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Pathogenesis and toxins

Role of obesity and adipose tissue-derived cytokine leptin during *Clostridium difficile* infection

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ABSTRACT

Obesity is among the most pressing health concerns in the world since it is increasingly common even in the developing world, and is clearly associated with increased risk for chronic debilitating diseases and death. Furthermore, obesity can influence the pathogenesis of infectious diseases by affecting the balance of pathogen clearance and pathological inflammation. The mechanisms that result in enhanced inflammation in obese individuals are poorly understood. *Clostridium difficile* is a major cause of nosocomial infections worldwide. Recent studies have shown that obesity is associated with increased risk of *C. difficile* infections. In this review, we will discuss our current knowledge of the role of obesity in determining risk of *C. difficile* infections, and focus on the role of the adipose tissue-derived cytokine leptin in *C. difficile* infections.

1. Obesity and infections

Obesity is an emerging health problem and worldwide >500 million people are obese (body mass index, BMI ≥ 30) and >1.4 billion are overweight (BMI 25–30) [1]. Obesity is clearly associated with a higher risk of cardiovascular diseases and metabolic syndrome, and high BMI is associated with increased all-cause mortality [2]. In terms of infectious diseases, observational studies have shown that obesity is associated with an increased risk of both nosocomial and community-acquired infections: increased surgical site infections [3], cellulitis [4] and community-acquired pneumonia [5]. Interestingly, while obese patients with community-acquired pneumonia have higher systemic inflammation – higher serum C-reactive protein levels (a marker of inflammation) and higher frequency of sepsis (based on systemic inflammatory response syndrome criteria) [6], the 30-day mortality is lower in obese patients [6,7]. In H1N1 influenza virus infections, both obesity (BMI ≥ 30) and morbid obesity (BMI ≥ 40) increases the risk of ICU admissions by two-fold, with morbid obesity (BMI ≥ 40) being associated with a higher risk of death [8]. The impact of obesity on infectious diseases outcomes is thus likely to be dependent on the specific pathogenic insult, the associated immune response and the degree of obesity.

While the mechanisms of obesity-mediated effects during infections are poorly understood, obesity-associated immune dysregulation likely plays an important role in infectious disease pathogenesis and outcomes. For example, in genetically obese mice (leptin deficient, *ob/ob* mice), infection with gram negative bacteria shows impaired macrophage responses, increased pathogen burden and higher mortality [9]. Mycobacterial infection of *ob/ob* mice is also associated with impaired immune response, as seen by decreased IFNγ production, impaired granuloma formation and higher pathogen burden [10]. In diet-induced obese mice, a model system that more closely resembles human obesity, H1N1 influenza virus infection leads to higher pro-inflammatory cytokine/chemokine production, severe pulmonary inflammation, lung injury and higher mortality [11] but impaired influenza-specific memory T cell function in obese animals [12]. Thus, obesity could have differential impact on the adaptive and innate immune systems.

1.1. Obesity and C. difficile infections

The epidemic of *C. difficile* has reached alarming proportions and

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is a leading cause of healthcare-associated infections in community hospitals in U.S. and Europe [13]. Antibiotic exposure, proton pump inhibitors and H2 blockers, old age, underlying chronic disease, recent hospitalization, gastrointestinal surgeries and tube feeds [14,15], have all been associated with increased risk of *C. difficile* infections. The role of BMI was unknown until recently when studies showed that high BMI and obesity is associated with a higher risk of acquiring *C. difficile* infections in hospitalized patients [16,17]. To the best of our knowledge, effects of obesity and BMI on disease severity or outcomes after *C. difficile* infection have not been studied.

The spectrum of *C. difficile*-associated disease extends from asymptomatic colonization to self-limited diarrheal illness, to severe colitis and sometimes death. This variability in disease course and outcomes suggests that host factors play an important role in disease pathogenesis. In fact, recent studies suggest that host inflammation is an important determinant of outcomes in *C. difficile* infections [18]. Since obesity is a state of persistent, low-grade inflammation [19], obesity-associated inflammation will likely have an impact on both the risk and outcomes in cases of *C. difficile* infections. Since obesity affects the composition of gut microbial communities [20,21] and disruption of gut microbiota is an essential first step in *C. difficile* infection, obesity-associated differences in host microbiome could be another factor affecting risk and outcomes of *C. difficile* infections.

2. Leptin and inflammation

While obesity is the result of dysregulation of multiple physiologic processes and no single molecule is causative, obesity-associated inflammation is believed to be both initiated and propagated by adipose tissue [19]. Adipose tissue secretes a number of adipocytokines (e.g. leptin, adiponectin, apelin, omentin, IL-6, TNF and MCP-1) during critical illness that affect both metabolism and host inflammation [22,23]. Leptin is one such adipocytokine that is elevated during early sepsis [24]. Leptin is a member of the IL-6 family and was initially discovered for its role in regulation of metabolism and satiety [25]. It is primarily secreted by fat cells (minor contributions from gut and placenta tissue) and leptin receptors are expressed on many different cell types: immune cells, epithelial cells and neurons [26]. Leptin has pleotropic functions and affects multiple physiological processes including angiogenesis [27], hematopoiesis [28], lipid [26], glucose [26] and bone metabolism [29], reproduction [30] and both adaptive and innate immune responses [31,32].

