most severe form of anthrax. Inhalational anthrax is only observed after an occupational/vocational exposure (i.e., wool mill/tannery workers, veterinarians, or drum makers-all of which involve the manipulation of potentially infected animal products) or after intentional exposure (i.e., those occurring in 2001 after exposure to spore laden letters in the USA). Due to non-specific symptomology, differential diagnosis can be very challenging. One of the main features of inhalational anthrax is a rather dramatic widening of the mediastinum as seen on chest films, a result of hemorrhage and necrosis attributed to both bacterial replication and toxin production within the mediastinal lymph nodes. It is important to mention that inhalational/pulmonary anthrax is not a true pneumonic disease. The lungs serve as the portal of entry but active infection occurs within lymphoid tissue [1]. Bacilli are not routinely found within the lungs until after systemic bacteremia is observed, and the vegetative bacteria are then released into the lungs through the capillary beds. If left untreated, inhalational anthrax has a fatality rate approaching 100% [1]. The events leading to inhalational anthrax have been examined for over a century but surprisingly there are still many facets of pathogenesis left unresolved. This review will attempt to discuss the events leading to inhalational anthrax.

2. The infectious particle: the spore

The infectious form of *B. anthracis* is the spore. Infections leading to inhalational anthrax are initiated by ungerminated/dormant spores which are inhaled by a host. The spore is an inherently highly stable particle which can withstand extreme conditions, ensuring survival of the bacterium and the infection of subsequent hosts. The properties that result in such unusual stability include a large number of spore coat proteins that form a thick shell which maintains spore dehydration and at the same time protects genomic material.

Spores of all species of *Bacillus* are built as a set of concentric shells (Fig. 1). The inner-most spore compartment is the core, which houses the chromosome. The chromosome is bound by small acid-soluble proteins (SASP) which protect the genetic material [4]. The cortex is a thick layer of peptidoglycan that surrounds the core [5]. The cortex, in turn, is surrounded by the inner and outer coats, proteinaceous layers composed of dozens of distinct proteins. Many spore coat proteins are conserved between *Bacillus subtilis* and *B. anthracis*, leading to an overlap in many of the mechanisms controlling coat assembly in the two species. Recent studies confirm that *B. anthracis* homologs of these critical *B. subtilis* coat proteins play important roles in *B. anthracis* coat assembly, albeit roles that may differ from their *B. subtilis* counterparts [6]. A number of spore proteins found in *B. anthracis* and *Bacillus cereus,* but absent in *B. subtilis*, are also important to spore formation [7,8].

For some *Bacillus* species, including *B. anthracis*, there is an additional layer that envelops the coat, called the exosporium, consisting of a basal membrane and a series of fine hair-like structures which project from the membrane and are described as a nap [9] (Fig. 1). This nap is primarily composed of the immunodominant protein *Bacillus* collagen-like protein A (BclA) [10]. BclA is thought to increase the hydrophobicity of the spore and prevent interactions with host matrices [11] and possibly act as an immune decoy. BclA is a complicated glycoprotein which contains a collagen-like region with multiple copies of a pentasaccharide side chain. This oligosaccharide possesses a unique sugar called anthrose, three rhamnose residues and a protein-bound *N*-acetylgalactosamine [12]. In addition, BclA contains internal tandem amino acid repeat regions, consisting primarily of GPT repeats containing most of the glycosylation sites, which serve as the primary anchor point for rhamnose oligosaccharides [10,12].

There has been much debate about the role of the BclA protein in virulence and pathogenesis. It has been demonstrated that this protein is not required for virulence in either an attenuated strain [10] or a fully virulent strain of *B. anthracis* [13]. Nonetheless, the BclA protein may be responsible for directing *B. anthracis* spores toward professional phagocytes [14]. This concept was demonstrated by genetically removing the BclA protein and the resulting *bclA* mutant spores were significantly more adherent to both epithelial and endothelial cells but not to macrophages [14,15].

The role of the exosporium structure in general continues to be somewhat enigmatic. While the BclA protein itself does not significantly alter infectivity, the role of the exosporium in pathogenesis continues to be debated. Mutant spores that lack the entire exosporium structure have been shown to be as virulent as wild-type spores [6]. This was shown to be the case for either parenteral or inhalational challenge routes using exosporium-deficient derivatives of fully virulent *B. anthracis*. However, spores lacking an exosporium may be more susceptible to sporicidal activities of professional phagocytes, as illustrated when the exosporium was physically removed by sonication from spores of the toxigenic nonencapsulated Sterne strain [16]. The exosporium does contain numerous proteins in addition to BclA that may play some role in combating host defenses. The exosporium contains superoxide dismutases, and their role in the resistance of spores to intracellular oxidative stress was demonstrated both in vitro and in vivo [17]. Additionally, alanine racemase has been identified within the exosporium. This enzyme may help to retard spore germination by limiting the amount of L-alanine that reaches the spore, until the spore is exposed to conditions favoring germination [18]. The alanine racemase achieves this by converting L-alanine (germination inducer) to D-alanine (incapable of inducing germination and can inhibit germination if present at high enough levels) [19]. This enzymatic function would be important during initial stages of infection in a mammal, but also during sporulation within the mother cell so that uniformly dormant spores can be produced and released into the environment.

3. Spore germination and disease

Once a *B. anthracis* spore is located within a suitable host organism and the appropriate nutrients are present, germination is initiated. Transition from a spore to the vegetative cell is essential for virulence. The small molecule signals for