Immunological Abnormalities in the Pathogenesis of Inflammatory Bowel Disease

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Crohn’s disease and ulcerative colitis represent two distinct forms of inflammatory bowel diseases (IBD). In this paper, we discuss how immunological mechanisms contribute to the pathogenesis of IBD. Intestinal homeostasis is sustained by various kinds of cells, such as epithelial cells, lymphocytes, antigen presenting cells, and other innate immune cells. We pay special attention to intestinal CD14+ macrophages. Intestinal macrophages play a central role in the regulation of immune responses against commensal bacteria. In the physiological condition, intestinal macrophages lack the expression of innate-immune receptor CD14 and do not produce proinflammatory cytokines. We identified a unique macrophage subset of IBD in the human intestine, which expressed both macrophage (CD14, CD33, CD68) and dendritic cell (DC) markers (CD205, CD209) and produced larger amounts of proinflammatory cytokines, such as interleukin (IL)-23 and tumor necrosis factor (TNF)-α. In addition, the CD14+ macrophages contributed to interferon (IFN)-γ production rather than IL-17 production by lamina propria mononuclear cells dependent on IL-23. We discuss herein this IL-23/IFN-γ-positive feedback loop in IBD patients. We also discuss IFN-γ and IL-17 production from mucosal T cells and natural killer (NK) cells. Here, we show our recent findings about the plasticity of T helper cells in colitis. Th 17 cells express T-bet, and finally lose the expression of retinoic acid-related orphan receptor (ROR)γt, the master regulator of Th 17 cells, and are differentiated ‘alternative Th 1 cells.’ In addition to Th 1 cells, mucosal NK cells are also important sources of IFN-γ. Some of our ideas may be provocative, but we hope this review paper will provide new and firm understanding of the pathogenesis of IBD. (Intest Res 2012;10:317-323)

Key Words: Macrophage; TNFα; IL-23; Mucosal NK cell; Th17

INTRODUCTION

IBD is classified to 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 pathogenesis. The gastrointestinal tract is continuously exposed to a variety of antigens including enteric bacteria and foods. However, homeostasis of the gut is maintained in the normal state without the development of intestinal inflammation, by suppressing excessive immune responses to foreign antigens. In both innate and acquired immunity, the disruption of regulatory mechanisms may lead to abnormal immune responses to enteric antigens and cause chronic intestinal inflammation. Here, we review the mechanistic aspects of IBD, especially focusing on intestinal macrophages, mucosal natural killer (NK) cells and pathogenic memory T cells.

INTESTINAL MACROPHAGES IN HOMEOSTASIS AND INFLAMMATION

1. Contribution of Intestinal Macrophages to Gut Homeostasis

As the intestinal mucosa is continuously exposed to numerous commensal bacteria, it is thought that the gut may possess innate and acquired immune system regulatory mechanisms to prevent excessive inflammatory responses against commensal bacteria. Macrophages are the major population of tissue-resident mononuclear phagocytes, and
play a key role in bacterial recognition and elimination, as well as in the polarization of innate and adaptive immunity. Besides these classical antibacterial immune roles, it has recently become evident that macrophages are also important for the maintenance of homeostasis, for example, inflammation dampening via the production of anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β, debris scavenging, angiogenesis, and wound repair. Recent studies have shown that M1 and M2 macrophages are functionally polarized in response to microorganisms and host mediators. M1 macrophages are characterized by the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-12, and IL-23, while M2 macrophages are characterized as IL-10 producing macrophages. Interestingly, it was previously reported that intestinal macrophages have immune-regulatory functions. In contrast to splenic macrophages, intestinal macrophages do not express innate response receptors, and although these cells retain their phagocytic and bactericidal functions, they do not produce pro-inflammatory cytokines in response to several inflammatory stimuli, including microbial components. Importantly, we and other groups have revealed that murine intestinal macrophages produce the anti-inflammatory cytokine, IL-10 (M2 macrophage) and contribute to maintain homeostasis of the intestinal immune system. Hirotani et al. demonstrated that wild type colonic lamina propria macrophages (LP-MΦs), were different from splenic macrophages, as they produced higher amounts of IL-10 in response to pathogen associated molecular patterns. Kamada et al. also demonstrated that in vitro macrophage colony-stimulating factor (M-CSF) differentiated macrophages isolated from bone marrow CD11b+ monocytes (M-MΦs) and intestinal CD11b+ macrophages produced abundant IL-10 in response to whole bacteria stimulation. In addition, the number of intestinal macrophages in M-CSF-deficient op/op mice was significantly decreased. Collectively, wild type mice intestinal macrophages have an IL-10 producing phenotype as well as in vitro differentiated M-MΦs. Thus, these results suggest that intestinal macrophages in wild type mice are of the IL-10 producing M2 type and may contribute to homeostasis maintenance. Uniquely, Takada et al. analyzed CD11b+ LP-MΦs from mice and demonstrated they could be divided to two sub-populations (LP-MΦ1 and LP-MΦ2) by flow cytometry analysis. LP-MΦ2 expressed CCR2 and produced large amounts of IL-10. Interestingly, MCP-1−/− mice contained fewer LP-MΦ2 cells resulting in the exacerbation of dextran sodium sulfate (DSS) induced colitis. Thus, LP-MΦs contribute to the maintenance of gut immune homeostasis by producing IL-10. LP-MΦs may have antigen presenting functions and induce the differentiation of FoxP3+ T regulatory cells that are dependent on IL-10 and retinoic acid. Thus, recent studies have suggested that macrophages located in the intestinal mucosa play important roles in the maintenance of intestinal homeostasis by protecting the host from foreign pathogens and negatively regulating excess immune responses to commensals.

