Agent-Based Network Modeling Study of Immune Responses in Progression of Ulcerative Colitis

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Ulcerative colitis, an inflammatory bowel disease, is a chronic inflammatory disorder that results in ulcers of the colon and rectum without known etiology. Ulcerative colitis causes a huge public health care burden particularly in developed countries. Many studies suggest that ulcerative colitis results from an abnormal immune response against components of commensal microbiota in genetically susceptible individuals. However, understanding of the disease mechanisms at cellular and molecular levels remains largely elusive. In this paper, a network model is developed based on our previous study and computer simulations are performed using an agent-based network modeling to elucidate the dynamics of immune response in ulcerative colitis progression. Our modeling study identifies several important positive feedback loops as a driving force for ulcerative colitis initiation and progression. The results demonstrate that although immune response in ulcerative colitis patients is dominated by anti-inflammatory/regulatory cells such as alternatively activated macrophages and type II natural killer T cells, proinflammatory cells including classically activated macrophages, T helper 1 and T helper 17 cells, and their secreted cytokines tumor necrosis factor-α, interleukin-12, interleukin-23, interleukin-17 and interferon-γ remain at certain levels (lower than those in Crohn’s disease, another inflammatory bowel disease). Long-term exposure to these proinflammatory components, causes mucosal tissue damage persistently, leading to ulcerative colitis. Our simulation results are qualitatively in agreement with clinical and laboratory measurements, offering novel insight into the disease mechanisms.

Key words: Network model, Agent-based method, Immune response, Ulcerative colitis

I. INTRODUCTION

Inflammatory bowel disease (IBD) includes two principal types: ulcerative colitis (UC) and Crohn’s disease (CD) [1]. Different from CD that can happen anywhere in the gastrointestinal tract, UC is a chronic disorder, which occurs in the colon and rectum with the primary symptoms of abdominal pain and diarrhea mixed with blood [2]. The etiology of IBD is unknown, current understanding of the pathogenesis suggests that a dysregulated immune response to intra-luminal antigens, which are of microbial origin (e.g., bacteria), leads to IBD including UC in a genetically susceptible host [1–3]. UC is characterized by chronic inflammation and epithelial injury but limited to the mucosa and submucosa with cryptitis and crypt abscesses [4, 5]. As the incidence of UC is generally increasing globally, there is significant morbidity and mortality associated with UC, which poses a major public health challenge worldwide particularly in developed countries [6]. Despite significant advances in understanding of the pathogenesis in recent years, therapeutic treatment, which is far from optimal, has made UC as well as CD a notorious area of unmet medical need [7]. Therefore, it is of great importance to understand the cellular and molecular mechanisms of immune responses in the progression of UC for development of effective therapies.

In the last few decades most studies of IBD immunopathogenesis have been concentrated on adaptive immunity [1, 8]. While CD has been designated to be a proinflammatory T helper (Th) 1- and/or Th17-type disease, UC has been characterized as a typical Th2 condition in which anti-inflammatory/regulatory cytokines, interleukin (IL)-13 and IL-5 rather than IL-4 predominate in the UC cytokine network [1]. However, recent clinical data have shown that proinflammatory immune cells such as Th17 and Th1, respectively, with their secreted cytokines IL-17 and interferon-γ (IFN-γ) were also increased to significant levels (but lower than those in CD patients), playing an important role in UC progression [1]. But it remains elusive how this seemingly controversial immune profile in UC patients is achieved. Recent advances have demonstrated that innate immunity has also an important role in UC [1, 8]. Indeed, an immune response in UC progression involves both innate and adaptive immunity regulated by an intrica-
te network that consists of multiple immune cell types, molecular mediators, and gut tissues. These elements are treated as the nodes of a network model in the discussion below. There exists a large body of literature regarding these individual network elements. However, little is known about combined interactions between these elements or the associated pathways [9]. In particular, UC progression is a dynamic and multistage process, studies on the temporal sequence of the immune response are lacking. Therefore, in spite of extensive research efforts, the underlying cellular and molecular mechanisms are not completely understood.

In this work, a network model based on our previous study [10] is expanded to describe the dynamics of immune response in the progression of UC. We aim to elucidate the detailed cellular and molecular mechanism of the disease development and address the issues mentioned above.

II. METHODS

A. Network Model

The UC-associated immune system is highly complex, providing a challenge to quantify the dynamics of immune response in UC progression. To reduce the complexity, a multi-scale network model is developed in this work by treating important cytokines, immune cells, and gut tissues as network nodes in a way similar to that in our previous study [10]. In this network model, two types of inputs are initiated from a node: a positive or an up-regulation input (denoted by “→”) represents that an increasing of the concentration of the tail node will result in an increasing of that of the head node or an up-regulation of the process when the input arrow ends at an edge between two nodes, and vice versa for a negative or a down-regulation (inhibition) input (denoted by “⊣”).

