Review

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New insights into gastrointestinal anthrax infection

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Bacterial infections are the primary cause of gastrointestinal (GI) disorders in both developing and developed countries, and are particularly dangerous for infants and children. *Bacillus anthracis* is the 'archetype zoonotic' pathogen; no other infectious disease affects such a broad range of species, including humans. Importantly, there are more case reports of GI anthrax infection in children than inhalational disease. Early diagnosis is difficult and widespread systemic disease develops rapidly. This review highlights new findings concerning the roles of the gut epithelia, commensal microbiota, and innate lymphoid cells (ILCs) in initiation of disease and systemic dissemination in animal models of GI anthrax, the understanding of which is crucial to designing alternative therapies that target the establishment of infection.

GI infection

Infectious colitis is caused by a variety of bacterial, viral, and parasitic organisms. However, bacterial infections are the primary cause of GI disorders, both in developing and developed countries [1], and diarrhea remains the second leading cause of death in children younger than 5 years of age, accounting for 1.3 million deaths every year worldwide [2,3]. Infection by pathogenic bacteria causes disease either by disturbing the homeostatic balance between the host and the gut commensal microbes, and/or by systemic dissemination. The gut has been characterized as the 'motor of critical illness' due to dysregulated crosstalk among the epithelia, immune system, and endogenous microflora of the gut [4], in which loss of balance between these tightly interrelated systems leads to the development of systemic manifestations of disease, reaching far beyond the intestine [5]. In this review, we focus on the current understanding of the synapses between GI anthrax infection and the protective innate populations of the GI tract. A better understanding of these interactions has important implications for the design of future studies and interventions that target establishment of infection by this deadly pathogen.

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Anthrax infection

B. anthracis is a sporulating Gram-positive bacterium that causes anthrax, an often fatal infection that occurs when endospores (see Glossary) enter the body through ingestion, inhalation, or abrasions in the skin. Regardless of the route of entry into the body, GI, inhalational (pulmonary), and cutaneous infections can rapidly progress to fatal systemic anthrax [6]. *B. anthracis* is not considered to be an invasive organism; under normal circumstances, the healthy integument, intestinal mucosae, and respiratory cilia put up efficient barriers to entry of anthrax spores into the body [7]. Recently, a new form of anthrax has also been recognized, 'injectional anthrax', because of its association with the injection of *B. anthracis*-contaminated heroin in Scotland from 2009 to 2010 [8,9]. During that time period, 47 patients had confirmed *B. anthracis* soft tissue infection, related to injection of contaminated heroin, with a fatality rate of 28%. Strikingly absent in most patients with injectional anthrax is the eschar formation typically associated with cutaneous *B. anthracis* infection. This lack of eschar development combined with the high fatality rate despite receipt of antimicrobial drugs support the notion that the pathogeneses of injectional and cutaneous anthrax differ [10–12].

All forms of anthrax infection can quickly become systemic, characterized by a toxemia caused by the secretion of lethal toxin (LT) and edema toxin (ET), and septicemia, which is associated with the bacterium's anti-phagocytic poly-D-glutamic acid capsule (Box 1) [13]. This anti-phagocytic capsule is produced by gene products encoded on the pXO2 plasmid and the tri-partite exotoxin is encoded on the pXO1 plasmid (Figure 1) [14]. Disease initiating spores are highly resistant to environmental conditions, including chemical disinfectants, heat, desiccation, ultraviolet and ionizing radiation, and extreme pressure [6]. These physical properties also render the spores highly resistant to killing by the host's immune system. Anthrax is the 'archetype zoonosis'; no other infectious disease affects such a broad range of species, including humans [7]. This disease is zoonotic to most mammals, and grazing herbivores are considered most susceptible, as infectious anthrax spores can remain dormant in the soil for decades [15]. Herbivores are also thought to provide most of the human exposure risk for anthrax [16]. GI anthrax is considered to be the primary