2. Role of Intestinal Macrophages in the Pathogenesis of Crohn’s Disease

Immune homeostasis in the gut is disrupted when intestinal macrophage function is dysregulated, resulting in chronic intestinal inflammation. IL-10 deficient (IL-10−/−) mice develop spontaneous chronic colitis and are widely used as an animal colitis model for human IBD. IL-10−/− mice develop 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. This suggests 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. Recent studies demonstrated that antigen presenting cells, such as macrophages and dendritic cells (DCs), from IL-10−/− mice were potent activators of Th1 responses, and importantly, depletion of macrophages prevented chronic colitis in IL-10−/− mice. These data suggest that macrophages and DCs play a key role in the pathogenesis of colitis in IL-10−/− mice. We previously demonstrated that in vitro LP-MΦs from IL-10−/− mice showed a paradoxical overproduction of IL-12p70 upon bacterial stimuli. These abnormal responses of intestinal macrophage subsets to enteric bacteria in IL-10−/− mice may contribute to a Th1 cytokine bias and the development of intestinal inflammation. Bone marrow derived macrophages (BM-MΦs) from wild type mice differentiated in vitro with M-CSF do not produce IL-12p70 or IL-23 in response to bacterial stimuli, while BM-MΦs from IL-10−/− mice produce robust IL-12p70 and IL-23, which mirrors the properties of LP-MΦs from WT and IL-10−/− mice. Importantly, IL-12p70 overproduction from bacteria-stimulated IL-10−/− M-CSF-induced macrophages was significantly reduced by exogenous IL-10 during differentiation. These results indicated that endogenous IL-10 inhibited IL-12p70 production and functionally regulated macrophages towards an anti-inflammatory phenotype. Interestingly, whole bacteria are potent inducers of macrophage IL-12p70 production compared with cell surface components such as lipopolysaccharide (LPS) in this model. Whole bacteria stimulation strongly induced signal transducers and activator of transcription (STAT)-1 activation. Significant repression of IL-12p70 production was achieved by inhibition of phagocytosis. These observations suggested that intracellular pathogen recognition and signaling are involved in the induction of IL-12p70 in IL-10−/− macrophages. Granuloma formation is a pathological characteristic of human Crohn’s disease. Mizoguchi et al. demonstrated that F4/80-positive immature CD11c+ DCs produce IL-23 and contribute to granuloma formation in a murine colitis model. Many studies have demonstrated the pathogenic contribution of intestinal macrophages in human Crohn’s disease.
The contribution of intestinal macrophages that express triggering receptor expressed on myeloid cells 1 (TREM-1) to the pathogenesis in a murine experimental colitis model and patients with IBD has been reported. We reported that LP-Mφs produce large amounts of IL-18 and promote Th1 immune responses in Crohn’s disease. Recently, we identified the infiltration of unique CD14+ intestinal macrophages (CD14+Mφs) in the mucosa during Crohn’s disease. This subset expressed both macrophage (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 macrophages. In patients with Crohn’s disease, the number of CD14+Mφs 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 in response to whole bacteria stimulation. Th1 immune responses predominate in Crohn’s disease, and CD4+ T cells in the LP of Crohn’s disease expressed T-bet and produced large amounts of interferon (IFN)-γ. Macrophage-derived IL-23 strongly enhanced IFN-γ production by lamina propria mononuclear cells (LPMCs) in Crohn’s disease. Thus, macrophage-derived IL-23 is a key cytokine for the predominance of Th1 responses in Crohn’s disease.

