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Role of the leukocyte response in normal and immunocompromised host after *Clostridium difficile* infection

Edwin Vargas*, Senu Apewokin, Rajat Madan

Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati, 231 Albert Sabin Way, MSB 6109, ML 0560, Cincinnati, OH 45267, USA

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ABSTRACT

Clostridium difficile is the leading cause of healthcare-associated infections in the United States. Clinically, *C. difficile*-associated disease can present as asymptomatic colonization, self-limited diarrheal illness or severe colitis (that may result in death). This variability in disease course and outcomes suggests that host factors play an important role as key determinants of disease severity. Currently, there are several scoring indices to estimate severity of *C. difficile*-associated disease. Leukocytosis and renal failure are considered to be the most important predictors of *C. difficile* disease severity in hosts with a normal immune system. The degree of leukocytosis which is considered significant for severe disease and how it is scored vary amongst scoring indices. None of the scores have been prospectively validated, and while total WBC count is useful to estimate the magnitude of the host response in most patient populations, in immune-compromised patients like those receiving chemotherapy, solid organ transplant patients or hematopoietic stem cell transplants the WBC response can be variable or even absent making this marker of severity difficult to interpret. Other cellular subsets like neutrophils, eosinophils and lymphocytes provide important information about the host immune status and play an important role in the immune response against *C. difficile* infection. However, under the current scoring systems the role of these cellular subsets have been underestimated and only total white blood cell counts are taken into account. In this review we highlight the role of host leukocyte response to *C. difficile* challenge in the normal and immunocompromised host, and propose possible ways that would allow for a better representation of the different immune cell subsets (neutrophils, lymphocytes and eosinophils) in the current scoring indices.

Clostridium difficile is a gram positive spore forming bacterium that is the leading cause of healthcare-associated infections in the U.S (https://www.cdc.gov/hai/organisms/cdiff/Cdiff_clinicians.html). The principal mode of *C. difficile* transmission is fecal-oral. Host factors including the magnitude of immune response play an important role in disease pathogenesis [1]. The degree of disease severity can vary amongst different type of hosts, usually with increased severity in immunocompromised populations. Clinical severity score indices for *C. difficile* infection have emerged as tools to stratify patients into mild or severe forms of disease presentation. By using clinical risk factors included into the scoring index the clinician is able to predict (to some degree) disease prognosis and decide what type of treatment is most appropriate. Currently, there are several scoring indices to estimate severity of *C. difficile*-associated disease (CDAD), however, none of them has been validated in a prospective manner. Most of the scoring indices take into account certain clinical and laboratory variables including total white blood cell count (WBC), serum creatinine and albumin levels and radiographic findings like ascites, ileus, colitis, bowel wall thickening, pneumatosis coli, etc. Notably, the total white blood cell count is part of all available scoring systems. While this is useful to estimate the magnitude of the host response in most patient populations, in certain cases of immune-suppression (cancer chemotherapy, solid organ and hematopoietic stem cell transplant recipients), the WBC response can be inappropriately suppressed or even absent making this marker of severity difficult to interpret.

Here we present a review of the current literature about the role of WBCs (and different cellular sub-populations) in *C. difficile* disease pathogenesis and outcomes in the normal and

* Corresponding author. Division of Infectious Diseases, University of Cincinnati, 231 Albert Sabin Way, MSB 6109, ML 0560, Cincinnati, OH 45267, USA.
E-mail address: vargasen@ucmail.uc.edu (E. Vargas).

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immunocompromised host. We also propose possible ways that allow for a better representation of the different immune cell subsets into the current available scoring systems.

1. Neutrophils

Neutrophils are the first cells recruited to the colon in response to *C. difficile*, and the neutrophil response is believed to be a determinant of disease severity. Initial migration of neutrophils from the bone marrow into peripheral circulation and recruitment of neutrophils to the site of *C. difficile* infection is mediated by production of neutrophil growth and recruitment factors (for example G-CSF, GM-CSF, IL-17, leptin, etc.) from the inflamed tissue [2–5]. Neutrophils have multiple mechanisms of controlling bacterial infections: release of neutrophil-specific granule components, reactive oxygen species (ROS) production, neutrophil extracellular traps production and phagocytosis to name a few. In the presence of *C. difficile* infection, neutrophils can be activated by *C. difficile* toxins, through the formyl peptide receptor-1 (FPR-1) and generate ROS [6]. Neutrophils can also perform phagocytosis of complement and antibody coated *C. difficile* at least *in vitro* [7,8]. However, despite of neutrophil bactericidal response, toxigenic strains of *C. difficile* have evolved mechanisms to resist neutrophil actions, for example, glutamate dehydrogenase secretion from *C. difficile* confers resistance to phagocytosis and neutrophil-induced oxidative stress [9].(See Table 1)

