Anaerobe 18 (2012) 254–259

Contents lists available at SciVerse ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Pathogenesis and toxins

Life-threatening clostridial infections

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ARTICLE INFO

Article history:
Received 12 October 2011
Accepted 4 November 2011
Available online 20 November 2011

Keywords:
Gas gangrene
Toxic shock syndrome
Clostridium
Myonecrosis

ABSTRACT

Life-threatening soft tissue infections caused by *Clostridium* species have been described in the medical literature for hundreds of years largely because of their fulminant nature, distinctive clinical presentations and complex management issues. The *Clostridium* species *perfringens, septicum* and *histolyticum* are the principal causes of trauma-associated gas gangrene and their incidence increases dramatically in times of war, hurricanes, earthquakes and other mass casualty conditions. Recently, there has also been an increased incidence of spontaneous gas gangrene caused by *Clostridium septicum* in association with gastrointestinal abnormalities and neutropenia. Similarly, over the last 15 years there has been increased recognition of a toxic shock-like syndrome associated with *Clostridium sordellii* in individuals skin-popping black tar heroin, in women undergoing childbirth or other gynecologic procedures including medically-induced abortion. Like their cousins *Clostridium tetanus* and *Clostridium botulinum*, the pathogenesis of these clostridial infections is largely the consequence of potent exotoxin production. Strategies to inhibit toxin production, neutralize circulating toxins and prevent their interaction with cells of the innate immune response are sorely needed. Recent studies have elucidated novel targets that may hold promise for newer therapeutic modalities.

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1. Introduction

1.1. Historical perspective

Necrotizing *clostridium* infections of the skin and soft tissues have been described in the medical literature for many centuries and are largely attributable to *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium histolyticum* and *Clostridium novyi*. Infections caused by *C perfringens* require extensive penetrating trauma whereas *C. septicum* and *C. sordelli* may initiate infection spontaneously or in association with minor trauma or child birth. Still all these species can cause clostridial myonecrosis or gas gangrene. Thus, the epidemiology of these infections is quite varied but closely tied to human evolution and, more specifically for gas gangrene, our inventions of weapons of destruction. For *C. septicum*, as a cause of spontaneous gas gangrene, the increased incidence is related to medical conditions such as neutropenia, gastrointestinal malignancy, radition therapy and chemotherapy. Recently, *C. sordellii* and *C. novyi* infections have been reported in association with skin-popping of black tar heroin, normal pregnancy and delivery, and medically-induced abortion.

During the Civil War, 50% of soldiers who were wounded ultimately died. If the soldier was not killed outright, the extensive destruction of tissue inflicted by the 45 caliber lead bullets used at the time predisposed soldiers to the development of gas gangrene largely due to the resultant vascular damage. Civil War surgeons had few resources such as anesthesia, intravenous fluids and antibiotics and thus “prophylactic amputation” became commonplace. During World War I, medical treatments had improved, however weaponry also became more sophisticated. In this period, the incidence of gas gangrene was as high as 10% of wounded [23]. Gas gangrene also claimed an estimated 100,000 German soldiers during this period. In World War II, medical evacuation, improved surgical debridement techniques in field hospitals, gas gangrene anti-toxin and antibiotics were available and reduced the overall incidence of gas gangrene. For example, death from gas gangrene in US casualties in the western front of Europe was 8 cases/1000 wounded, for the Free French Forces it was 12.3/1000 and among prisoners of war, 51.9/1000 wounded. The much higher rate among prisoners was attributed to a delay in definitive surgical treatment which was 1 day for US casualties and 3.5 days for prisoners [23]. In addition, the incidence of gas gangrene was higher in European theaters than in desert operations, likely because the fertile valleys of Europe were heavily contaminated with *C. perfringens* (50,000 per gram of soil), whereas they were rarely recovered in the Sahara Desert. Uniforms, too, were found to be contaminated with enteric

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1075-9964/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.anaerobe.2011.11.001

clostridia and *C. perfringens* was isolated from 24% of shirts and 44% of trousers [20–22].

In the Korean, Vietnam, Gulf and Afganistan wars, rapid evacuation, thorough (but not radical) surgical debridement, vascular reconstruction, improved supportive measures and greater antibiotic efficacy and availability have greatly reduced the incidence of *C. perfringens* gas gangrene. Gas gangrene continues to be problematic in both military theaters as well as the civilian sectors, again always associated with traumatic injuries. For instance, among nearly 2800 patients hospitalized following the 2008 Wenchuan earthquake in China, over 2.4% developed gas gangrene [14].

Interestingly, *C. septicum* as a cause of non-traumatic gas gangrene has increased in civilian populations in recent years and necrotizing infections associated with skin-popping black tar heroin have been attributed to *C. perfringens*, *C. novyi* and *C. sordellii*. Finally, *C. sordellii* has been reported not only in trauma cases but also associated with pregnant females undergoing normal vaginal delivery, ceasarian sections or medical abortions.

2. Major exotoxins of the histotoxic clostridia

Traditionally, the major lethal toxins of the clostridia are given Greek letters with the letter "α" always used to designate the most potent or most significant lethal factor. A marvelous review can be found in the monograph by Smith [29] but are discussed in brief below.

