Review Article

Intestinal macrophage, “a double-edged sword” for homeostasis and inflammation in the gut

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Gut is always exposed to enteric bacteria and food antigens, but maintains its homeostasis without the development of acute or chronic inflammation in normal situations. In contrast, abnormal innate immunity to enteric flora may develop intestinal inflammation in inflammatory bowel disease (IBD). This paper reviewed recent studies regarding intestinal macrophage (Mφ) function in gut. Intestinal Mφ contributes to the elimination of pathogens and gut immune homeostasis. However, it also causes chronic inflammation by producing proinflammatory cytokines. Thus, intestinal Mφ is “a double-edged sword” for individuals. Dysregulation of this mucosal immune system may induce abnormal responses to commensal and food antigen resulting in development of IBD.

Rec./Acc.9/10/2010, pp412-418

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Key words autophagy, Crohn’s disease, flora, IFNγ, IL-23, inflammatory bowel disease, innate immunity, intestinal macrophages, TNFα

Introduction

Inflammatory bowel disease (IBD) is classified into two typical phenotypes, namely ulcerative colitis and Crohn’s disease. Although the precise etiologies of IBD remain obscure, several reports have indicated that dysfunctions of the mucosal immune system play important roles in its pathogenesis9. Especially, the innate immunity has been highlighted in the pathogenesis of IBD. The gastrointestinal tract is always exposed to a variety of antigens including enteric bacteria and foods; however, the homeostasis in the gut is maintained without the development of intestinal inflammation by suppressing excessive immune responses to these foreign antigens in the normal state. Unbalance of this homeostatic regulation of innate immunity may cause abnormal inflammatory responses to the enteric antigens.

Macrophage (Mφ), the major population of tissue-resident mononuclear phagocytes, plays key roles in recognition of bacteria, elimination of pathogens, and regulation of the innate and adaptive immunity. Mφs are activated by microbial pathogen associated molecular patterns (PAMPs) through pattern-recognition receptors such as toll like receptors (TLRs)2,3, and produce the type 1 cytokines such as IL-12 and IL-23, which are critical cytokines for the induction of Th1 immune response4,5. Besides the these classical anti-bacterial immune roles, it has become evident in recent years that Mφs also play important
roles in maintaining tissue homeostasis, such as dampening inflammation, scavenging debris, angiogenesis, and wound repair.\(^4\)

Since the intestinal mucosa is always exposed to luminal antigens, activation of innate immune cells must be precisely regulated to prevent excess immune response to antigens. In this regard, intestinal M\(\phi\)s exhibits unique anti-inflammatory phenotypes compared with other tissue phagocytic cells. On the other hands, dysfunction of intestinal M\(\phi\)s causes abnormal immune response to commensal bacteria and leads to development of IBD.

Here, we review intestinal M\(\phi\)s as “a double-edged sword” in gut homeostasis and pathogenesis of IBD.

**Classification of M\(\phi\) subsets**

In general, M\(\phi\)s are the major population of tissue-resident mononuclear phagocytes, and play key roles in bacterial recognition and elimination, as well as in the polarization of the innate and adaptive immunity. Besides these classical antibacterial roles, M\(\phi\)s also play important roles in the maintenance of tissue homeostasis, for example, inflammation dampening via the production of anti-inflammatory cytokines such as IL-10 and TGF-\(\beta\)\(^4\) (Fig. 1). Recent studies have shown that M1 and M2 M\(\phi\)s are two functionally distinct subsets in terms of response to microorganisms and production of immune mediators. M1 M\(\phi\)s is characterized by its capacity to produce pro-inflammatory cytokines such as TNF-\(\alpha\), IL-12, IL-23, while M2 M\(\phi\)s is characterized as its anti-inflammatory properties such as production of IL-10.\(^9\)

**Intestinal M\(\phi\) contributes to the immune homeostasis in the gut**

It is well known that M\(\phi\)s and DCs play essential roles in host innate immunity toward foreign pathogens to prevent infectious disease. Recent studies have demonstrated that intestinal M\(\phi\)s have a unique regulatory potential and contribute to the immune homeostasis in the gut. Murine intestinal M\(\phi\)s produces immunosuppressive cytokine, IL-10, but not IL-12 or IL-23 in response to enteric bacteria antigens\(^9,10\).

Intestinal M\(\phi\) phenotype has similar phenotypes to *in vitro* monocyte-derived M\(\phi\) induced by M-CSF in terms of production of high IL-10, but no IL-12 or IL-23.\(^11,12\) Indeed, abundant M-CSF mRNA transcripts can be detected in the intestine of the human as well as mouse\(^13,15\). Consistent with these observations, the number of intestinal M\(\phi\)s in op/op mice, which lack M-CSF signaling, was significantly decreased\(^13,14\). Based on the anti-inflammatory properties of the intestinal M\(\phi\), they are considered to belong to M2 subset.