CD14+Mφs also have antigen presenting functions and can stimulate the differentiation and proliferation of naïve CD4+ T cells obtained from peripheral blood. Importantly, although in vitro differentiated DCs cannot induce the differentiation of peripheral blood naïve CD4+ T cells to Th17 cells, lamina propria CD14+Mφs from Crohn’s disease patients can strongly induce T cell differentiation to both Th1 and Th17 cells by whole bacteria stimulation. The differentiation of Th17 cells is dependent upon IL-1β and IL-6, but not IL-23, produced by CD14+Mφs. These findings are consistent with previous observations that showed the difference in requirement of cytokines to promote the polarization of Th17 cells between humans and mice. TL1A/TNFSF15, a member of the TNF superfamily was identified as a susceptibility gene between humans and mice. CD14+ macrophages in Crohn’s disease express membrane binding of TL1A/TNFSF15 that acts synergistically with IL-23 to promote the production of IFN-γ and IL-17 by LPMCs. Both T cells and mucosal NK cells in Crohn’s disease have the potential to produce IFN-γ. Thus, CD14+Mφs are central in the promotion of an inflammatory cytokine network at mucosal sites and are involved in the pathogenesis of Crohn’s disease (Fig. 1).

3. Conditioning and Control of Differentiation of Innate Immune Cells

As well as T cells, innate immune cells such as macrophages and DCs are a therapeutic target in IBD. Especially in Crohn’s disease, the conditioning or regulation of differentiation of innate immune cells to a regulatory or a proinflammatory cytokine hypo-producing phenotype could be a therapeutic target. IL-12 and IL-23 are key cytokines that promote Th1 and Th17 immune response. Therefore, suppression of production of these cytokines is a promising therapy for Crohn’s disease as well as molecular targeting therapy by monoclonal antibodies. Tetomilast (OPC-6535), originally developed to inhibit superoxide production, suppressed the infiltration of unique CD14+ intestinal macrophages (CD14+Mφs) in the mucosa during Crohn’s disease.
TNF-α and IL-12 but not IL-10 production from LPS-stimulated human monocytes and ameliorated chronic colitis in IL-10−/− mice. Histidine inhibited LPS-induced TNF-α and IL-6 production by mouse macrophages and dietary histidine ameliorated colitis in a IL-10−/− mouse transfer colitis model. Interestingly, amino acid profiling revealed decreased levels of plasma histidine concentration in the patients with active Crohn's disease.

We have demonstrated that several molecules can affect the differentiation of monocytes to DCs. Am80, a synthetic retinoic acid receptor (RAR) agonist, promotes the differentiation of monocytes to an IL-12p70 hypo-producing DC phenotype with a reduced Th1 polarizing ability. Am80 treatment ameliorated DSS-induced colitis in mice. Bile acids and a synthetic agonist can also induce the differentiation of monocytes to IL-12p70 hypo-producing DCs by signaling through TGR5, a member of the rhodopsin-like superfamily of transmembrane G-protein coupled receptors. These molecules may be candidate therapeutic targets for Th1 dominant inflammatory disorders such as Crohn's disease.

