Anaerobe 41 (2016) 79–84

Contents lists available at ScienceDirect

Anaerobe

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

The microbiota and immune response during *Clostridium difficile* infection

Erica L. Buonomo [a], William A. Petri Jr. [a, b, c, *]

[a] Department of Microbiology, Immunology and Cancer, University of Virginia Charlottesville, VA, 22908, USA
[b] Department of Medicine, University of Virginia, USA
[c] Department of Pathology, University of Virginia, USA

ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form
9 May 2016
Accepted 16 May 2016
Available online 20 May 2016

Handling editor: Elisabeth Nagy

Keywords:
Clostridium difficile
Colitis
Immune response
Microbiota
Fecal transplant
Innate
Inflammation

ABSTRACT

Clostridium difficile is a gram-positive, spore forming anaerobe that infects the gut when the normal microbiota has been disrupted. *C. difficile* infection (CDI) is the most common cause of hospital acquired infection in the United States, and the leading cause of death due to gastroenteritis. Patients suffering from CDI have varying symptoms which range from mild diarrhea to pseudomembranous colitis and death. The involvement of the immune response to influence disease severity is just beginning to be investigated. There is evidence that the immune response can facilitate either protective or pathogenic phenotypes, suggesting it plays a multifaceted role during CDI. In addition to the immune response, the microbiota is pivotal in dictating the pathogenesis to CDI. A healthy microbiota effectively inhibits infection by restricting the ability of *C. difficile* to expand in the colon. Thus, understanding which immune mediators and components of the microbiota play beneficial roles during CDI will be important to future therapeutic developments. This review outlines how the microbiota can modulate specific immune mediators, such as IL-23 and others, to influence disease outcome.

1. Introduction

Clostridium difficile is a spore forming, Gram-positive, toxin-producing anaerobe that infects the gut when the natural flora has been disrupted, primarily through use of antibiotics. It is currently the leading cause of nosocomial infection in the United States, surpassing methicillin resistant *Staphylococcus aureus* (MRSA) [1–3]. The Centers for Disease Control and Prevention (CDC) lists *C. difficile* as one of three urgent threats in the United States and it is estimated to cause approximately 453,000 infections per year with 29,300 related deaths [4]. Moreover, a 30-day mortality rate has been observed in up to 15% of *C. difficile* patients [5]. Disease can range from asymptomatic colonization to mild diarrhea, pseudomembranous colitis, and life threatening toxic megacolon. Treatment for *Clostridium difficile* infection (CDI) costs the US health care system an estimated $4.8 billion annually in acute health settings alone, with an additional substantial burden seen in long-term care facilities [6]. Despite therapy, recurrent disease is seen in 10–35% of patients after initial infection and secondary relapses are observed in 35–65% of patients after primary reoccurrence [7–9] Risk factors include antibiotic exposure, acute or long term care facility exposure, advanced age, comorbidities such as inflammatory bowel disease, and use of proton pump inhibitors [6,10]. The prevalence of *C. difficile* cases have been increasing annually in both health care and community settings and hypervirulent strains of *C. difficile*, most notably BI/NAP1/Ribotype 027 strains, are also becoming more common [11]. Additionally, the ability of *C. difficile* spores to survive in harsh conditions including resistance to alcohol-based cleaners contributes to disease transmissibility. In the past ten years, there has been a five-fold rise in disease incidence in the North American population, emphasizing the need for better treatment and management strategies [12,13].

2. Pathogenesis of CDI

Disruption of the host's endogenous microbiota, a state called dysbiosis, provides an ideal environment for CDI to occur. Several

* Corresponding author. University of Virginia Health System, PO Box 801340, Charlottesville, VA, 22908-1340, USA.
E-mail addresses: elb3ea@virginia.edu (E.L. Buonomo), wap3g@virginia.edu (W.A. Petri).

http://dx.doi.org/10.1016/j.anaerobe.2016.05.009
1075-9964/

components of a healthy microbiota contribute to preventing host susceptibility to infection, outlining the importance of commensal bacteria to combat *C. difficile*. Bacterial spores are transmitted through the fecal-oral route and germinate into vegetative cells in the intestine of susceptible hosts. These cells infiltrate the mucus layer surrounding the epithelial cell layer and adhere to its surface [14]. Once adhered, the bacteria produce toxins that mediate a robust inflammatory response. Toxin A (TcdA) and toxin B (TcdB) are the primary virulence factors of *C. difficile* and are released during the late log phase and stationary phase of vegetative growth [15]. TcdA and TcdB are able to glucosylate and inactivate Rho and Ras family small GTPases causing disruption of the actin cytoskeleton, cell rounding, inhibition of cell division and cell death [16,17]. This process is especially harmful to the integrity of the epithelial barrier. The breakdown of the epithelium causes permeability of the barrier and allows for both pathogenic and commensal bacteria to translocate into the lamina propria. Collectively, these actions induce the release of proinflammatory mediators from epithelial and immune cells in the lamina propria that subsequently recruit additional immune effector cells to the site of infection [16,18–20]. Neutrophils are the hallmark cell subset recruited to the intestinal barrier during infection and are found in pseudomembranous lesions during severe disease. However, the role of the immune response during infection remains incompletely understood as there is evidence to support both protective and pathogenic roles during CDI. The dual role of the immune response coupled with the knowledge that a healthy microbiota prevents infection demonstrates the importance of both commensal bacteria and the host inflammatory response during CDI.

