Combating the Insidious Enemy: Epidemiology, Pathophysiology, and Treatment of Clostridial Gas Gangrene

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Abstract

Clostridial gas gangrene is a life-threatening condition having dreaded features like myonecrosis, gas production, and sepsis and is usually caused by *Clostridium perfringens* which exists in the soil and as part of the gastrointestinal flora of humans. The disease is a noncommunicable one and the infection generally occurs in traumatic wounds with soil contamination and surgery involving the bowel; nonetheless, a spontaneous gangrene is also reported. Common characteristics of inciting events include contamination of the site with *Clostridium* sp. and devitalization of tissue. Areas with low oxygen concentration are ideal for these infections; anaerobic conditions impel *Clostridium* sp. to convert itself from spore to the vegetative form which produces toxins, say α-toxin and θ-toxin which in a row are responsible for tissue damage and systemic manifestations. On successful infection, it destroys RBCs, platelets, and polymorphonuclear leukocytes (PMNs), causing widespread cell membrane and capillary damage which establish the typical pathophysiology. Initially the wound becomes swollen and the affected skin primarily appears to be blistered with hemorrhagic bullae, red and feels warm with pain upon touching before progressing to a bronge, brown or black color. Foul-smelling brown-red or bloody discharge drip from the affected tissues or wound and with time signs of sepsis, toxemia, septic shock, and multiorgan failure become evident. For managing the infected patients, an early diagnosis is crucial. Other successive indispensible measures include aggressive resuscitation, surgical debridement, antibiotic therapy, and supportive intensive care. Delays in doing so increase tissue loss and mortality which are directly proportional to time of intervention.

Introduction

Gangrene is a serious and life-threatening condition that occurs when a considerable mass of body tissue dies by means of necrosis. It is caused by a loss of blood supply due to an underlying illness, injury, and/or infection. Fingers, toes, and limbs are affected most often, but gangrene can also occur inside the body, damaging organs and muscles. There are different types of gangrene with different symptoms, such as dry gangrene, wet gangrene, gas gangrene, internal gangrene, and necrotizing fasciitis (Carol 2007).

Gas gangrene is a bacterial infection that produces gas within tissues. It is a deadly form of gangrene usually caused by a bacterial exotoxin producing clostridial species, mostly found in soil, and other anaerobes (e.g., *Bacteroides* and anaerobic streptococci). The three most common species responsible for myonecrosis are *Clostridium perfringens*, *Clostridium septicum*, and *Clostridium novyi* (Fernandez and Gluck 1994). These environmental bacteria may enter the

muscle through a wound and subsequently proliferate in necrotic tissue and secrete powerful toxins. These toxins destroy nearby tissue, generating gas (5.9 % hydrogen, 3.4 % carbon dioxide, 74.5 % nitrogen, and 16.1 % oxygen), which was reported in one clinical case (Chi et al. 1995).

These infections occur in both military and civilian settings. The disease can be traumatic/postoperative, the most common form accounting for 70 % of the total cases worldwide or spontaneous/nontraumatic gangrene. This life-threatening local and systemic complication frequently occurs mainly due to bacterial infection at the site of penetrating and perforating wounds. Infection spreads rapidly as the gases produced by bacteria expand and infiltrate healthy tissue in the vicinity. The hallmarks of this disease are rapid onset of myonecrosis with muscle swelling, severe pain, gas production, and sepsis. Progression to toxemia and shock is often very rapid (Moustokas et al. 1985). Because of its ability to quickly spread to surrounding tissues, gas gangrene should be treated as a medical emergency. Treatment options include debridement (or, in severe cases, amputation) of the affected body parts, antibiotics, vascular surgery, maggot therapy, and hyperbaric oxygen therapy.

Types of Gangrene

In general, clostridial soft-tissue infections may be categorized into three classes: gas gangrene, anaerobic cellulitis, and superficial contamination. The most fatal is gas gangrene that requires prompt, often severe treatment. Frequently encountered clostridia infections are much less acute and require much less radical treatment; however, they may share some similarities with gas gangrene which complicates differential diagnosis. Gas gangrene producing clostridia primarily damages muscle and impairs blood supply resulting in discolored and edematous tissues with a pungent foul smell and gas bubbles. On accumulation of exudates and increased gas production, the clostridia multiply within the necrotic tissue, releasing more toxins into the tissue and the systemic circulation. On the other hand, anaerobic cellulites is a condition where clostridia invade only tissue that is already dead and the infection does not spread to healthy, undamaged tissue. The least severe type of clostridia infections is the superficial contamination, which involves infection of necrotic tissue where the pain experienced is minimal and wound healing is rapid. Typically, two major types of gangrenes are encountered among patients: dry gangrene and wet gangrene.

Dry gangrene: More common in people with diabetes and autoimmune diseases, dry gangrene usually affects the hands and feet. It develops when blood flow to the affected area is impaired, usually as a result of poor circulation. Unlike other types of gangrene, infection is typically not present in dry gangrene. However, dry gangrene can lead to wet gangrene if it becomes infected.

Wet gangrene: Unlike dry gangrene, wet gangrene almost always involves an infection. Injury from burns, or trauma where a body part is crushed or squeezed, can rapidly cut off blood supply to the affected area, causing tissue death and increased risk of infection. It is called "wet" because of pus formation. Infection from wet gangrene can spread quickly throughout the body, making wet gangrene a very serious and potentially life-threatening condition if not treated quickly.

Types of wet gangrene include the following:

Internal gangrene: If gangrene occurs inside the body, then it is referred to as internal gangrene. This is usually related to an infected organ such as the appendix or colon. Colon cancer is one of the predisposing factors for developing internal gangrene.

Fournier's gangrene: Also a rare condition, Fournier's gangrene is caused by an infection in the genital area. Men are affected more often than women. If the infection gets into the bloodstream, a sepsis-like condition develops which can be life threatening.

Gas gangrene: Gas gangrene is rare, but dangerous. It occurs when infection develops deep inside the body, such as inside muscles or organs, usually as a result of trauma. The bacterium that causes gas gangrene, called clostridia, release dangerous toxins or poisons that wreak havoc throughout the body along with gas which can be trapped within body tissue. Gas gangrene warrants immediate medical treatment. Without treatment, death can occur within 48 h.