A previous study demonstrated that monocytes in mice can be classified into two distinct subsets, based on expression of CCR2, CD62L, Ly6C and CX3CR1; CCR2+CD62L+CX3CR1mid and CCR2-CD62L+CX3CR1hi.\(^6,7\) The CCR2+CD62L+CX3CR1mid monocyte subset can migrate towards inflammatory lesions in response to locally produced monocyte chemoattractant protein-1 (MCP-1). Therefore, this subset has been called “inflammatory monocytes”, which infiltrate inflammatory sites. However little is known about their functional roles in the regulation of inflammation. These infiltrated inflammatory monocytes are also involved in wound repair.

We reported that MCP-1 spontaneously produced by epithelial cells and intestinal M\(\phi\)s recruited by MCP-1 play an important role in the immune homeostasis in the gut. We found that lamina propria (LP) M\(\phi\)s could be classified into two subsets, LPM\(\phi\)1 and 2, according to the fluorescence intensity of side-scattered plots on flow cytometric analysis. The LPM\(\phi\)1 subset, which expresses CCR2 (MCP-1 receptor), was diminished in MCP-1\(^+\) mouse LP, even in the normal state. Intestinal M\(\phi\)s in MCP-1\(^+\) mice produced a lesser amount of IL-10 compared with that in wild type mice. Analysis of cytokine production of LPM\(\phi\)1 and 2 subsets revealed that LPM\(\phi\)2 produced a larger amount of IL-10 than LPM\(\phi\)1 did (Fig. 2). Furthermore, MCP-1\(^+\) mice showed high susceptibility to dextran sodium sulfate-induced colitis\(^18\). These data suggest that LPM\(\phi\)2 is an intestinal M\(\phi\) subset that is recruited by MCP-1, and may contribute to the gut homeostasis by producing IL-10 in both normal and inflammatory conditions.

It has recently become evident that intestinal M\(\phi\)s and dendritic cells (DCs) contribute to the polarization of the acquired immunity. CD11b+F4/80+CD11c- intestinal M\(\phi\)s, which produce IL-10, induce Foxp3+ T regulatory (Treg) cells, while LPDCs promote Th17 immunity. Induction of Foxp3+ Treg by intestinal M\(\phi\)s is independent on IL-10, retinoic acid and TGF-\(\beta\).\(^19\)

Although intensive research on intestinal innate immune cells has been performed in murine models, data on human intestinal M\(\phi\)s are limited. Smythies et al. reported that human intestinal M\(\phi\)s are hyporesponsive in their cytokine production in response to bacterial stimuli, but maintained their abilities to phagocytose and digest bacteria\(^10\). Schenk M, et. al reported that triggering receptor expressed on myeloid cells-1 (TREM-1) amplifies the inflammatory response by augmenting the secretion of pro-inflammatory cytokines from M\(\phi\)s. However, normal intestinal M\(\phi\)s generally lack TREM-1 expression\(^21\).

Collectively, intestinal M\(\phi\)s exhibits unique immunoregulatory
phenotypes and contributes to the gut homeostasis by eliminating pathogens, dumping debris, and helping tissue repair without causing excess immune response.

Dysregulation of intestinal $M\Phi$ causes intestinal inflammation

The immune homeostasis in the gut is disrupted when the intestinal $M\Phi$ function is dysregulated, resulting in chronic intestinal inflammation. For example, IL-10 deficient (IL-10$^{-/-}$) mice develop spontaneous chronic colitis and widely used as an animal colitis model for human IBD$^{20}$. IL-10$^{-/-}$ mice show Th1 polarized immunity in response to the intestinal microbiota evidenced by the observation that IL-10$^{-/-}$ mice do not develop intestinal inflammation in germ-free conditions$^{20}$. These facts suggest that enteric bacteria play an essential role in the onset and development of colitis in IL-10$^{-/-}$ mice, which may also be the case in human IBD. APCs, such as $M\Phi$s and DCs, mediate the chronic intestinal inflammation observed in IL-10$^{-/-}$ mice. Recent studies demonstrated that APCs from IL-10$^{-/-}$ mice were potent activator of Th1 responses$^{20}$, and importantly, depletion of $M\Phi$ prevented the chronic colitis in IL-10$^{-/-}$ mice$^{23}$. These data suggest that $M\Phi$s and DCs play a key role in the pathogenesis of colitis in IL-10$^{-/-}$ mice.

We previously demonstrated that in vivo LPM$\Phi$s from IL-10$^{-/-}$ mice showed paradoxical overproduction of IL-12p70 upon bacterial stimuli. These abnormal responses of intestinal M$\Phi$ subsets in IL-10$^{-/-}$ mice to enteric bacteria may contribute to the Th1 bias and the development of intestinal inflammation. To study this in vivo LPM$\Phi$ phenomenon, we established an in-vitro system. Wild type mice bone-marrow derived M$\Phi$s (BMMs) differentiated under the influence of M-CSF do not produce IL-12 p70 or IL-23 in response to bacterial stimuli, while BMMs from IL-10$^{-/-}$ mice produce robust IL-12 p70 and IL-23, which mirrors the properties of LPM$\Phi$s from WT and IL-10$^{-/-}$ mice. Importantly, IL-12p70 overproduction from bacteria stimulated IL-10 deficient M-CSF induced M$\Phi$s was significantly reduced by supplemental IL-10 during differentiation process. These results indicated that endogenous IL-10, which was induced in process M$\Phi$ differentiation, functionally regulated M$\Phi$ to anti-inflammatory, especially IL-12 hypoproduced, phenotype$^{10}$. The pathological characteristics of human Crohn’s disease represent “granuloma” formation. Mizoguchi et al. identified that F4/80-positive immature CD11c+ DCs produce IL-23 and contribute to granuloma formation in a murine colitis model$^{10}$.