Although the exact pathogenesis of IBD (including UC) is not fully understood, it is generally accepted that genetic and environmental factors induce impaired epithelial barrier function (i.e., epithelial cell damage) that allows the translocation of commensal bacteria and microbial antigens from the gut lumen into the lamina propria, leading to immune cell activation and cytokine production [1, 9]. The innate immune cells such as macrophages and dendritic cells provide the first line of defense against any invading pathogens. Macrophages (M0) and dendritic cells (DC0) are located mostly in the intestinal lamina propria in close proximity to the epithelial monolayer. While resident macrophages and dendritic cells in the healthy intestine display an anergic and tolerogenic phenotype mediating tolerance to commensal bacteria (Bc) [11, 12], pro-inflammatory macrophages (M1) and dendritic cells (De) are activated in response to pathogenic bacteria (Bp) in IBD progression [1]. M1 and De cells produce molecular mediators including IL-1, IL6, IL-12, IL-23, and tumor necrosis factor-α (TNF-α), which can activate M1 and Th1 cells through binding TNF receptor 2 [13, 14], as well as reactive oxygen/nitrogen (ROS/RNS) to initiate inflammatory responses [13, 15]. While M1 macrophages elicit a protective immune response, they also can cause tissue damage (TD), for example, by releasing ROS/RNS leading to oxidative stress, proteases such as metalloproteases (MMPs) to ingest pathogens and apoptotic cells, and chemokines to recruit neutrophils into intestinal mucosa. The damaged tissue can also recruit monocytes (precursors of macrophages, M0) into the intestinal mucosa [10]. These M0 cells are further differentiated into M1. Therefore, a positive loop, M1→TD→M1 (loop 1) is formed. In particular, TNF-α is a major cytokine that drives the death of intestinal epithelial cells and paneth cells, resulting in TD through further impairment of the barrier function [1, 14]. Furthermore, TD also contributes to the TNF-α production [1]. Therefore, another positive feedback loop, TNF-α→TD→TNF-α (loop 2), is created.

TD is also an important early source of IL-4 production [9] that leads to alternatively activated macrophages (M2) and Th4, respectively [16]. M2 can release IL-10 and transforming growth factor, TGF-β, to down-regulate the inflammatory process. Moreover, damaged epithelial cells in TD can activate type II natural killer T cells (NKT) cells to secrete IL-13, which activates M2 [16] and also induces tissue damage further [17], resulting in a positive feedback loop, IL-13→TD→NKT→IL-13 (loop 3).

Dendritic cells (DCs) are specialized antigen-presenting cells that orchestrate innate and adaptive immune responses. DCs (DC0) can be activated to incite a proinflammatory response (De) or to induce immune tolerance (Dt) in different local environments [12]. Mature DCs migrate to mesenteric lymph nodes and present antigens to naïve lymphocytes [12]. Naïve, quiescent T cells (Th0) cannot enter the gut mucosa. Once activated by matured DC, they can move into the lamina propria and differentiate into effector T cells (predominantly Th1, Th2 and Th17 cells) and regulatory T cells T-regulatory (Treg) cells in their corresponding cytokine environments. For example, in the presence of IL-12 secreted by M1 (as well as De), naïve CD4+ T cells (Th0) differentiate into T helper 1 (Th1) cells [18]. As Th1 cells secrete IFN-γ to activate M1 [8, 18], a multi-node positive feedback loop, M1→IL12→Th1→IFN-γ→M1 (loop 4), is thus created. In contrast to Th1, Th2 is polarized from Th0 in the presence of IL-4. Th2 produces IL-13, IL-5 and IL-4, release of which further enhances the production of IL-10 and transforming growth factor-β (TGF-β) by M2. In the presence of TGF-β, Th0 cells differentiate into Treg, which secrete IL-10 [12]. TGF-β and IL-6 together induce Th17 differentiation, leading to the production of IL-17 (IL-17A) [19–21], which acts primarily on non-hematopoietic cells such as epithelial cells [22]. As Th17-type cytokines represented by IL-
FIG. 1 Schematic depiction of interactions between various nodes that represent immune cells, cytokines and intestinal tract tissues.