2.1. C. perfringens

The major *C. perfringens* extracellular toxins are alpha toxin and theta toxin. Alpha toxin has both phospholipase C and sphingomyelinase activities. Active immunization of mice with purified recombinant protein consisting of the C-terminal alpha toxin domain (amino acids 247–370) provided protection against lethal challenge with *C. perfringens* [40]. Alpha toxin is also a potent platelet agonist. In vivo, intravascular activation of platelets by alpha toxin leads to platelet aggregation [10,39] and formation of occlusive thrombi that completely and irreversibly occlude capillaries, venules and arterioles [10,11]. Without adequate tissue perfusion, the anaerobic niche is extended and rapid destruction of viable tissue, so characteristic of clostridial gas gangrene, ensues. Alpha toxin also has direct deleterious effects on cardiomyocyte function [3].

Theta toxin from *C. perfringens* (also known as perfringolysin O, PFO) is a member of the cholesterol-dependent cytolysins (CDCs) that includes streptolysin O from group A streptococcus, pneumolysin from *Streptococcus pneumoniae* and several others. Upon contact with cell membrane cholesterol, theta toxin monomers oligomerize and insert into the membrane, forming a pore and resulting in cell lysis [28]. Theta toxin, in sublytic amounts, also contributes to the pathogenesis of gas gangrene via its ability to modulate the inflammatory response to infection [9,38].

2.2. C. septicum

C. septicum produces four main toxins: alpha toxin (α, lethal, hemolytic, necrotizing activity); beta toxin (β, DNase); gamma toxin (γ, hyaluronidase); and delta toxin (Δ, septicolysin, an oxygen-labile hemolysin), as well as a protease, and a neuraminidase [29]. Unlike the alpha toxin from *C. perfringens*, the *C. septicum* alpha toxin does not possess phospholipase activity. Active immunization against alpha toxin significantly protects against challenge with viable *C. septicum* [5].

2.3. C. sordellii

Pathogenic strains of *C. sordellii* produce up to 7 identified exotoxins. Of these, lethal toxin (LT) and hemorrhagic toxin (HT) are regarded as the major virulence factors. LT and HT are members of the large clostridial cytotoxin (LCC) family, all having molecular weights between 250 and 308 kDa. Other members include the *Clostridium difficile* toxins A and B and *C. novyi* α-toxin. All LCCs possess remarkable amino acid similarity, with identities ranging between 26 and 76%. LT and *C. difficile* toxin B have the highest homology with amino acid sequences being 76% identical and 90% homologous to one another. All LCCs possess glycosyltransferase activity and modify signaling molecules controlling cell cycle, apoptosis, gene transcription and the structural functions of actin such as cell morphology, migration and polarity. Once modified, these proteins become inoperative. Modification of actin cytoskeletal assembly and organization presumably leads to the massive capillary leakage characteristic of *C. sordellii* infection. The *C. sordellii* neuraminidase has been shown to contribute to the leukemoid reaction, in part, by enhancing proliferation of granulocyte progenitor cells [1]. Other exotoxins include an oxygen-labile hemolysin, DNase, collagenase and lysolecithinase however their roles in pathogenesis have not been extensively investigated.

3. Clinical features

3.1. Traumatic gas gangrene

C. perfringens myonecrosis (gas gangrene) is one of the most fulminant Gram-positive infections of humans. Predisposing conditions include crush type injury, laceration of large or medium sized arteries and open fractures of long bones which are contaminated with soil containing the bacterial spores. Gas gangrene of abdominal wall and flanks occurs after penetrating injuries such as knife or gunshot wounds sufficient to compromise intestinal integrity with resultant leakage of bowel contents into the soft tissues. In the last few years, cutaneous gas gangrene caused by *C. perfringens, C. novyi* type A and *C. sordellii* have been described in the United States and Northern Europe among drug abusers injecting "black tar heroin" subcutaneously [6,7,12,13,19].

Clostridial gas gangrene is characterized by the sudden onset of excruciating pain at the infection site [23] and rapid development of a foul smelling wound containing a thin serosanguinious discharge and gas bubbles. Brawny edema and induration develop and give way to cutaneous blisters containing bluish to maroon colored fluid. Later such tissue may become liquified and slough. The margin between healthy and necrotic tissue often advances several inches per hour despite appropriate antibiotic therapy [23], and radical amputation remains the single best life-saving treatment. Shock and organ failure frequently accompany gas gangrene and when patients become bacteremic, the mortality exceeds 50%.

Clinical diagnosis is not difficult because 1) the infection always begins at the site of significant trauma; 2) is associated with gas in the tissue and 3) is rapidly progressive. Gram stain of drainage or tissue biopsy is usually definitive demonstrating large gram-positive rods and an absence of inflammatory cells.

3.2. Pathogenesis

The mere presence of *C. perfringens* in wounds is not sufficient to cause gas gangrene. In fact, in World War II nearly 80% of wounds were contaminated with *C. perfringens*, yet only 8/1000 (0.8%) developed gas gangrene. Thus, the pathogenesis of gas gangrene is complex and can be considered in 4 separate stages.