Etiology

In humans, few clostridia species normally exist in the gastrointestinal tract and in the female genital tract, although they occasionally are isolated from the skin or the mouth. Of the known species of the genus *Clostridium*, at least 30 have been isolated from human infections. Like several other pathogenic anaerobic bacteria species, clostridia are quite aerotolerant, but they do not grow on artificial media in the presence of oxygen. Clostridia characteristically produce abundant gas in artificial media and form subterminal endospores. *Clostridium perfringens*, one of the most important species, is encapsulated and nonmotile and rarely sporulates in artificial media; the spores can usually be destroyed by boiling. Clostridia are present in the normal colonic flora at concentrations of 10^9–10^{10}/g. Of the $\geq$30 species that normally colonize humans, *C. ramosum* is the most abundant and is followed in frequency by *C. perfringens*. These organisms are universally present in soil at concentrations of up to 10^4/g. *C. perfringens* strains are classified (on the basis of their production of several lethal toxins) into five types, designated A through E. Type A predominates in fecal flora of humans as well as in soil, whereas the habitats of types B through E are thought to be the intestinal tracts of other animals. Although clostridia are gram-positive organisms, many species may

appear to be gram-negative in clinical specimens or stationary-phase cultures. Therefore, the result of Gram's staining of cultures or clinical material should be interpreted with great care. *C. perfringens* is the most common clostridia species isolated from tissue infections and bacteremias; next in frequency are *C. novyi* and *C. septicum*. In the category of enteric infections, *C. difficile* is an important cause of antibiotic-associated colitis, and *C. perfringens* is associated with food poisoning (type A) and enteritis necroticans (type C) (Borriello 1995; Lorber 2000). The complex etiology of this disease may also be confounded by multiple types of strains that play different roles during the disease process. There still remains many questions regarding these complicated diseases and the organisms that cause them.

Predisposing Risk Factors

Age – Gangrene is much more common in older people. People older than 60 years of age are diagnosed with gangrene more often than younger people. As people gets older, their heart and blood vessels change. Blood may have a harder time getting to the tissues that need it most. This raises the risk for gangrene.

Diabetes – The high blood sugar levels, which are common in diabetes, may eventually damage the nerves, especially in the feet. When the nerves are damaged, the patient does not feel pain and will not know if he/she has an injury. The patient may continue walking without protecting the wound. The wound may get worse and develop into a foot ulcer. High blood sugar levels may also damage blood vessels, resulting in poor blood supply to the area. Less blood means less nutrients and oxygen for the tissue cells and fewer white blood cells and T cells to fight off infection. The ulcer becomes infected; the infection grows rapidly and gangrene develops. The oxygen-/nutrient-deprived cells are weak and rapidly die.

Obesity – Obesity is linked to health factors related to gangrene including diabetes and vascular disease. The stress of extra weight may constrict arteries which also reduces blood flow. Reduced blood flow increases the risk of infection and causes wound to heal poorly.

Vascular diseases – Diseases of the blood vessels, such as atherosclerosis (narrowed arteries), and blood clots can result in poor blood flow to various parts of the body.

Injury or surgery – Anything which wounds the skin and tissues below will raise the risk of gangrene. People with underlying conditions which may affect blood flow who also wound their skin run an even higher risk. Approximately 40 % of wet gangrenes are caused by infections that occur during surgery and about 50 % are caused by serious traumatic injuries. Gangrene from frostbite and gunshot wounds are less common than from automobile accidents, crush injuries, burns, and industrial accidents.

Weakened immune system – People with weakened immune systems, such as those with AIDS, patients receiving chemotherapy or radiotherapy, as well as organ transplant recipients who are on immune suppressants, are more susceptible to the complications of infection, which include gangrene.

Smoking – Smoking causes the blood vessels to narrow, resulting in less blood flow.

Other factors – Reynaud's disease, trauma, blood clots, appendicitis, hernia, animal bites, compound fractures, foreign bodies, frostbite, thermal or electrical burns, subcutaneous or intravenous drug use, drinking alcohol, pressure sores, motor vehicle crashes, postoperative, gastrointestinal tract surgery, genitourinary tract surgery, abortion, amputation, tourniquets, casts, bandages, or dressings applied too tightly may also add to the risk of developing gangrene. The disease process must include tissue inoculation and a low oxygen tension environment. More than 50 % of cases are preceded by trauma. Other cases occur spontaneously or in patients after operative procedures (Davoudian and Flint 2012).

Pathogenesis

Mode of Transmission

Bacterial infection of the soft tissues occurs in a variety of roots, viz., soil contamination of the wounds, surgery involving the bowel or biliary system, unhygienic injection of medications, or by the intake of contaminated food. Food-borne illness acquired by ingestion of large number of *C. perfringens* vegetative cells present in the food. Food sources are usually cooked meat, contaminated pork meat, vegetables, fish, or poultry dishes which have been stored at ambient temperatures for a long time after cooking. Infection can occur through contamination of wounds (fractures, bullet wounds) with dirt or any foreign material contaminated with *C. perfringens* with an incubation period of 8–24 h (Fig. 1) (Ryan 2004). It is not communicable from person to person but can be transmitted from animals to humans only through food. Spores can survive in soil, crevices, food, decaying vegetation, marine sediments, internal cavities, and in the anaerobic conditions inside the meat rolls, animal carcasses, feces, and dehydrated and cooked food. Spores are resistant to most disinfectants, heat, and gamma-irradiation; when susceptible, they require longer contact time. Enterotoxin is heat labile and can be inactivated by heat treatment at 60 °C for 5 min. Vegetative cells can be rapidly killed by dry heat at 160–170 °C for 1–2 h or moist heat at 121 °C for 15–30 min. There is no known vector for the transmission of the disease (Songer 2010).

Bacteriology of Pathogenicity

The microbiology of gas gangrene is an exceptionally intricate problem, far from being entirely implicit till date. *Clostridium perfringens* was first described by Welch and Nuttall in 1892 and is at once the commonest, the most important, and the best known of the gas gangrene clostridia. In a smear made from an acute case, *C. perfringens* appears as a short, plump, gram-positive rod with rounded ends and a well-marked capsule (MacLennan 1962). Histotoxic clostridium is a

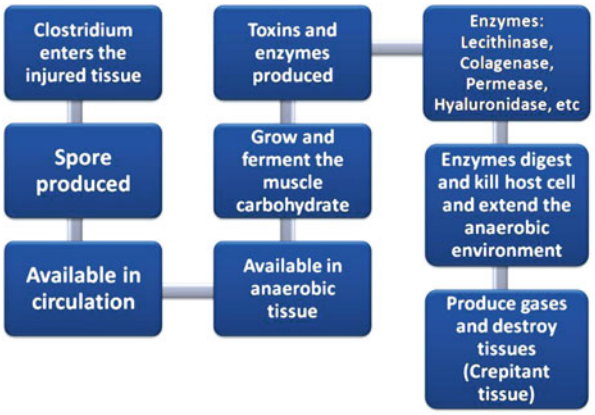

Fig. 1 Mode of bacterial entry into the host tissue and its pathogenicity. The causative form of gas gangrene enters into the body through the mechanically injured site and enters in the tissue. It produces a large number of spores in tissue mass and subsequently enters into the blood stream. In deep tissue, an anaerobic physiological environment will help them to start fermentation using muscle carbohydrate. At this stage, it produces a large number of toxins with different enzymatic activities which digest and kill the adjacent cells and tissues simultaneously with producing gas and enhancing the anaerobic environment

Table 1 Involvement of *C. perfringens* types and its connection with animal and human diseases