17 were found to mediate proinflammatory responses, they also can induce TD in which the activated epithelial cells secrete IL-6. Therefore a positive feedback loop, IL-6 → Th17 → IL-17 → TD → IL-6 (loop 5), is formed [23]. However, IL-17 signaling in intestinal epithelial cells was also found to promote expression of genes involved in tight junction formation [22]. Moreover, studies showed that IL-23 produced by M1 activates Th17 to secrete IL-17, which causes TD in intestinal mucosa [23]. As such, one more positive feedback loop, M1 → IL-23 → Th17 → IL-17 → TD → M1 (loop 6), is constituted. In addition, while IFN-γ can suppress the differentiation of Th17 cells [19], IL-6 can down-regulate the activation of Treg that secretes IL-10 to inhibit Th17 [24]. Consequently, a positive feedback loop, IL-6 → Treg → IL-10 → Th17 → IL-17 → TD → IL-6 (loop 7), is constructed.

The cytokines, immune cells, and TD discussed above are treated as the network nodes, whose interactions are then integrated into the network model shown in FIG. 1. In this network model, M1, De, Th1, and Th17 with their associated cytokines, TNF-α, IL-6, IL-12, IFN-γ, and IL-17 form multiple proinflammatory pathways, whereas M2, Th2, Treg and type II NKT with their related cytokines, IL-4, TGF-β, IL-10, and IL-13, form anti-inflammatory/regulatory pathways. These two types of pathways are interconnected with each other through several nodes representing molecular mediators such as IL-6, TGF-β, IL-10, and IL-13 (FIG. 1). These pathways eventually converge at the TD node that represents the tissue damage. Here, we focus on the immunologic aspect of UC and the TD node is highly coarse-grained, involving neutrophil-induced tissue damage, epithelial cell injury and extracellular matrix degradation etc. As TD is a major feature of UC, the dynamics of TD is used to measure UC progression in this work

B. Agent rules and model dynamics

In this work, an agent-based network modeling (ABNM) method is used to study dynamics of the network discussed above. In this agent-based method (ABM) [25–27], TD and cytokines are treated as patch variables whereas the immune cells are treated as agents [27]. The TD patches have a local parameter denoted as tissue life associated with tissue damage. This parameter is set between 0 and 100 in which 0 represents a completely destroyed patch where the value of TD=100%, and 100 means full health (TD=0%). Agents represent individual entities that can move from patch to patch [27]. The agents in the present model represent multiple types of cells. In the following discussion, 11 agent variables are used for Bc and Bp, and the different immune cell types. Netlogo5.3 (Center for Connected Learning and Computer Based Modeling, Northwestern University) is applied to perform the ABNM simulations.

Model dynamics starts with an entry of Bc and Bp bacteria into intestinal lamina propria by overcoming epithelial barrier, triggering a cascade of immune responses. The ABNM simulations are initiated by ran-
randomly placing a certain number of Bc and Bp in lamina propria. Here, 5 units of Bc and 25 units of Bp are used for the first time. The same amount of Bc and Bp can be placed at every later time point for continual simulations of the immune response. ABNM dynamics is governed by a set of rules that describe the interactions between the network nodes (agents and patches) shown in FIG. 1. The associated rules are given in Table S1 in supplementary materials. The model environment comprises 2500 patches on a square arranged in a grid (50 patches × 50 patches). The model is initiated with 10 units of M1, M2, De, and Dt cells, 13 units of Th1 and Th17, and 40 units of Th2, NKT and Treg cells which are randomly distributed in this environment.

III. RESULTS AND DISCUSSION

A. ABNM dynamic simulations

The time courses of the changes in the immune cells, cytokines in response to Bc and Bp are presented in FIG. 2–5, respectively. Seen in FIG. 2, the population of M1 along with M1-produced TNF-α, IL-6 and IL-12 (FIG. 3(b)) ascends relatively quickly then goes down until the 180th day as M2, which secretes IL-10 (FIG. 4(b)) to down-regulate M1, goes up, showing an acute phase of inflammatory response in UC progression. During this phase, type II NKT (FIG. 5) is activated by Bp, leading to a large increase of IL-13 (FIG. 4(b)). As these network components work together, TD (FIG. 6) thus rises up but rather slowly. After 180 days of exposure to invading pathogens, the M2 population is raised quickly, and then gradually goes to a steady state with a level much higher than that of M1, leading to high levels of IL-10 and TGF-β. Although M2 predominates over M1 in the later immune response, M1 still remains at a certain level, so do M1-produced TNF-α, IL-6, IL-12 and IL-23 (see FIG. 3). As such, Th1 and Th17 (FIG. 5) along with IFN-γ and IL-17 also remain at certain levels. Consequently, M1 along with IL-13, IL-17, and IFN-γ together drives TD to a steady state (FIG. 6). Our results in qualitative agreements

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with experiments demonstrates that M2 dominates M1 in UC progression. However, M1 macrophages and the M1-secreted proinflammatory cytokines, TNF-α, IL-12, IL-23, IL-6 still remain at certain levels mainly due to the persistent invasions of bacterial antigens (Bp) into the luminal propia. As such, Th1 and Th17 are persistently activated to produce proinflammatory cytokines, e.g., IL-17 and IFN-γ. Consequently, these proinflammatory components along with type II NKT-producing IL-13 consistently cause TD, which is counteracted by a wound-healing mechanism, leading to a steady UC state.