In terms of inflammation and immunity, leptin has a pro-inflammatory mode of action. Leptin induces inflammatory cytokine and chemokine production, increases neutrophil chemotaxis, enhances NK cell cytotoxicity and activates dendritic cells, neutrophils and NK cells [32–36]. Leptin also regulates adaptive immunity by suppressing regulatory T cells (Tregs) and increasing Th1 cellular responses [31,32]. Presumably via increasing the inflammatory response, leptin enhances the host defense during infections. Thus, humans with leptin deficiency have a higher incidence of infections [37] and mice deficient in leptin (*ob/ob*) and leptin receptor (*db/db*) are more susceptible to pneumonia [9,10], listeriosis [38] and amebic colitis [39]. Congruent with leptin's pro-inflammatory role, mice lacking leptin (*ob/ob* mice) have significantly less inflammation and colonic cytokine production as compared to wildtype control mice in models of inflammatory colitis (DSS- and TNBS-induced colitis) [40]. In the gastro-intestinal tract, leptin also increases mucin production by activating Protein Kinase C-, phosphatidylinositol 3-kinase- and MAP kinase-dependent pathways [41,42], enhances epithelial cell turnover [43] and maintains intestinal epithelial barrier integrity [44].

In humans, serum leptin levels are proportional to the amount of adipose tissue [45] and thus obesity is a state of hyper-leptinemia [45]. Interestingly, the high serum leptin levels in obese patients fails to control their body weight, a condition termed as “leptin resistance” [46]. While this phenomenon is clinically observed, due to the varied effects of leptin on multiple physiologic processes, the underlying molecular mechanisms of leptin resistance have not been defined. Specifically the affect of high leptin levels on immune cells in obese individuals and in turn response to infectious and inflammatory challenge is not known.

2.1. Leptin and infections

Alterations in leptin signaling pathway do play a role in influencing the risk and outcomes of infectious diseases. We have shown that a particular single nucleotide polymorphism (SNP rs1137101) located at amino acid position 223 in leptin receptor, encoding for arginine (223R) instead of glutamine (223Q) increases the risk of amebiasis in children in Bangladesh (OR = 3.91; $p = 0.007$) [47]. The homozygous 223R allele is also associated with increased susceptibility to amebic liver abscess in an independent cohort of adults [47]. Other studies have shown that homozygosity for 223R allele is linked to increased risk of death in cases of non-appendicular secondary peritonitis [48] and increased risk of developing chronic bronchitis [49]. While the functional consequences of *LEPR* Q223R polymorphism have not been completely elucidated, our studies have shown that the polymorphism affects downstream STAT3 signaling with the wild type 223Q allele having a more robust STAT3 signal after leptin stimulation as compared to the mutant 223R allele [50], without any affects on the binding kinetics of leptin to it's receptor [51]. Recently, we have shown that the odds of having *C. difficile* infection are increased in individuals homozygous for the 223R allele as compared to heterozygous or those homozygous for the 223Q allele (OR = 3.03; $p = 0.01$) [52].

Previous studies have investigated the role of leptin during *C. difficile* disease pathogenesis and showed an increase in both serum leptin levels and gut mucosal leptin receptor mRNA expression in mice after Toxin A challenge [53]. Mechanistically, both leptin- and leptin receptor-deficient (*ob/ob* or *db/db* respectively) mice were resistant to toxin A-induced ileal fluid secretion [53]. The role of leptin signaling during infection (rather than intoxication) with *C. difficile* had not been studied until our recent work, which demonstrated that leptin signaling leads to increased inflammation and clinical signs of disease acutely after *C. difficile* infection [52]. This heightened response to infection was associated with better control of pathogen burden during acute infection (Day 2, peak disease) as measured by levels of Toxin A/B and *C. difficile*-specific glutamate dehydrogenase (GDH) in the cecal contents [52]. Notably, mice with uncoupled leptin receptor-STAT3 signaling had diminished colonic inflammation, decreased colonic chemokine and immune cell recruitment, and less severe clinical signs of disease [52]. Thus leptin-signaling acting via STAT3 promotes an early inflammatory response after *C. difficile* infection that manifests as worse clinical disease, but also enhances bacterial clearance during acute infection. The role of leptin signaling later during the course of *C. difficile* disease has not yet been defined.

3. Possible mechanisms of leptin-mediated effects during *C. difficile* infection

The tempo, vigor and character of the inflammatory response to noxious challenge is an important determinant of immune homeostasis and disease course. Early (or delayed) inflammation or an over-exuberant inflammatory response can thus be critical in determining the balance between protection and disease. Leptin is