Immunology Letters 162 (2014) 77–81

Contents lists available at ScienceDirect

Immunology Letters

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

Review

Emergence of fecal microbiota transplantation as an approach to repair disrupted microbial gut ecology

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ARTICLE INFO

Article history:
Available online 10 August 2014

Keywords:
Fecal microbiota transplantation
Clostridium difficile infection

ABSTRACT

In the recent years fecal microbiota transplantation (FMT) has emerged as an effective therapeutic option for patients with refractory *Clostridium difficile* infection that is not responding to antibiotic therapy. It results in implantation of donor microbiota into recipients and restoration of normal distal gut microbial community structure. We anticipate that this form of therapy represents merely the first entry into a new class of therapeutics. There is great interest in application of FMT or defined microbial consortia to treatment of many diseases associated with dysbiosis. However, many challenges remain in development as our understanding of microbial ecology within the human body and microbiota–host interactions remain limited. Future advances in this field will be critically depending on detailed mechanistic understanding.

1. Introduction

The germ theory of disease has been the foundation of some of the largest successes in medical history, which throughout recorded time has been dominated by infectious disease. While the development of antibiotic therapies allowed these diseases to be combated, such treatments focused exclusively on pathogens with little consideration of any effects on the resident microbial communities in the human host. Until recently, these commensal microbiota were largely neglected in clinical practice and mostly inaccessible to investigation. In the absence of data to suggest otherwise, host microbial communities were thought to be extremely resilient, and it was assumed that antibiotics could be used with impunity. However, there is now growing concern that changes in development and composition of host microbiota caused by pervasive use of antibiotics and dietary changes have contributed to the emergence of new diseases such as metabolic syndrome, autoimmunity, atopic conditions, and many others.

Nowhere is the relationship between antibiotics, host microbiota, and human disease more clear than in the treatment of refractory *Clostridium difficile* infection (CDI) with fecal microbiota transplantation (FMT). Recent investigations of patients treated with FMT demonstrate that there are limits to the resilience of host microbiota to antibiotic exposure, and that entirely new microbial communities can be established in adult patients by direct implantation [1–3]. The remarkable clinical success of this procedure in extinguishing *C. difficile* bacteria and achieving resolution of associated gastrointestinal symptoms has encouraged many investigators and patients to imagine that FMT can result in similarly successful outcomes when applied to many other conditions [4]. However, while some small observational studies do support a degree of optimism, it is clear that moving forward requires deeper mechanistic understanding of how microbial communities are held together, how they interact with the host, and what activities of the microbiota are needed for therapeutic effects in specific diseases.

2. Current uses of FMT in treatment of CDI

Although fecal enemas were introduced as early as 1958 by Eiseman et al. in the treatment of pseudomembranous enterocolitis [5], a condition that is now typically associated with CDI, the practice has been relatively sparse until recently. Vancomycin, an antibiotic that suppresses vegetative forms of *C. difficile*, was introduced into clinical practice in 1959 [6], and antibiotic-refractory cases of CDI were relatively uncommon for several decades. However, new, more virulent strains of *C. difficile* emerged in the early 1990s, associated with broader antibiotic resistance, greater capacity for toxin

Abbreviations: BMT, bone marrow transplantation; CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; RCDI, recurrent *Clostridium difficile* infection.

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http://dx.doi.org/10.1016/j.imlet.2014.07.016
0165-2478/

production, and increased sporulation efficiency [7–9]. This has been accompanied by greater incidence, morbidity, and mortality associated with CDI. CDI today is the most common cause of nosocomial diarrhea in the US, and has increasingly become an important cause of community-acquired diarrhea [10,11]. Although the Centers for Disease Control conservatively attributes approximately 14,000 deaths to CDI annually, some estimates of CDI-associated mortality are >100,000 per year [12].

Antibiotics constitute the major risk factor for CDI, which is acquired by ingestion of *C. difficile* spores. Antibiotic-induced suppression of the host microbiota creates an environment favorable to *C. difficile* spore germination and growth of the vegetative form of the bacteria [13,14]. Clinical symptoms, which vary from fecal urgency and diarrhea to cessation of bowel movements in toxic megacolon, are caused by *C. difficile* enterotoxins that damage the colonic mucosa and produce inflammation. Common clinical challenges associated with CDI include recurrent infection (RCDI), severe or complicated infection refractory to antibiotic therapy, and CDI associated with underlying inflammatory bowel disease (IBD). FMT appears to be effective in all these presentations, but there are important nuances associated with each.