Glossary

Anthrax toxin receptor 1 (ANTXR1): a type I transmembrane protein that binds to PA. This protein is also known as tumor endothelial marker-8 (TEM8) because it is a tumor-specific endothelial marker that appears in be involved in colorectal cancer.
Anthrax toxin receptor 2 (ANTXR2): a type I transmembrane protein that binds to PA, also known as capillary morphogenesis gene 2 (CMG2).
B-1 cells: are considered innate immune cells that produce the majority of IgM and IgA, which are largely encoded by germline immunoglobulin genes. B-1 cells predominate during fetal and neonatal development, self-renew, and localize mostly to the peritoneal and pleural cavities. Subsets of B-1 cells can be delineated by differential expression of CD5.
B-2 cells: are considered 'conventional B cells', and are continually generated from bone marrow precursors, and circulate throughout the blood and secondary lymphoid tissues. B-2 cells can undergo class switching, somatic hypermutation, and generate memory cells.
Cholesterol-dependent cytolysins (CDCs): a family of β-barrel pore-forming exotoxins that are secreted by Gram-positive bacteria. The presence of cholesterol in the target membrane is required for pore formation, but is not required by all CDCs for binding.
Dysbiosis: a breakdown in the balance between protective intestinal bacteria versus harmful intestinal bacteria.
Dysphagia: difficulty in swallowing.
Edema factor (EF): part of the three-protein anthrax exotoxin that is a calmodulin-dependent adenylate cyclase that inhibits the immune response.
Edema toxin (ET): refers to the protein complex of edema factor (EF) bound to protective antigen (PA).
Endospores: dormant, hardy, non-reproductive structures produced by certain bacteria, especially Gram-positive bacteria.
Eschar: a piece of necrotic tissue that is sloughed from the surface of the skin; is characteristically thick, dry, and black dead tissue seen with cutaneous anthrax infection.
Germinal center (GC) B cells: within germinal centers (GC) of lymphoid tissue, B cells proliferate, undergo affinity maturation of their B cell receptor genes, class switch for antibody production, and differentiate into longer-lived plasma cells or memory B cells.
Hematemesis: vomiting of blood.
Innate lymphoid cells (ILC): a group of immune cells that belong to the lymphoid lineage but do not respond in an antigen specific manner. This relatively newly described group of cells has different subtypes with different physiological functions, some of them analogous to helper T cells, while also including cytotoxic natural killer (NK) cells.
Lethal factor (LF): part of the three-protein anthrax exotoxin that inactivates neutrophils so they cannot phagocytose bacteria.
Lethal toxin (LT): refers to the protein complex of LF bound to PA.
Melena: black, 'tarry' stool that is associated with upper GI bleeding.
Meningoencephalitis: inflammation of the brain and the meninges, the membranes that surround the brain and spinal cord.
Microbiota: the entire resident microbe population of a certain organ or organ system; often refers to the flora of the GI tract.
Nod-like receptors (NLRs): Nod-like receptors (nucleotide-binding oligomerization domain receptors) are pattern recognition receptors (PRRs) and play key roles in regulation of the innate immune response.
***Nlrp1b*:** the gene encoding NACHT, LRR, and PYD domains-containing protein 1, which is a component of the inflammasome pathway.
Occludins: integral plasma membrane proteins that together with the claudin group of proteins are the main component of tight junctions.
pXO1: a plasmid in *B. anthracis* that controls the production of ETs and LTs, which are made of three proteins, EF, PA, and LF.
pXO2: a plasmid in *B. anthracis* that codes for the capsule, a layer of polysaccharides outside of the cell wall that protects the bacteria against phagocytosis.
Protective antigen (PA): part of the three-protein anthrax exotoxin that is a cell binding protein that binds to two surface receptors on the host cell.
Peyer's patches: aggregated nodules of lymphatic tissue that play a central role in intestinal immunosurveillance. They are similar to LNs in structure, except that they are not surrounded by a connective tissue capsule.
Transepithelial electrical resistance (TEER): an *in vitro* method for evaluating the permeability of epithelial cells by measuring the electrical physical resistance.
Toll-like receptors (TLRs): play a critical role in the early innate immune response to invading pathogens by recognizing highly conserved structural motifs known as pathogen-associated microbial patterns (PAMPs), which are exclusively expressed by microbial pathogens.
Transcytosis: the transport of macromolecules or bacteria from one side of a cell to the other side via the interior of the cell.
Zoonosis: a disease that can be passed from animals to humans or vice versa.

route of infection for livestock and can also occur in humans through the ingestion of contaminated food [17]. Ingested spores germinate within the herbivore host to produce the vegetative forms, which proliferate and produce their virulence factors (toxins and capsule) [6]. While anthrax has been well managed in developed countries, this disease persists in areas of sub-Saharan Africa, Southeast Asia, and parts of the former Soviet Union with weakened public health systems [18].

Clinical signs of GI anthrax

GI anthrax can present clinically as either intestinal or, less commonly, oropharyngeal infection. The incubation period is typically between 1 and 6 days, and the means by which the bacteria establish infection at the mucosae are unclear. Both GI forms involve epithelial barrier breakdown and ulceration, and the mortality rate is variable, but may approach 100% depending on the outbreak [15], or ≤40% with appropriate antibiotic treatment [17]. Oropharyngeal anthrax is characterized by mucosal ulcerations, sore throat, enlargement of cervical lymph nodes (LNs), soft tissue edema, and dysphagia [16]. Intestinal anthrax is caused by infection of the stomach or bowel [19], and primarily manifests with ulceration of the ileum and/or cecum [20]; this should not be confused with the non-ulcerative hemorrhagic lesions associated with the septicemia that eventually results from anthrax infection [17,21]. Illness begins with anorexia, nausea, vomiting, and fever, progressing to severe abdominal pain, hematemesis, melena, and/or frank blood in the stool [17].

There have been more case reports of GI anthrax in children than inhalational disease, and the clinical presentation can differ from that of adults. A review of cases from 1900 to 2005 found that none of the children presented with hematemesis, and only one case reported bloody stool. However, seven out of 20 children developed secondary meningoencephalitis, which is usually associated with inhalational anthrax in adults, likely from hematologic dissemination [22]. Locations endemic for anthrax exist on every continent that contains subtemperate or tropical regions, and GI anthrax fatalities have been reported in India, Iran, Turkey, Thailand, Uganda, Zimbabwe, and Gambia [7,16]. During an outbreak in Uganda, 155 villagers became ill after consuming the meat of a zubu. Within 15–72 hours, 91% of the villagers had GI complaints, 9% had oropharyngeal edema, and nine victims, all children, died within 48 hours of disease onset [17,23]. GI anthrax has been reported in the US [24], but protection of the food supply has been credited for its rarity [17].

Cutaneous anthrax is the most common route of infection (95% of cases) and is easily diagnosed, as the black eschar lesions are always accompanied by significant edema. By contrast, disease onset can be insidious in the inhalational (5%) and GI (<1%) forms, with nonspecific flu-like symptoms, fever, or mild gastroenteritis [6]. Thus, early diagnosis can be difficult, and widespread systemic disease develops rapidly, resulting in circulatory shock, respiratory failure, sepsis, and death [25]. This has led some investigators to propose that GI anthrax is underreported and underestimated, as this form of the disease tends to occur in rural areas of developing countries, and mild GI signs are nonspecific and attract little