Neutrophil-mediated inflammation can act as a double-edged sword and neutrophil actions themselves can lead to immune-mediated damage of host tissues. In animal models of *C. difficile*, ablation of neutrophil response can either have beneficial or deleterious effects [2,10]. In case of *C. difficile* Toxin A-induced intoxication, depleting neutrophils decreases edema and colonic disease at the microscopic levels [11]. However, in a mouse model of *C. difficile* infection, depletion of neutrophils while associated with decreased colonic inflammation was associated with higher mortality, likely due to inability to control translocation of commensal gut microbes [2]. Similar dichotomy is seen in patients with *C. difficile* colitis as well: while leukocytosis (albeit without discrimination of cellular components) has been associated to increased mortality, neutropenia has also been associated with an increased incidence and recurrence of *C. difficile* associated diarrhea [12–14]. Thus, a well-balanced and controlled neutrophil response is needed for best outcomes after *C. difficile* infection. Neutrophils are also known to set stage for eventual disease resolution [15], by clearance of bacteria and secretion of anti-inflammatory and pro-resolving intermediates. However, the role of neutrophil-mediated disease resolution after *C. difficile* infection has not been well studied in either animal models of in patient cohorts.

Interestingly, clinical factors like age, steroids and chemotherapy, which have been associated to *C. difficile* infection are also known to modify the normal neutrophil response. Thus, while most of the clinical studies and scoring indices focus on total WBC count, we think that further studies should be focused on studying neutrophils as disease modifying mediators. We postulate that the magnitude of the neutrophil response varies amongst hosts, and could be a good predictor of the *C. difficile* clinical outcomes. It is also important to consider how such clinical scoring indices should be adjusted for neutropenic populations. Current studies would suggest that neutropenia should also be considered a risk factor similar to leukocytosis.

2. Eosinophils

Eosinophils are granulocyte leukocytes that at homeostasis are present in the gut [16]. Eosinophils are involved in presentation of antigens through MHC II, expression of pattern recognition receptors like TLR2, NOD1, NOD 2, response to immunomodulatory mediators like IL-33, IL-25, TGF β and IL-17A and secretion of IL-10 and TGF β [16]. Eosinophils from the Lamina Propia in the intestinal mucosal surface have been shown to induce differentiation of regulatory T cells [17], as well as to be important for development and maintenance of mucosal IgA plasma cells. Thus, a protective and regulatory role for Lamina Propia eosinophils has been proposed [18]. Normal eosinophil counts in the blood vary from 50 to 350 cells/microliter (0–6% of total WBCs). In patients with *C. difficile*, a recent meta-analysis comparing Vancomycin vs Fidoxamicin for the treatment of CDAD, showed that low eosinophil counts in the blood was an independent predictor of persistent diarrhea and death in the first 12 days of therapy. The same effect was not observed later in the course of disease (days 13–40) [19]. In animal studies, Buonomo et al. demonstrated a protective role of tissue eosinophils in mice, and this effect was mediated by IL-25 secretion. In this study, restoration of IL-25 levels in a murine model of *C. difficile* infection led to reduced mortality, whilst eosinophil depletion resulted in loss of the protection mediated by IL-25 [20]. In another study by the same group, *C. difficile* Binary toxin CDT (*C. difficile* transferase) was able to induce inflammation in a murine model by binding to eosinophil Toll-like receptor 2 (TLR-2), resulting in activation of NFκB and suppression of eosinophil response via indirect induction of eosinophil apoptosis. This experiment suggests a protective role from eosinophils in *C. difficile* infection [21].

Eosinophils are known to play an important role in asthma inflammatory response as well as a protective role in helminth infections [22,23]. But so far, there is lack of evidence that suggests that they offer *in vivo* antibacterial activity against *C. difficile*. The exact mechanisms by which eosinophils provide protection against *C. difficile* are not clearly understood, and further study of

Table 1
Leukocyte cut-off values for the commonly used *C. difficile* severity score indices. Each severity index (left column) assigns different leukocyte values (middle column) to determine disease severity. The weight of leukocyte values into each scoring index is represented in points, which are the values in parenthesis. After adding up the points given to different clinical variables (additional clinical variables not represented on this table) the clinician is able to define severe disease (right column). Severe disease is associated with worse clinical outcomes. (SHEA) Society for Healthcare Epidemiology of America, (IDSA) Infectious Disease Society of America, (UPMC) University of Pennsylvania medical center.

Severity Index	White blood cell counts (cells/mm^3)	Score defining severe disease
UPMC [56]	<1500 or >20,000 (1 point)	≥2 points
Hines VA [57]	≥15,000 to < 30,000 (1 point) or ≥ 30,000 (2 points)	≥3 points
Beth Israel [58]	>20,000 (1 point)	>4 points
University of Illinois [59]	≥15,000 (1 point)	≥2 points or Pseudomembranous Colitis on endoscopy or treatment in intensive care unit.
SHEA-IDSA [43]	≥15,000	≥15,000 cells/mm^3 or a creatinine level greater than or equal to 1.5 times the premorbid level.