Antibiotic therapy alone fails to clear the infection in a significant fraction of patients (~20–30%), and each round of antibiotic treatment for CDI further increases the risk of recurrence by approximately 20% [15]. The reason for recurrence of CDI may be failure to clear the *C. difficile* spores with antibiotic treatment, which further suppresses the normal microbiota and perpetuates the underlying problem. Ultimately, a significant fraction of patients develop recurrent *C. difficile* infection syndrome, a condition characterized by an indefinite series of antibiotic treatments and relapses. Analysis of fecal samples in such patients shows marked contraction of the normally dominant members of the Bacteroidetes and Firmicutes phyla, accompanied by dramatic expansion of γ-Proteobacteria [2,3,16,17]. Infusion of fecal material taken from a healthy donor promptly leads to establishment of donor-like composition of fecal bacteria in the recipient and normalization of gastrointestinal symptoms. Clinical efficacy of FMT, as defined by abrogation of CDI recurrence over 1–2 months following cessation of antibiotics, is approximately 90% in multiple case series of consecutive patients treated [18]. Furthermore, FMT is confirmed to be effective over vancomycin in a randomized, controlled trial [3].

Reports of FMT in severe and complicated CDI refractory to antibiotic therapy remain sparse, and the standard therapy continues to be surgical colectomy. However, surgical therapy accompanied by modern supportive care is still associated with ~50% mortality [19,20]. The prognosis is only marginally better than ~75% mortality associated with pseudomembranous enterocolitis in 1950s, when Eiseman's team first reported use of fecal enemas [5]. Isolated case reports of successful use of FMT in treatment of severe and complicated CDI suggest that this approach should be investigated further [21–25]. Commonly these patients have multiple serious co-morbidities and constitute a very challenging study population. In this situation FMT is introduced during active infection when vegetative forms of *C. difficile* are still present. Nevertheless, in our own experience the response to FMT in the treatment of active, complicated CDI can be very prompt and measureable in mere hours [26]. However, we find that sequential administration of FMT is needed to achieve sustained recovery [26].

CDI is a common complication in patients with underlying IBD [27–29], and is always an important diagnostic consideration when the patient presents with a flare of IBD activity. In this context one generally does not see pseudomembranes during an endoscopic examination of the colon, and failure to make the diagnosis can lead to a delay in providing appropriate antibiotic treatment and escalation of immunosuppressive therapy that by itself may worsen the disease. FMT appears to be comparably effective in clearing the infection in RCDI patients with and without underlying IBD [30].

3. FMT and immune mediated colonization resistance in CDI

Despite its efficacy, the mechanisms of FMT are poorly understood, though the concept behind the procedure seems intuitively straightforward. Even the earliest investigators using FMT in the 1950s recognized the importance of gut microbiota in the normal function of the gastrointestinal tract, and made a connection between the use of antibiotics and the clinical syndrome caused by the infection [5]. They hypothesized that restoration of the normal microbial gut ecology by transfer of entire microbial communities from healthy donors could be curative in this disease. Recent experiments have largely validated this hypothesis, although it remains unclear how these transferred microbiota are able to combat the infection. Potential mechanisms include competition for limiting resources by other microorganisms within the same ecological niche in the intestinal tract, direct elimination of *C. difficile* or interference with its pathogenicity by microbial products, restoration of secondary bile acid metabolism in the colon, and induction of immune-mediated colonization resistance. These various mechanisms were reviewed recently in several articles [31,32], and this review will focus primarily on immune-mediated colonization resistance.

It is well-established that the commensal microbiota provide both tonic stimulation in the mucosa, critical for epithelial tissue protection and repair, and maintenance of both innate and adaptive immune defenses. Treatment of mice with a mix of antibiotics that includes metronidazole and vancomycin, which are routinely used in the care of patients with CDI, results in downregulation of intestinal expression of RegIIIγ, a secreted C-type lectin that kills Gram-positive bacteria [33]. Human α-defensins produced by intestinal Paneth cells also neutralize *C. difficile* toxin B, which is largely responsible for CDI-associated pathology [34]. Expression of many defensive antimicrobial proteins and peptides, including RegIIIγ and α-defensins, is dependent on MyD88 stimulation [33,35]. Notably, MyD88-deficient mice suffer markedly increased mortality following CDI [36]. While it is not yet clear which MyD88-mediated protective mechanisms may be operative in CDI, one mechanism is likely to be expression of CXCL1. This chemokine recruits neutrophils, which play an important role in suppressing lethality of CDI in mice and dominate the histopathology of pseudomembranous colitis [36]. In fact, neutrophil recruitment is further potentiated by IL-1β-mediated secretion triggered by translocating commensals through the damaged intestine [37]. Therefore, decreased MyD88 stimulation following antibiotic treatment may contribute to an environment where *C. difficile* can grow and produce toxins, and makes the host vulnerable to septic complications.

Patients with RCDI are subjected to prolonged courses of antibiotics, which may affect these immune responses indirectly by altering the diversity and composition of the colonic microbiota. An average patient with RCDI in our clinical practice has had >5 antibiotic courses for the infection [30], and prolonged tapered or pulsed courses of vancomycin or similarly long sequential treatments with several antibiotics, e.g., vancomycin and rifaximin, constitute standard practice approaches in RCDI. The marked loss of microbial diversity that is observed in these patients, therefore, is not surprising. It is not uncommon for these patients to develop progressive diarrheal symptoms despite ongoing antibiotic treatment and absence of other pathology such as inflammatory bowel disease (IBD). Although explanations for this diarrhea may include alterations in bile acid composition, it is also possible that