Type	Major toxins	Disease link
A	α, θ	Animal and human gas gangrene, fowl necrotic enteritis, bovine enterotoxaemia, human food poisoning, horse colitis, hemorrhagic gastroenteritis
B	α, β, ε	Dysentery, enterotoxaemia of lamb, sheep, goats
C	α, β	Human necrotic enteritis, sheep and calves enterotoxaemia
D	α, ε	Enterotoxaemia of sheep, goats, cattle, and human
E	α, ι	Enteritis of rabbit, enterotoxaemia of calves and lambs

spore-forming, saprophyte, nonmotile organism which is invariably found where decaying organic matter is present, commonly found in soil and in the intestines of humans and other animals. The species has been divided into five distinct types, A–E. Of these subgroups, *C. perfringens* type A causes the majority of human infections. Although classified as an anaerobe, *C. perfringens* is somewhat aerotolerant, one may say it a facultative type of bacterium. Under optimal conditions, its generation time can be as little as 8–10 min, and growth is accompanied by abundant gas production (Stevens and Bryant 2002). The ability of the bacterium to cause infection is ascribed chiefly to the production of an array of potent extracellular protein toxins. The so-called major toxins (a-, b-, e-, and i-toxins) are not necessarily produced in large quantities, but the differential production of these toxins is used to assign strains into one of five biotypes. These biotypes are associated with different diseases of humans and animals (Table 1) (Titball 2005).

Mode of Pathogenicity

In spite of the isolation of clostridia species from many serious traumatic wounds, the prevalence of severe infections due to these organisms is low. Two factors that appear to be essential to the development of severe disease are tissue necrosis and a low oxidation-reduction potential. *C. perfringens* requires about 14 amino acids and at least six supplementary growth factors for optimal growth. These nutrients are not found in appreciable concentrations in normal body fluids but are present in necrotic tissue. When *C. perfringens* grows in necrotic tissue, an area of tissue damage due to the toxins elaborated by the organism allows progressive growth. In contrast, when only a few bacteria leak into the bloodstream from a small defect in the intestinal wall, the organisms do not have the opportunity to multiply rapidly because blood as a medium for growth is relatively deficient in certain amino acids and growth factors. Therefore, in a patient without tissue necrosis, bacteremia is usually benign.

C. perfringens possesses at least 17 possible virulence factors, including 12 active tissue toxins and enterotoxins. The enterotoxins include four major lethal toxins: α, β, ε, and ι. The α-toxin is a phospholipase C (lecithinase) that splits lecithin into phosphatidylcholine and diglyceride (Fig. 2). It has been associated with gas gangrene and is known to be hemolytic, to destroy platelets and polymorphonuclear leukocytes (PMNs), and to cause widespread capillary damage. When injected intravenously, it causes massive intravascular hemolysis and damages liver mitochondria. The α-toxin may be important in the initiation of muscle infections that may progress to gas gangrene. Experimentally, the higher the concentration of α-toxin in the culture fluid, the smaller the dose of *C. perfringens* required to produce infection. The protective effect of antiserum is directly proportional to its content of α-antitoxin. Studies suggest that θ-toxin, a thiol-activated cytolysin that is also called perfringolysin O, may also play an important role in pathogenesis by promoting vascular leukostasis, endothelial cell injury, and regional tissue hypoxia. The resulting perfusion defects extend the anaerobic environment and contribute to rapidly advancing tissue destruction. A characteristic pathologic finding in gas gangrene is the near absence of PMNs despite extensive tissue destruction. Experimental data indicate that both α- and θ-toxins are essential in the leukocyte aggregation that occurs at the margins of tissue injury instead of the expected infiltration of these cells into the area of damage. Genetically altered strains induce less leukocyte aggregation when α-toxin is absent and none when θ-toxin is missing. The other major toxins, β, ε, and ι, are known to increase capillary permeability (Kasper and Madoff 2005; Stevens and Bryant 2002).

Epidemiology

Clostridia species are ubiquitous and widely distributed in the soil, especially in cultivated land. The density of clostridia in the soil is a contributing factor in the development of trauma-related gas gangrene. In the USA, civilian cases of gas

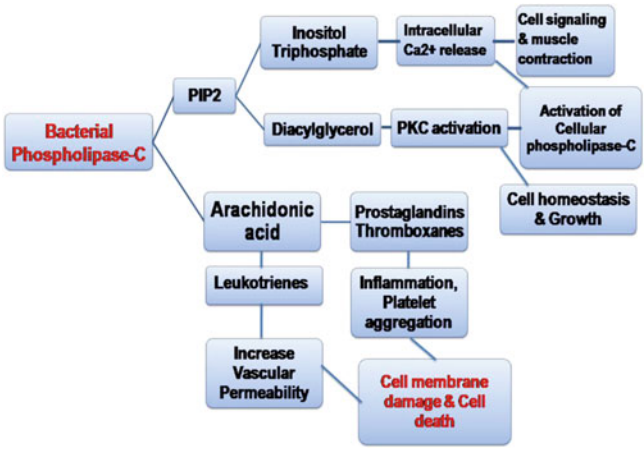

Fig. 2 Role of alpha-toxin on eukaryotic cell signaling and cell death. At sublytic concentrations, *C. perfringens* alpha-toxin causes accumulation of diacylglycerol leading to activation of protein kinase C (*PKC*) which subsequently activates cellular phospholipase C and ultimately can lead to partial cell membrane damage. Clostridial phospholipases C also able to stimulate the arachidonic acid cascade. Arachidonic acid in one hand produces leukotrienes which increase vascular permeability; it also produces prostaglandins and thromboxanes which start inflammatory cascade of reactions which ultimately leads to cell damage and cell death

gangrene are more common, with approximately 3,000 cases per year. Gas gangrene can be classified as posttraumatic, postoperative, or spontaneous. Posttraumatic gas gangrene accounts for 60 % of the overall incidence; most cases involve automobile collisions (Brown and Kinman 1974). Approximately 50 % of wet gangrene cases are the result of a severe traumatic injury, and 40 % occur following surgery. Car and industrial accidents crush injuries, and gunshot wounds are the most common traumatic causes. Because of prompt surgical management of wounds with the removal of dead tissue, the incidence of gangrene from trauma has significantly diminished. Approximately two-thirds of cases affect the extremities, and the remaining one-third involves the abdominal wall. According to the US Agency for Healthcare Research and Quality, 45,400 Americans were hospitalized for gangrene in 2003, compared to 21,000 in 1991. From 1998 to 2002, *C. septicum* was implicated in causing serious infections in recipients of contaminated musculoskeletal-tissue allograft (Fisher et al. 2005).