3. The role of the microbiota during *Clostridium difficile* infection

Antibiotic exposure remains the leading risk factor of disease, stressing the beneficial role of the microbiota in host protection [21]. Disruption of a 'healthy' microbiota or the reduction of its diversity is directly linked to host susceptibility to CDI. The microbiota of patients in the hospital are commonly in a dysbiotic state due to increased incidence of antibiotic treatment, modulation of diet, and other treatments such as chemotherapy. Dysbiosis coupled with enhanced exposure to *C. difficile* spores in the hospitals may explain why the majority of CDI cases are associated with health care facilities. The loss of disease resistance associated with alterations of the endogenous flora is an important initial step in the pathogenesis of CDI (Fig. 1). The necessity of antibiotic pretreatment to render mice susceptible to CDI has since been established in mouse models [22]. The microbiota has been shown to protect against infection through a process called colonization resistance, which involves commensal microbes outcompeting the pathogen for space and nutrients in the intestine [23]. Wilson and colleagues originally described colonization resistance by demonstrating that the transfer of cecal contents from an untreated hamster to a vancomycin-treated hamster effectively prevented susceptibility to CDI [24]. It was later shown that bacteria with similar nutrient and spatial demands are capable of excluding *C. difficile*. In fact, a series of experiments demonstrated that pre-infection with a non-toxigenic strain of *C. difficile* was capable of successfully protecting hamsters from subsequent infection with a toxigenic *C. difficile* strain [25]. Although, the immune response to non-toxigenic *C. difficile* was not examined as a potential mechanism of disease prevention, the authors conclude that similar niche requirements utilized by non-toxigenic *C. difficile* results in protection from CDI [24]. Furthermore, recent studies have identified that alterations in the microbiota in response to antibiotic treatment induced spikes in succinate and sialic acids which are then exploited by *C. difficile* to facilitate its expansion in the gut [26,27]. Together, this data supports a role for the microbiota to outcompete *C. difficile* resulting in inhibition of infection.

In addition to colonization resistance through competition for space and nutrients, the microbiota has also been observed to regulate primary and secondary bile salts to inhibit *C. difficile* outgrowth. The primary bile salt taurocholate was identified as an *in vivo* germinant of *C. difficile* spores into vegetative cells that cause disease [28]. It was later shown that derivatives of cholate activate spore germination when combined with glycine, whereas derivatives of chenodeoxycholate suppress germination, supporting a role for bile salts in regulating the outgrowth of *C. difficile* [29,30]. Interestingly, antibiotic treatment and subsequent changes in the endogenous microbiota lead to increased taurocholate in the cecum and reduced levels of the inhibitory secondary bile salt deoxycholate, which is toxic to vegetative cells [31]. Thus, antibiotic treatment supports the outgrowth of *C. difficile* by inducing bile salts that enhance germination and reducing bile salts that suppress the expansion of vegetative cells. In fact, transfer of bacteria from the cecal contents of antibiotic treated mice supported expansion of *C. difficile in vivo*, while transfer of contents from untreated mice increased host resistance to infection [32]. Buffie et al. associated protection observed in mice receiving cecal contents from untreated mice with the presence of enhanced secondary bile acids [32]. The elevation of secondary bile acids in protected mice could be achieved by transferring a cocktail of four specific bacteria, with a primary role for bacterium *Clostridium scindens* [32]. This study supports the hypothesis that specific components of the microbiota have the ability to protect against CDI.

In addition to its well-defined role in preventing host susceptibility, there is emerging evidence for the ability of the microbiota to resolve active CDI. Fecal microbiota therapy (FMT) involves the transfer of 'healthy' donor stool to *C. difficile* infected patients with the goal of restoring bacterial diversity in the colon and expelling *C. difficile*. Studies in mice are now beginning to explore the ability of a transferred microbiota to clear CDI from mice and prevent relapsing disease. The transfer of six phylogenetically diverse intestinal bacteria was sufficient to clear CDI in mice [33]. Moreover, Tvede and Rask-Madsen successfully performed FMT in six CDI patients in 1989, although the treatment has not received mainstream attention until recent years [34]. A recent study from the New England Journal of Medicine observed an approximate 90% cure rate in relapsing CDI patients [35], indicating that the microbiota has a beneficial role to play at both the resistance and resolution stages of disease. Furthermore, restoration of microbiota through FMT has shown more promise in preventing disease reoccurrence then vancomycin treatment [35]. This may be due to the ability of vancomycin to target beneficial members of the microbiota in addition to *C. difficile* and prevent the reestablishment of a healthy microbiota and patient recovery.

4. The dual role of the immune response during *Clostridium difficile* infection

The role of the immune response during *C. difficile* infection remains controversial as there is evidence to support protective and pathogenic phenotypes during disease. In animal models of *C. difficile* infection, the absence of an intact immune response is disadvantageous to the host. The importance of neutrophils is supported by evidence that mice infected with *C. difficile* that lack the capability to recruit neutrophils to the gut suffered from enhanced mortality compared to controls [36,37]. $TLR4^{-/-}$ and $MyD88^{-/-}$ mice experienced enhanced morbidity, likely through the observed decrease in MyD88-dependent neutrophil