**TH17 CELLS IN INTESTINAL HOMEOSTASIS AND INFLAMMATION**

1. Are Th17 Cells Pathogenic in Gut Mucosal Immunology?

The hypothesis of a Th1/Th2 cytokine balance has been used to explain the pathogenesis of chronic inflammatory disorders such as IBD. However, the recent discovery of a new class of Th cells, Th17 cells, which produce IL-17 family cytokines, raised a new paradigm that Th17 cells are an essential T cell subpopulation in the development of chronic inflammatory disorders in humans and mice. It is thought that each Th subpopulation is independently generated in the presence of specific transcription factors. In mice, Th17 cells are generated from naïve T cells in the presence of TGF-β and IL-6 and express a specific transcription factor, retinoic acid-related orphan receptor (ROR)γt. Recent reports suggest that the combination of IL-6, IL-23, and IL-1β effectively induce IL-17 production in naïve T cells, independently of TGF-β. In humans, the mechanism of Th17 development is more complicated. It is difficult to induce Th17 cells from naive peripheral blood T cells using in vitro differentiated conventional DCs even in the presence of TGF-β and IL-6. Acosta-Rodriguez et al. reported that IL-1β and IL-6 are essential for the development of Th17 cells in humans. However, Manel et al. succeeded in inducing Th17 cells from naïve T cells obtained from cord blood in the presence of TGF-β, IL-1β, and IL-21 or IL-23.

Th17 cells play a significant role in the pathogenesis of murine colitis models. IL-23p19 transgenic mice spontaneously developed chronic colitis. In IL-10−/− mice, which develop chronic Th1/Th17 dominant colitis, IL-23 administration exacerbated intestinal inflammation. The transfer of IL-17A-producing Th17 cells from C3Bir mice caused severe chronic colitis in the severe combined immunodeficiency mice, which was inhibited by anti-IL-23p19 monoclonal antibody (mAb) treatment. Recombination activating gene (RAG)-1−/− mice transferred with CD4+ CD25− T cells from RORγt−/− mice did not develop colitis. These findings suggest that Th17 cells may contribute to the pathogenesis of chronic intestinal inflammation. However, it is still controversial whether IL-17A plays a pathological role in colitis. Administration of neutralizing anti-IL-17A mAb did not reduce the severity of colitis in an adoptive transfer model of CD4+CD45RBhigh T cells. However, the administration of neutralizing anti-IL-17A mAbs exacerbated DSS-induced colitis. Furthermore, RAG-1−/− mice reconstituted with CD45RBhigh T cells from IL-17 receptor-deficient mice exhibited an accelerated wasting disease. In addition, IL-17A induced colitis in RAG-1−/− mice transferred with CD4+CD25− T cells from RORγt−/− mice. IL-17 receptor-deficient mice were protected from acute trinitrobenzene sulfonic acid-induced colitis. Thus, the contribution of Th17 cells in the pathogenesis colitis remains unresolved. It has become evident that Th17 cells can be classified in several subpopulations according to their cytokine profile. McGeachy et al. reported that Th17 cells could be divided to two types: Th17 cells producing IL-17 and IL-10. Th17 cells developed under IL-23 stimulation producing IL-17 and pro-inflammatory cytokines. Furthermore, recent studies have suggested interactions between Th1 and Th17 cells. IL-17A suppressed a murine model of colitis by blocking the development of Th1 cells. We demonstrated the existence of interference between colitogenic Th1 and Th17 cells in vivo. We co-transferred CD4+ T cells from colitic RAG-2−/− mice with CD4+ CD45RBhigh cells from colitic IL-10−/− mice into naïve RAG-2−/− mice. The proportions of IFN-γ and IL-17A producing CD4+ T cells in co-transferred mice were significantly decreased compared with single-cell transferred mice. To date, although it is thought that each Th subpopulation is generated independently, the current progress of immunology research has highlighted the plasticity between these Th cell lineages. Lee et al. demonstrated that Th1 cells could be generated from IL-17F-expressing Th17 cells in the presence of IL-23 in vitro. To date, IL-17A+IFN-γ+ double positive T cells have not been observed, although the existence of this unique subpopulation was demonstrated in several mice models of chronic inflammatory disorders including a CD4+CD45RBhigh T cell transfer colitis model. We investigated the plasticity between Th1 and Th17 cells in vivo. When RAG-2−/− mice were transferred with CD4+ CD45RBhigh T cells derived from RORγt-green fluorescent protein (GFP) reporter mice, they developed colitis, but less than 10% of the CD4+ T cells were GFP+ (RORγt+), suggesting this model would be Th1 dominant. GFP+ cells isolated from inflamed mucosa consisted of only IL-17A+IFN-γ+ Th1 cells, while GFP− cells consisted of IL-17A+IFN-γ− Th17 cells, IL-17A+IFN-γ− Th17/Th1...
cells, and IL-17A IFN-γ+ Th1 cells. Both GFP+ and GFP− cells expressed Tbx21, the gene product of T-bet, suggesting that GFP+ cells included RORγt+ T-bet+ cells, and RORγt− T-bet− cells. When GFP+ cells were isolated and re-transferred into new RAG−/− mice, approximately half of the cells lost GFP expression. Most GFP expressing cells were IL-17A IFN-γ− cells, and only a few GFP+ cells were IL-17A IFN-γ+ Th17 cells and IL-17A IFN-γ+ Th17/Th1 cells. This suggests that GFP+ (RORγt+) cells are induced to express T-bet and differentiate to IL-17A IFN-γ− alternative Th1 cells via IL-17A IFN-γ+ Th17/Th1 cells and IL-17A IFN-γ+RORγt+ Th1-like cells. Collectively, these results indicate a distinct developmental pathway from Th17 to alternative Th1 cells via Th17/Th1 and Th1-like cells during colitis (Fig. 2).