Several users of injection drugs in Scotland, Ireland, and England developed serious clostridia infections (*C. novyi* and *C. perfringens*) complicated by a high mortality rate (97 %). Most of these patients reported injecting heroin intramuscularly within the previous 2 weeks. With more than 200,000 liposuctions performed in Germany in 2003, several serious complications had been reported. Necrotizing fasciitis and gas gangrene were the most frequent, major, and lethal complications observed in a review of 72 cases of complications caused by liposuction performed in Germany between 1998 and 2002 (Lehnhardt et al. 2008). A tsunami ravaged Indonesia in December 2004 and killed more than 200,000 Indonesians. Soaking in

contaminated water, several injured persons later died of tetanus or gas gangrene. In May 2008, the Sichuan earthquake in China caused more than 70,000 deaths and approximately 400,000 injuries; several injured persons developed gas gangrene and later underwent amputations. Among 2,131 survivors admitted to a public hospital in the Sichuan area, at least 19 patients (0.9 %) developed gas gangrene (Wang et al. 2010).

Gangrene occurs equally in men and women. Although age is not a prognostic factor in gas gangrene, advanced age and comorbid conditions are associated with a higher likelihood of mortality. Type I necrotizing fasciitis occurs most commonly in patients with diabetes and patients with peripheral vascular diseases. It is the most common form of necrotizing fasciitis in the general population. Type II necrotizing fasciitis has an annual incidence of 5–10 cases per 100,000 in the USA. *C. perfringens* is one of the most common causes of food poisoning in the USA and Canada. Contaminated meats contained in stews, soups, and gravies are usually responsible for outbreaks in developed countries and cause about 250,000 cases of food-borne illness every year in the USA. Approximately, half of the cases of streptococcal necrotizing fasciitis occur in young and previously healthy people. The incidence of gas gangrene in the USA is nearly 3,000 cases annually. Severe penetrating trauma or crush injuries associated with interruption of the blood supply are the usual predisposing factors. *C. perfringens* is the most common cause of trauma-associated gas gangrene with very high mortality rates (Stevens et al. 2005). Spontaneous gas gangrene caused by *Clostridium septicum* may be more common than trauma-associated gangrene, caused by other *Clostridium* species. Disease frequency is common among drug abusers and patients with atherosclerosis, antiphospholipid syndrome (9 %), and malignancy affected by paraneoplastic acral vascular syndrome. The annual incidence of atheroembolism that leads to ischemic gangrene ranges from 0.3 % to 3.5 % overall, although after a vascular procedure it can rise to 30 %. Most patients with Reynaud's phenomenon are women aged between 20 and 40 years, in whom lesions develop during the cold months (Baker and Bick 2008; Hirschmann and Raugi 2009). Gas gangrene is undoubtedly an infection that carries a very high mortality rate. The reported mortality rates vary widely, with a rate of 25 % in most recent studies. The mortality rate approaches 100 % in individuals with spontaneous gas gangrene and in those with delayed treatment (Stevens et al. 2005).

Retrospective population-based surveillance for clostridia bacteremia was conducted among all residents of the Calgary Health Region (population 1.2 million) during 2000–2006. One hundred and thirty-eight residents had incident *Clostridium* species bacteremia (1.8 per 100,000/year); 45 (33 %) were nosocomial, 55 (40 %) were healthcare-associated community onset, and 38 (28 %) were community acquired. Older age and a number of underlying conditions were risk factors for acquiring *Clostridium* species bacteremia most importantly hemodialysis (relative risk (RR) 212.3; 95 % confidence interval (CI) 106.5–385.5), malignancy (RR 40.2; 95 % CI 27.6–58.1), and Crohn's disease (RR 11.2; 95 % CI 3.0–29.4). *Clostridium perfringens* was most commonly identified with 58 (42 %)

isolates followed by *Clostridium septicum* (19; 14 %), *Clostridium ramosum* (13; 9 %), *Clostridium clostridioforme* (8; 6 %), and *Clostridium difficile* (7; 5 %). Reduced susceptibility to penicillin occurred in 14/135 (10 %), to metronidazole in 2/135 (1 %), and to clindamycin in 36/135 (27 %) isolates. The median length of stay was 12.7 days and 39/130 (30 %) patients died in hospital for mortality rate of 0.5 per 100,000/year (Leal et al. 2008).

Gas gangrene was responsible for around 6 % of open fractures and 1 % of all open wound infections during World War I and 0.7 % during World War II. Early recognition and adequate therapy lowered the mortality rate in a high percentage of cases. On the other hand, this observation of low reports can be delineated only to urban areas and developed countries. Though a few gas gangrene cases have been reported from many parts of developing and underdeveloped countries, these reports merely suggest an approximate estimate of the disease incidence; the actual numbers may significantly be higher, as, very often, many cases go unreported due to lack of awareness among the people, inadequate surveillance, and poor epidemiologic and pathologic expertise.

Pathophysiology

Gangrene is necrosis and subsequent decay of body tissues caused by infection or thrombosis or lack of blood flow. Gas gangrene fabricates a severe life-threatening pathophysiological condition which has the following features: muscle necrosis, gas production, sepsis, and ultimately death. It is usually the result of critically insufficient blood supply sometimes caused by injury and subsequent contamination mainly with clostridium class of bacteria.

Clostridia are gram-positive, anaerobic, spore-forming bacilli commonly found throughout nature (with the exception of the North African desert). Cultivated rich soil has the highest density of organisms. In addition, clostridia have been isolated from normal human colonic flora, skin, and the vagina. More than 150 *Clostridium* species have been identified, but only six have been demonstrated to be capable of producing the fulminant pathological condition known as clostridial gas gangrene. A study by Méndez et al. suggests that sugar may inhibit the production of alpha- and theta-toxins that trigger the gas generation (Méndez et al. 2012). *Clostridium perfringens*, previously known as *Clostridium welchii*, is the most common cause of clostridial gas gangrene (80–90 % of cases). Other clostridia species responsible for the condition include *Clostridium novyi (40 %)*, *Clostridium septicum (20 %), Clostridium istolyticum (10 %), Clostridium bifermentans (10 %)*, *and Clostridium fallax (5 %)*. The two most commonly isolated species have been *C. perfringens and C. septicum*. *Clostridium perfringens* is ubiquitous in nature, commonly found in the soil and gastrointestinal tracts of warm-blooded animals. *C. septicum* is also virulent, sometimes referred to as the malignant edema bacillus, but poorly understood pathogen that is recognized as the causative agent of atraumatic myonecrosis. It also produces α-toxin, distinct from *C. perfringens*, which acts as a pore-forming

Table 2 Different key exotoxins produced by *Clostridium perfringens* and their biological effects

Key exotoxins	Biological effects
Alpha-toxin	Lecithinase, necrotizing, hemolytic, cardiotoxic
Beta-toxin	Necrotizing
Epsilon-toxin	Permease
Iota-toxin	Necrotizing
Delta-toxin	Hemolysin
Phi-toxin	Hemolysin, cytolysin
Kappa-toxin	Collagenase, gelatinase, necrotizing
Lambda-toxin	Protease
Mu-toxin	Hyaluronidase
Nu-toxin	Deoxyribonuclease, hemolytic, necrotizing

cytolysin and is essential for virulence (Kennedy et al. 2005). *Clostridium perfringens* gas gangrene is, without a doubt, the most fulminant necrotizing infection that affects humans. Infections are characterized by a very low level of host inflammation in response to organism-associated exotoxins. In fact, it is more of a response to the exotoxins than a classic immune response to invading organisms. Purulence is often absent. The process of myonecrosis can spread as fast as 2 cm/h. This results in systemic toxicity and shock that can be fatal within 12 h. Overwhelming shock with accompanying renal failure usually leads to death (Stevens and Bryant 2002).