**B. Knockout simulations**

In recent years, targeting cytokines for UC therapies have become an important therapeutic strategy for UC treatment [6]. To identify important cytokines in UC progression, *in silico* knockout simulations for TNF-α, IL-23, IL-12, IL-13, IL-17, and IFN-γ were performed in this study. Knockout calculations are executed via deletions of these molecular mediators, respectively, and the associated interactions with the other nodes of the
network (FIG. 1) in the following discussion and the results are represented in FIG. 7.

As seen in FIG. 7, TNF-α knockout simulations show that there is significant reduction in TD. This result is consistent with clinical data in which anti-TNF-α drugs have significantly improved treatment of UC [28, 29]. Interestingly, while IL-12 deletion has no obvious effect on TD production, IL-23 knockout significantly reduces TD from ~50% to ~20%, qualitatively consistent with mice experiments in which IL-23 rather than IL-12 is required for IL-10 knockout enterocolitis and T-cell transfer-induced UC [22]. Our modeling study in this work also provides a rationale for anti-IL-23/12 monoclonal antibodies such as Ustekinumab for effective UC treatment. Our simulations demonstrate that IFN-γ knockout has little effect on TD reduction, which is in line with that anti-IFN-γ agents (e.g., Fontolizumab) failed to show efficacy in clinic [30]. From our in silico simulations, deletion of IL-17 has a significant effect on TD dynamics similar to that of IL-23, showing that IL-23-driven IL-17 can be a promising target for the immuno-suppressive treatment of UC. Intriguingly, IL-13 knockout also results in a significant TD reduction, in line with clinical and mice experiments [31–33]. However, two recent clinical trials failed, crushing the enthusiasm for anti-IL-13 treatment in UC [34]. As mice experiments showed that the UC outcome is determined by IL-13-utilized signaling pathway, a direct blockade of IL-13 may be still a potential therapeutic strategy for a subset of UC patients who have elevated IL-13 production in tissue [33].

IV. CONCLUSION

As discussed above, UC is chronic inflammatory disease caused by an abnormal immune response against persistent invasions of commensal bacteria in genetically susceptible subjects. Despite significant advances in understanding of UC immuno-pathogenesis, the immunological profile in UC patients still remains controversial. UC has been classically characterized as an atypical Th-2 type disease in which anti-inflammatory/regulatory cytokines such as IL-13 and IL-5 are predominantly enhanced at high levels, but with low expression of IL-4 [1, 31, 32]. However, experiments showed that pro-inflammatory immune cells, M1, Th17 and Th1 with the associated cytokines TNF-α, IL-23, IL-17 and IFN-γ were also enhanced to certain levels (but lower than those in CD patients), playing an important role in UC progression. Indeed, the crucial roles of these pro-inflammatory components have been supported by the efficacies of Infliximab and Ustekinumab, an anti-TNF-α and IL-23 monoclonal human antibody in the treatments of UC patients [22, 29]. However, how this seemingly controversial immune profile in UC patients is achieved remains largely illusive. In other words, the precise mechanism of the UC immunopathogenesis is not completely clear.

To address the above issue, a network model is developed in this work based on our previous study but in different context [10]. An agent-based network modeling method (ABNM) is then applied for computer simulations of the dynamics of immune response in UC progression. Our modeling study demonstrates that the immune response in UC progression is mainly anti-inflammatory/regulatory, but pro-inflammatory cells and their associated molecular mediators still remain at certain levels (generally lower than those with CD). Specifically, from our simulations anti-inflammatory/regulatory cells, M2 and type II NKT, dominate proinflammatory M1 cells. However, M1 and the M1-producing cytokines, TNF-α, IL-12, IL-23, and IL-6 are increased and remain at levels high enough to activate Th1 and TH17 cells to secrete IL-23-driven IL-17 and IFN-γ at significant levels, respectively. Similar to those demonstrated in our previous study [10], the knockout simulations via deletions of key components in the positive feedback loops show that TNF-α→TD→TNF-α (loop 2), M1→IL-23→Th17→IL-17→TD→M1 (loop 6) are particularly of great importance for the enhanced pro-inflammatory signals at certain levels in UC progression. Long-term exposure to these pro-inflammatory components causes persistently the damage of mucosal tissues, leading to UC. Our calculations are in good agreement with clinical and laboratory experiments (see Table S2 in supplementary materials), providing novel insight into the cellular and molecular mechanisms of UC progression.

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Supplementary materials: Table S1 lists agent-based rules.