2. Th17 Cells in Human IBD

The pathological role of Th17 cells in human IBD remains unclear. To increase our understanding, we should consider the difference in the functional roles of Th17 cells between mice models and human diseases as reviewed by Steinman. Fujino et al. reported that IL-17A expression is upregulated in CD3− T cells and CD68+ macrophages in the inflamed mucosa of patients with IBD. We previously reported that CD14+ Mφ in the inflamed mucosa of Crohn’s disease patients produced IL-23, IL-6, TNF-α, and TLL1/TNFSF15 in response to whole bacteria stimulation and induced the differentiation of naïve T cells to both Th1 and Th17 cells. In Crohn’s disease especially, IL-23 strongly induced IFN-γ production from T cells and NK cells. In contrast, we also demonstrated that IL-23 enhanced Th17 immunological responses in ulcerative colitis. To date, the contribution of Th17 cells to human IBD pathogenesis is controversial. Indeed, the efficacy of anti-IL-17A mAb has not been proven in patients with IBD.

3. Mucosal NK Cells as a Novel Factor in IBD Pathogenesis

NK cells play an important role in systemic immunosurveillance to protect hosts from neoplasms and infections. NK cells can rapidly detect and dispose of target cells such as tumor cells and infected cells. NK cells also contribute to immunity by producing several types of cytokines such as IFN-γ and TNF-α. Recently, mucosal NK cells were shown to be involved in homeostasis at mucosal sites. We previously indentified that numbers of mucosal NK cells were increased in the LP of Crohn’s disease. We found increased numbers of c-kit+ lineage markers (lin) cells in human adult intestine. These intestinal immune precursors expressed CD34, CD38, CD14, IL-2Rα, and IL-7Rα. The lin+ c-kit+ precursors mainly differentiated to CD56+ c-kitdim cells in vitro corresponding to intestinal NK cells, which are clearly distinguished from peripheral blood NK cells by expression patterns of surface molecules and cytokine production. These cells produced higher amounts of IFN-γ, while their cytotoxic activity was relatively low. Interestingly, both c-kitdim cells and NK cells were increased in the inflamed mucosa of Crohn’s disease. Further analysis showed that intestinal mucosal CD3-CD56−NK cells could be classified into two subpopulations: NKP44+NKP46− (NKP44+) and NKP44−NKP46+ (NKP46+) cells. In contrast to recent studies in mice, NKP46+ cells expressed RORγt and produced IL-22, whereas human intestinal NKP46+ did not express RORC and produced higher amounts of IFN-γ. Importantly, the balance of NKP44+/NKP46− cells was disrupted in the intestinal mucosa of patients with Crohn’s disease, where IFN-γ producing NKP46+ cells were dominant. These findings suggest that T cells and mucosal IFN-γ producing NK cells may contribute to excessive Th1 immune responses and the pathogenesis of Crohn’s disease.

CONCLUSIONS

Intensive research in intestinal mucosal immunology has recently suggested several new paradigms such as the importance of Th17 immunity, the identification of several subpopulations of intestinal antigen presenting cells, and the identification of a new immune cell population. However, we should also recognize that we still have an incomplete picture of the pathogenesis of IBD. There is still a long way to go, but we confidently expect that the recent advances in

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our understanding of mucosal immunology will contribute to understanding the etiology and the development of new therapeutics of IBD.

REFERENCES