Infection requires two conditions to coexist. First, organisms must be inoculated into the tissues. Second, oxygen tension must be low enough for the organisms to proliferate. These organisms are not strict anaerobes; 30 % oxygen tension in the tissues allows for free growth of these bacteria, but 70 % oxygen tension restricts their growth. Inoculation of organisms into low oxygen tension tissues is followed by an incubation period that usually ranges from 12 to 24 h. However, this period can be as brief as 1 h or as long as several weeks. The organisms then multiply and produce exotoxins that result in myonecrosis. Although not very well understood, exotoxins appear to be tissue-destructive soluble antigens produced by clostridia. They include lecithinase, collagenase, hyaluronidase, fibrinolysin, hemagglutinin, and hemolysin toxins (Table 2). *C perfringens* produces at least 17 identifiable exotoxins that are used for species typing (e.g., type A, type B, type C) (Titball 2005).

Theta-toxin causes direct vascular injury, cytolysis, hemolysis, leukocyte degeneration, and polymorphonuclear cell destruction. These effects on leukocytes may explain the relatively minor host inflammatory response that is observed in tissues of patients with clostridial myonecrosis. Kappa-toxin, also produced by *C perfringens*, is a collagenase that facilitates the rapid spread of necrosis through tissue planes by destroying connective tissue. Alpha-toxin is produced by most clostridia and has phospholipase C activity. This potent lecithinase causes lysis of red blood cells, myocytes, fibroblasts, platelets, and leukocytes. It also may

decrease cardiac inotropy and trigger histamine release, platelet aggregation, and thrombus formation (Monturiol-Gross et al. 2012).

Considerable variation exists among clostridia species as to the mechanism of action of the alpha-toxin. In *C. septicum*, the alpha-toxin forms pores and induces necrosis by causing the rapid loss of intracellular potassium and depletion of adenosine triphosphate (ATP). Strains that do not produce alpha-toxin are less virulent, underscoring its importance (Knapp et al. 2010). In mice models, alpha-toxin-induced lethality was inhibited by the pre-administration of erythromycin. Erythromycin resulted in a reduction of the release of cytokines tumor necrosis factor-alpha (TNF-alpha), interleukin 1 (IL-1), and interleukin 6 (IL-6). Additionally, TNF-alpha-deficient mice were resistant to *C. perfringens* alpha-toxin, suggesting that TNF-alpha is an important contributor to the toxic effects of clostridial proteins (Oda et al. 2008). Moreover, very recently, it has been found that, at lytic concentrations, *C. perfringens* PLC (α-toxin) causes membrane disruption, whereas at sublytic concentrations, this toxin causes oxidative stress and activates the MEK/ERK pathway, which contributes to its cytotoxic and myotoxic effects. The results demonstrate that the toxin induces reactive oxygen species (ROS) production through PKC, MEK/ERK, and NFκB pathways, the latter being activated by the MEK/ERK signaling cascade. Inhibition of either of these signaling pathways prevents cytotoxic effect of α-toxin (Monturiol-Gross et al. 2014).

Genetic regulation of clostridia exotoxin production is under the control of several different regulatory systems, including the global VirR/VirS 2-component signal transduction system and the RevR. The VirR, a membrane-bound external sensor, and the VirS, a gene response regulator, together transmit and receive signals from the environment to the inside of the cell. The VirR/VirS system uses RNA intermediates to control 147 distinct genes and their associated operons (Ohtani et al. 2010). The phi-toxin is a hemolysin. Even though it does not directly suppress myocardial function in vitro, it contributes to myocardial suppression in vivo, possibly by increasing the synthesis of secondary mediators that do suppress myocardial function in vitro. At higher concentrations, the phi-toxin can cause extensive cellular degeneration and direct vascular injury. The kappa-toxin produced by *C perfringens* is a collagenase responsible for destruction of blood vessels and connective tissue. Other toxins include a deoxyribonuclease and hyaluronidase (Monturiol-Gross et al. 2012).

Traumatic gas gangrene and surgical gas gangrene occur through direct inoculation of a wound. With a compromised blood supply, the wound has an anaerobic environment that is ideal for *C. perfringens*, the cause of 80–95 % of cases of gas gangrene. Spontaneous gas gangrene is most often caused by hematogenous spread of *C. septicum* from the gastrointestinal tract in patients with appendicitis or colon cancer or through other gateway. Neutropenic and immunocompromised patients are also at risk. The organism enters the blood via a small break in the gastrointestinal mucosa and subsequently seeds muscle tissue. Unlike *C. perfringens*, *C. septicum* is aerotolerant and can infect normal tissues.

Glucose fermentation causes gas production in gas gangrene where the major components are nitrogen (74.5 %), followed by oxygen (16.1 %), hydrogen (5.9 %), and carbon dioxide (3.4 %). Production of hydrogen sulfide and carbon dioxide gas begins late and dissects along muscle bellies and facial planes. These all provide favorable conditions for spread of infection. Low oxidation/reduction and the necessary enzymes provided by the necrotic tissue result in the spore germination. Spores germinate to form the vegetative cells which produce exotoxins which result in tissue necrosis, thrombosis, and edema. With *C. perfringens*, the local and systemic manifestations of infection are due to the production of potent extracellular protein toxins by the bacteria. These are most notably alpha-toxin (a phospholipase C) and theta-toxin (a thiol-activated cytolysin). These toxins hydrolyze cell membranes, cause abnormal coagulation leading to microvascular thrombosis (further extending the borders of devascularized and thus anaerobic tissue), and have direct cardio-depressive effects. Furthermore, the products of tissue breakdown, including creatine phosphokinase, myoglobin, and potassium, may cause secondary toxicity and renal impairment (Titball 2005; Moustoukas et al. 1985).

Significant and refractory anemia may also be present in patients with gas gangrene. This effect is a direct consequence of toxin-mediated hemolysis of RBCs when significant amounts of alpha-toxin are released into the bloodstream. Alpha-toxin has negative inotropic effects on cardiac myocytes contributing to the severe, refractory hypotension seen in some cases of gas gangrene. Theta-toxin causes a cytokine cascade, which results in peripheral vasodilation similar to that seen in septic shock. These secreted exotoxins might also result in hemolysin which causes low hemoglobin and hypertension and may cause acute tubular necrosis and renal failure. It has been reported that alpha-toxin and theta-toxin which are lipophilic in nature are bound to tissue plasma membrane and may result in secretion of secondary metabolites (Fiorentini et al. 1999; Moustokas et al. 1985).