In human studies, so many studies have demonstrated the participation of intestinal M$\Phi$ in IBD pathogenesis. TREM-1 expression on intestinal M$\Phi$s was upregulated in a murine experimental colitis model and patients with IBD$^{21}$. We reported that LPM$\Phi$s produce large amount of IL-18 and promote Th1 immune response in Crohn’s disease$^{27}$.

Thus, functional alteration of intestinal M$\Phi$ is a key in the pathogenesis of human Crohn’s disease.
Unique CD14+ intestinal Mφ plays a central role in the pathogenesis of Crohn’s disease

We identified a unique Mφ subset in human intestine (Fig.3). This subset expressed both Mφ (CD14, CD33, CD68) and DC markers (CD205, CD209) and produced larger amounts of proinflammatory cytokines, such as IL-23, TNF-α, and IL-6, than typical intestinal resident Mφs (CD14-CD33+ Mφs). In patients with Crohn disease (CD), the number of these CD14+ macrophages was significantly increased compared with normal control subjects. In addition to increased numbers of cells, these cells also produced larger amounts of IL-23 and TNF-α (Fig.4). In addition, the CD14+ Mφs contributed to IFN-γ production by lamina propria mononuclear cells (LPMCs) dependent on IL-23 and TNF-α. IFN-γ triggered further abnormal Mφ differentiation with an IL-23-hyperproducing phenotype. Furthermore, TLR1/2/4, a member of the TNF superfamily identified as a Crohn’s disease susceptible gene was produced by CD14+ intestinal Mφs. TLR1A and IL-23 synergistically promoted the production of IFN-γ and IL-17 by LPMC. Not only T cells, but also mucosal NK cells have the potential to produce IFN-γ. In deed, we previously indentified that number of mucosal NK cells is increased in LP of Crohn’s disease. Collectively, IL-23/ IFN-γ-positive feedback loop induced by abnormal intestinal Mφs is involved in the pathogenesis of chronic intestinal inflammation in patients with Crohn’s disease.

Consistent with the expression of both Mφ and DC markers, LP CD14+ Mφ has antigen presenting function as well as conventional DCs. This unique Mφ subset induced proliferation.
and differentiation of naïve CD4+ T cells. Moreover, LP CD14+ Mφ subset strongly evoked differentiation of Th1 and Th17 cells.[34]

Thus, LP CD14+ Mφ that we identified may act a key player in both local immunity and promotion of acquired immunity in the pathogenesis of Crohn’s disease (Fig. 5).

Autophagy and Crohn’s disease

Recent advances through the genome-wide scanning approach have involved the identification of novel Crohn’s disease susceptibility genes, namely autophagy-related gene 16-like 1 (ATG16L1) and IRGM, which are associated with “autophagy”.[35-38]. Autophagy is the mechanism for maintaining cellular homeostasis. Autophagy means “to eat oneself” or “self-cannibalization”. Apoptosis is a cell death pathway, while autophagy is involved in recycling cellular organelles for cell survival. Autophagy is also considered to be important for host defense against intracellular microorganisms. The association of autophagy-associated genes with Crohn’s disease strongly supports the hypothesis that abnormal innate immune responses to intracellular pathogens by Mφs or DCs is involved in the pathogenesis of Crohn’s disease.

Clinical implication of intestinal Mφs in IBD therapeutics

Recently, development of biologics such as anti-TNFα mAb, gives us the splendid benefit in the treatment of IBD. Although precise mechanisms of anti-TNFα mAb remain unclear, the efficacy of inhibition of TNFα signaling pathway is widely accepted as being beyond doubt in several chronic inflammatory disorders such as IBD and rheumatoid arthritis. Following in success of anti-TNFα mAb, anti-IL-12/23 p40 mAb is under the clinical trial in IBD. Thus, intestinal Mφs or cytokines produced by them are now highlighted as a molecular target for IBD treatment.

Abbreviations used in this paper

DC, dendritic cell; GM-CSF, granulocyte macrophage colony stimulating factor; IBD, inflammatory bowel disease; MCP-1, monocyte chemotactic protein-1; M-CSF, macrophage colony-stimulating factor; Mφ, macrophage; LPMC, lamina propria mononuclear cells; Treg, T regulatory; TREM-1, triggering receptor expressed on myeloid cells-1

Acknowledgements

This study was supported in part by Grants-in-Aid from the Japanese Ministry of Education, Culture and Science, the Japanese Ministry of Health, Labor and Welfare, Keio University and the Keio Medical Foundation, Tokyo, Japan.

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