Role of Alpha- and Theta-Toxin

Among all the exotoxins produced by this organism, the alpha-toxin, α (phospholipase C, PLC), and theta-toxin, θ (perfringolysin O, PFO), are its major virulence factors. Although the role of PFO in the pathogenesis of gas gangrene is somehow controversial, the part played by the α-toxin is beyond doubt in causing the disease. However, on the other hand, the PFO have been shown to facilitate the growth of its corresponding bacterium within mammalian phagocytic cells. Furthermore, experimental animal studies have demonstrated the protective efficacy of several antibody preparations against these toxins (Bryant and Stevens 1997). PLC and PFO each contribute to the morbidity and mortality of gas gangrene by uniquely different mechanisms. PLC is hemolytic, is cytotoxic to platelets and leukocytes, and increases capillary permeability effects that are likely related to its ability to cleave sphingomyelin and the phosphoglycerides of choline,

ethanolamine, and serine present in eukaryotic cell membranes. PLC requires calcium for optimal activity. It gets that Ca^{2+} by hydrolyzing the intracellular phosphoinositide (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG), a potent second messenger that in turn activates several other cell signaling mechanisms including the one leading to the generation of thromboxane A2 (Fig. 2).

As mentioned earlier, the A-type *C. perfringens* chiefly produces the exotoxin-α that has a hemolytic activity and is the main causative agent of clostridial myonecrosis or gas gangrene. The toxin is a 370-residue, zinc metalloenzyme that has phospholipase C activity and can bind to membranes in the presence of calcium. In fact, this was the first bacterial toxin to be identified as an enzyme. During that time people started to accept a convention that all phospholipase C's are toxic. Further studies proved, however, that not all PLCs are toxic (*Clostridium novyi* PLC, e.g., is toxic, while that *Bacillus cereus* PLC is not) and that therefore enzymatic activity alone is not sufficient for toxicity. Studies of α-toxin have shown that it is the key virulence determinant of *C. perfringens* in gas gangrene. Specific mutants of *C. perfringens* that do not produce α-toxin are unable to cause disease, and vaccination with a genetically engineered toxoid has been shown to induce protection against gas gangrene (Williamson and Titball 1993). An emerging theme is that toxicity can be ascribed to the ability of the enzyme to interact with membrane phospholipids, thereby perturbing host cell metabolism and promoting the effects outlined above which allow the spread of the bacteria into otherwise healthy tissues. To fully understand the molecular basis of toxicity, the crystal structure of α-toxin has been determined (Naylor et al. 1998). This reveals a two-domain protein. The α-helical amino-terminal domain contains three zinc ions, located within a cleft. These zinc ions appear to play roles both in stabilizing the structure of the protein and in the phospholipase C catalytic activity (Titball and Rubidge 1990) (Fig. 3). There are several pieces of evidence indicating that this cleft is the phospholipase C active site. Firstly, when the surface of the protein is modeled, a phospholipid molecule can be accommodated within the charged cleft (Naylor et al. 1998). More importantly, when recombinant amino-terminal domain is produced in E. coli, the purified polypeptide retains phospholipase C activity. However, the amino-terminal domain lacks the toxicity and the cytolytic activity of the holotoxin (Titball et al. 1991).

Although the amino-terminal domain alone is nontoxic, it is clear that its enzymatic activity is required for toxicity. The site-directed mutagenesis of zinc-binding ligands in the amino-terminal domain simultaneously abolishes phospholipase C activity and other toxic and cytolytic activities (Nagahama et al. 1997). This finding suggests that the carboxy-terminal domain confers cytolytic and toxic activities on the enzyme. The isolated carboxy-terminal domain has no detectable toxic or cytolytic activity, but mixing the purified amino- and carboxy-terminal domains together in solution restores hemolytic activity, presumably because these domains can associate via the hydrophobic face between these domains in the holotoxin (Titball et al. 1993).

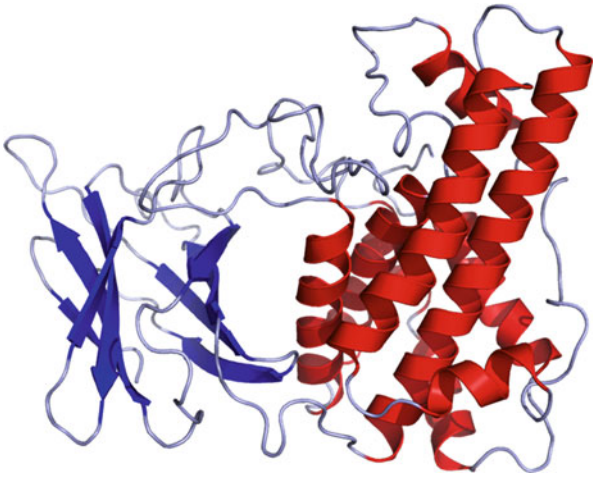

Fig. 3 The crystal structure of "*Clostridium perfringens*" alpha-toxin published in the Protein Data Bank (*PDB*: 1CA1). Alpha-helical amino-terminal domain (*red*) contains three zinc ions binding cleft. The carboxy-terminal domain (*blue*) binds calcium and allows the toxin to bind to the phospholipid head groups on the cell surface (Source: http://en.wikipedia.org/wiki/File:Clostridium_perfringens_Alpha_Toxin.rsh.png; Created from PDB 1CA1 and rendered by Ramin Herati (2006) using Pymol)

Precise Mechanism of Action of Alpha-Toxin

The alpha-toxin is a zinc metalophospholipase, requiring zinc for activation. First, the toxin binds to a binding site on the cell surface. The C-terminal C2-like PLAT domain binds calcium and allows the toxin to bind to the phospholipid head groups on the cell surface (Fig. 3). The C-terminal domain enters the phospholipid bilayer. The N-terminal domain has phospholipase activity. This property allows hydrolysis of phospholipids such as phosphatidylcholine, mimicking endogenous phospholipase C. The hydrolysis of phosphatidylcholine produces diacylglycerol, which activates a variety of second messenger pathways. The end result includes activation of arachidonic acid pathway and production of thromboxane A2 and production of IL-8, platelet-activating factor, and several intercellular adhesion molecules (Fig. 2). These actions combine to cause edema due to increased vascular permeability that in turn allows the growth of the obligatory anaerobe, although it is capable of growing in slightly aerobic condition (Titball et al. 1993; Titball 2005).

Symptoms and Complications

Gas gangrene may result generally from predisposing factors like blood loss, trauma, hemorrhage, or bone fracture near the wound and by accidental contact of soil or fecal matter in wound. The earliest symptoms of gas gangrene are intense pain, low-grade fever, edema, and a sweet-odorous discharge which occurs several

hours to a few days (6 h to 3 days) after injury. Within few hours, the entire region may become markedly edematous. Gas produced by clostridia may produce a crackling sensation when the affected area is pressed. Crepitus follows gas production; at times, crepitus may not be detected with palpation owing to brawny edema. It becomes swollen and the swollen skin may initially be blistered with hemorrhagic bullae, red, and warm to the touch before progressing to a bronze, brown, or black color. Foul-smelling brown-red or bloody discharge drips from the affected tissues or wound. In advanced stages, all the signs of profound damage-like severe pain in the affected area are followed by numbness, rapid breathing (tachycardia), a grayish/yellowish complexion, etc. if the toxins spread into the bloodstream. Symptoms typically begin suddenly and quickly worsen. If the patient is not treated at the initial stage, the infection from gangrene gets into the blood; it may develop sepsis and go into septic shock which is life threatening. Symptoms of sepsis may include low blood pressure, rapid heartbeat (tachycardia), shortness of breath, change in body temperature, light headedness, body pain and rash, confusion, and cold, clammy, and pale skin (Kasper and Madoff 2005).

If the patient remains untreated for long time, in the advance stage, few other medical complications like massive hemolysis, disseminated intravascular coagulation, acute renal failure, jaundice with liver damage, acute respiratory distress, coma, delirium, and shock are very common. Gangrene can lead to scarring which sometimes requires reconstructive surgery. Sometimes, the amount of tissue death is so extensive that the body parts or extremities may need to be surgically removed.

Diagnosis

The diagnosis of clostridial disease, in association with positive cultures, must be based primarily on clinical findings. Because of the presence of clostridia in many wounds, their mere isolation from any site, including the blood, does not necessarily indicate severe disease. Smears of wound exudates, uterine scrapings, or cervical discharge may show abundant large gram-positive rods as well as other organisms. Cultures should be placed in selective media and incubated anaerobically for identification of clostridia. The diagnosis of clostridial myonecrosis can be established by frozen-section biopsy of muscle. Histopathological findings in gas gangrene consist of widespread myonecrosis, destruction of other connective tissues, and a paucity of neutrophils in the infected area. Leukocyte aggregates are found in the border regions. The urine of patients with severe clostridial sepsis may contain protein and casts, and some patients may develop severe uremia. Profound alterations of circulating erythrocytes are seen in severely toxemic patients. Patients have hemolytic anemia, which develops extremely rapidly, along with hemoglobinemia, hemoglobinuria, and elevated levels of serum bilirubin. Spherocytosis, increased osmotic and mechanical red blood cell fragility, erythrophagocytosis, and methemoglobinemia have been described. In patients with severe sepsis, Wright's or Gram's staining of a smear of peripheral blood or buffy coat may demonstrate clostridia. X-ray examination sometimes provides an

important clue to the diagnosis by revealing gas in muscles, subcutaneous tissue, or the uterus. However, the finding of gas is not pathognomonic for clostridial infection; other anaerobic bacteria too, frequently mixed with aerobic organisms, may produce gas (Kasper and Madoff 2005).

CT scanning is also helpful, especially in abdominal cases of gas gangrene. A recent study with the new-generation CT scanners reported 100 % sensitivity to detect necrotizing soft-tissue infections; however, it excluded patients taken to surgery prior to CT scanning and did not explore surgically all clinically suspected cases (Zacharias et al. 2010). Studies on MRI to detect necrotizing soft-tissue infection have reported lower sensitivity (80–90 %) and limited specificity. In addition, MRI is time consuming and is not always available. Ultrasound, although attractive as rapid bedside test, has not been well studied in this clinical scenario. In a cadaveric model of soft-tissue gas, it showed excellent sensitivity in detecting gas and its localization (Butcher et al. 2011). Rapid detection of alpha-toxin or sialidases or neuraminidases in infected tissues through enzyme-linked immunosorbent assay (ELISA) is not widely available but represents a potential diagnostic tool. ELISA can provide results in as little as 2 h when the test is applied to wound exudate, tissue samples, or serum.

Treatment

Treatment for gangrene involves removing the dead tissue, treating and preventing the spread of infection, and treating the condition that caused gangrene to develop. Owing to fast progression of the disease, early management of gas gangrene plays a critical role in saving the patient. Three main classes of therapy are recommended in general: surgical debridement of necrotic tissue, use of antibiotics, and hyperbaric oxygen therapy. The combination of aggressive surgical debridement and effective antibiotic therapy is the determining factor for successful treatment of gas gangrene. In case of advanced bacterial toxemia, blood transfusions and supportive therapy may also be required to manage pain and secondary pathophysiological conditions.

Debridement

Several studies have shown that the most important factor affecting mortality is timing and adequacy of initial surgical debridement. Delayed or inadequate debridement dramatically increases mortality. Radical debridement may necessitate limb amputation. It removes the source of infection and toxins, and furthermore, removal of infarcted tissue improves the subsequent penetration of antibiotics. Profuse washing should be performed with sterile normal saline solutions and/or 3 % liquid hydrogen peroxide. Debridement of all wounds should be performed as soon as possible, with removal of badly damaged, contaminated, and necrotic tissue, especially in patients who might have been contaminated by soil,

farm land, or dirty water. The infection is rarely eradicated after a single debridement, and serial debridements are almost always needed. Optimally, three debridements spaced 12–36 h apart are required to obtain control of gross infection. Debridement may result in significant intraoperative blood loss and inability to close surgical wounds. Vacuum-assisted dressings and skin expansion devices may have a role. Reconstructive surgery should be considered only when the patient has been stabilized and the infection fully eradicated (Davoudian and Flint 2012).

Antibiotic Therapy

In animal models, prompt treatment with antibiotics significantly improves survival rates. Historically, penicillin G in dosages of 10–24 million U/day was the drug of choice. Currently, a combination of penicillin and clindamycin is widely used (Stevens et al. 2005). A number of studies showed that protein synthesis inhibitors (e.g., clindamycin, chloramphenicol, rifampin, tetracycline) may be more effective because they inhibit the synthesis of clostridial exotoxins and lessen the local and systemic toxic effects of these proteins. In spite of increasing clindamycin resistance among anaerobes, cases of clindamycin-resistant *C. perfringens* are exceptional (Khanna 2008). A combination of clindamycin and metronidazole is a good choice for patients allergic to penicillin. A combination of penicillin and metronidazole may be antagonistic and is not recommended. Because other nonclostridial bacteria are frequently found in gas gangrene tissue cultures, additional antimicrobial coverage is indicated. Although approved for treating complicated skin and soft-tissue infections, newer antibiotics such as daptomycin, linezolid, and tigecycline have not been studied in patients with gas gangrene; therefore, they should not be used as primary antibiotics for treating this condition (Stevens et al. 2005). Supportive medication should be given to manage pain and other secondary pathophysiological conditions.

Hyperbaric Oxygen (HBO) Therapy

Since the 1960s, HBO therapy has been used in the USA for the treatment of gas gangrene; however, its use remains controversial. Controlled prospective studies on human subjects have not evaluated the impact of this treatment on survival. One reason for this is the low number of patients with gas gangrene. In addition, the therapeutic effect of HBO is difficult to reliably assess because of a lack of well-designed comparative studies. Many retrospective studies report increased survival in patients when HBO therapy is added to treatment with surgery and antibiotics. However, HBO therapy failed to show a survival advantage in two retrospective multicenter studies of the treatment of major necrotizing infections (George et al. 2009). Studies of animal models show conflicting reports about enhanced survival associated with HBO therapy. Studies indicate that HBO therapy has a direct bactericidal effect on most clostridia species, inhibits alpha-toxin production,

and can enhance the demarcation of viable and nonviable tissue prior to surgery. For these reasons, some authors recommend the use of HBO therapy before the initial debridement, if possible. The most common regimen for HBO therapy involves administration of 100 % oxygen at 2.5–3 absolute atmospheres for 90–120 min 3 times a day for 48 h and then twice a day as needed. In view of the frequent catastrophic outcomes in patients with gas gangrene, HBO therapy is an important adjunct to surgery and antimicrobial therapy, despite the lack of convincing clinical efficacy. Potential risks in patients undergoing HBO therapy include pressure-related trauma (e.g., barotraumatic otitis, pneumothorax) and oxygen toxicity (e.g., myopia, seizures). Other common adverse effects include claustrophobia. Most adverse effects are self-limiting and resolve after termination of HBO therapy (Tibbles and Edelsberg 1996; Kindwall 1993).

Intensive care should be taken as the patients with gas gangrene frequently have end-organ failure and other concomitant serious medical conditions that require intensive supportive care. Monitoring serum calcium may need special attention when large areas of necrotic fat may lead to its deposition (Ustin and Malangoni 2011).

Antitoxin and Vaccines

Gas gangrene has a relatively shorter incubation period and hence antitoxin is of little benefit in treatment. If the target animal is pre-immunized continually with formalinized antigens (toxins), it may be helpful to prevent infection. Chiefly, antitoxins may be administered to prevent the growth and to neutralize the trace amounts of toxins that are latent in the tissue/wound in a longer duration. The usefulness of gas gangrene antitoxin is currently a disputed matter. Some healthcare experts suggest that the advanced medical care currently available would be sufficient to beat any uses of the polyvalent antitoxins while some believe that the latter should be administered systematically and locally in the salvaged tissue for a complete cure. Currently there are no licensed vaccines suitable for use in humans which protect against gas gangrene. However, vaccines being developed for use in animals have the potential to be developed for use in humans in the future (Titball 2009). In 2005, *Clostridium perfringens* type A toxoid was developed by a US-based company and was the first commercial product for cattle to receive a conditional license by the US FDA to aid in the control of disease syndromes caused by the alpha-toxin of *C. perfringens*. But this toxoid is strictly prohibited for human utilization.

Fragments of the alpha-toxin of *C. perfringens*, a major virulent factor responsible for gas gangrene, were produced using genetic manipulation techniques, and the recombinant proteins were immunized into mice. Anti-$Cpa_{1–249}$ serum neutralizes phospholipase C activity but not hemolytic activity of the toxin. Anti-$Cpa_{247–370}$ serum neutralizes both the phospholipase C and hemolytic activities (Williamson and Titball 1993). Chen et al. developed a new strategy using group II intron-based Target-Tron technology and inactivated the *plc* gene (alpha toxin gene) in *C. perfringens* (Chen et al. 2005). Stevens et al. immunized mice with

C-terminal of alpha-toxin and they found in histopathological studies demonstrating limited muscle necrosis reduced microvascular thrombosis and enhanced granulocytic influx to the infected site (Stevens et al. 2004).

Maggot Therapy (Biotherapy)

Maggot therapy is a nonsurgical way of debridement to remove dead tissue. When used to treat gangrene, certain type of maggots from fly larvae (specially bred in a sterile laboratory conditions) are placed on the wound, where they consume the dead and infected tissue without harming healthy tissue. They also help fight infection and speed up healing by releasing substances that kill bacteria. During maggot therapy, the tiny maggots are put on to the wound and covered with gauze, under a firm dressing. After a few days, the dressing is cut away and the maggots, often 10 times bigger after eating the dead tissue, are then flushed away. A number of medical studies have shown maggot therapy can achieve more effective results than surgical debridement. However, due to the nature of this type of treatment, many patients are reluctant to try it (Dumville et al. 2009; Opletalova et al. 2011).

Conclusion and Future Directions

Considering the multiple toxins and organisms involved in gas gangrene etiology and the ever-increasing abuse of antibiotics and unavailability of world-class medical setup and disproportionate illiteracy rate in developing countries can raise serious concerns regarding the cure of this disease. Explicit major threats, posed by gas gangrene, are as follows: firstly, the involvement of multiple pathogens in the onset of the disease can be a problem in early detection and treatment of the disease. Coinfection of multiple pathogens like *E. coli* and *S. aureus* along with clostridia in the wounds would mask the isolation of the latter as the former pathogens grow rapidly on synthetic and semisynthetic media that are generally used to isolate clostridia. The second concern is due to the extensive antibiotic abuse in developing countries. As a result of this problem, many clostridia already gained antibiotic resistance genes and the spectrum of this resistance pattern is ever increasing. Due to this unsettled problems, a nonantibiotic therapeutic molecule or vaccine is to be developed; it should be a subunit vaccine incorporating multiple protective domains. Effective antibody development against the toxins is essentially important for not only fighting against the clostridial exotoxin but also limiting the systemic cytokine release associated with systemic inflammatory response syndrome. Care should be taken to consider all the possible variant of antigens (alpha-toxins) from different necrotizing clostridia species of different geographical sources during multivalent antibody development which should be working effectively and universally. Careful structure-function relationship and functional domain analysis and interactions with bodily available natural ligands should be helpful to develop perfect antitoxin molecules which could have the

ability to neutralize these toxins at multiple sites. The calcium binding is important at C-terminal domain of alpha-toxin which allows the toxin to bind to the phospholipid head groups on the cell surface, and N-terminal domain has phospholipase activity which hydrolyses the phospholipids of the membrane. Therefore, it is a time to understand all the possible structure-function using modern bioinformatics tools in such a challenging situation. Due to debridement of necrotic tissues, the portions that require regeneration may need stem cell therapy and regenerative medicine that will be helpful for complete tissue recovery. The viral vector-based antitoxin gene therapy may be another future option. Lastly, the scientific world should acquire sufficient global antitoxin weapons against the deadly clostridia toxins to get ready in fighting such bioterrorism.

Cross-References

- ▶ Challenges in Developing Biotoxin Inhibitors
- ▶ Evolutionary Traits of Toxins
- ▶ Immunosensors: Using Antibodies to Develop Biosensors for Detecting Pathogens and Their Toxins
- ▶ The Public Health Response to Potential Bioterrorism by Toxin Attack

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