Macrophages are leukocytes of myeloid origin that display avid phagocytic capacity, and that contribute to the immune response via antigen presentation and strong secretory potential. Together with dendritic cells, they are part of a group of cells known as mononuclear phagocytes, which contain two of the three main phagocytic cells (the third being the neutrophil, or polymorphonuclear phagocyte). Once thought to originate solely from monocytes, it is now known that macrophages may be monocyte- (and therefore bone marrow-) derived, or of embryonic origin, originating from yolk sac and fetal liver-derived progenitors (74). In contrast to their postmitotic monocyte-derived counterparts, embryonic-derived macrophages comprise the majority of tissue-resident macrophages (88) and exhibit proliferative capacity (41). This review will focus on the colonic macrophage (a monocyte-derived, tissue-resident macrophage) and the role it plays in health and disease, specifically in inflammatory bowel disease (IBD) and colorectal cancer (CRC). Special emphasis will be given to macrophage polarization, or phenotype, in these different states. Potential macrophage-targeting strategies will also be discussed.

Macrophage Polarization and Nomenclature

Macrophages are a heterogeneous immune cell population, with diverse origins and functions (102). The concept of macrophage polarization is a useful approach to classify macrophages according to their activation state (66). This classification scheme emerged when Stein and colleagues (95) demonstrated that murine macrophages stimulated in vitro with interleukin (IL)-4 adopted a phenotype distinct to that of interferon-γ (IFN-γ)-stimulated macrophages, or classically activated macrophages. They observed that the alternatively activated macrophages exhibited higher scavenging potential in the form of increased mannosylated-BSA degradation and mannose receptor (CD206; MRC1) transcript expression and decreased proinflammatory cytokine production, evidenced by lower tumor necrosis factor-α (TNF-α) transcript levels. These alternatively activated macrophages also had an elongated, fibroblast-like morphology, in contrast to the mostly round/oval morphology of classically activated macrophages. Mills and colleagues (61) contributed to the classification scheme of macrophage polarization by coining the M1/M2 terminology to designate proinflammatory and anti-inflammatory macrophages, respectively, similar to the Th1/Th2 polarization of helper T cells. They further proposed that M1 and M2 macrophages could be distinguished from one another on the basis of arginine metabolism, where M1 macrophages preferentially metabolize arginine to nitric oxide via the inducible nitric oxide synthase (iNOS; NOS2), whereas M2 macrophages preferentially metabolize arginine to ornithine via arginase-1. Subsequently, the term M1 macrophage was employed to refer collectively to classically activated, proinflammatory, and M1 macrophages, and the term M2 macrophage was applied to alternatively activated, anti-inflammatory, and M2 macrophages (56). Figure 1 summarizes notable differences between M1 and M2 macrophages.

Address for reprint requests and other correspondence: R. A. Isidro, Dept. of Basic Sciences, Ponce Health Sciences Univ.-Medical School and Ponce Research Institute, 395 Zona Industrial Reparada 2, Ponce, Puerto Rico 00716 (e-mail: tonyisidro@me.com).
The M1/M2 terminology for describing macrophage polarization was further expanded by Mantovani and colleagues (56), who added subcategories for M2 macrophages. Thus macrophages alternatively activated in vitro by 1) IL-4 or IL-13 were designated as M2a macrophages; 2) immune complexes (IC), in combination with either Toll-like receptor (TLR) or IL-1 receptor (IL-1R) agonists, as M2b; and 3) IL-10 or glucocorticoids (GC), as M2c. Most recently, a panel of experts has recommended that macrophages polarized in vitro should be named based on their activator (66). According to this naming scheme, M1 macrophages would be subcategorized and named as M(LPS), M(LPS + IFN-γ), or M(IFN-γ), and M2 macrophages would be subcategorized and named as M(IL-4), M(lc), M(IL-10), M(GC + transforming growth factor (TGF)-β), or M(GC). Although this nomenclature parallels somewhat that of M1 and M2a/b/c, it has the advantage of specifically indicating how the particular macrophage population under study has been generated, which is particularly useful when one type of macrophage can be generated in different ways (e.g., M1 macrophages can be generated with LPS, IFN-γ, or a combination of the two, and gene and marker expression can vary between these) (66). Several defining characteristics have been described for M1 and M2a/b/c macrophages generated in vitro; however, assigning macrophages from in vivo models to one of these categories, particularly the M2a/b/c subcategories, can prove challenging. Therefore, for the purposes of this review, rather than relying strictly on the in vitro characteristics of M1 and M2 macrophages, we will describe in vivo macrophages as M1 macrophages when evidence indicates that these cells promote inflammation and/or type 1/Th1/Th17 immune responses, and as M2 macrophages when evidence indicates that these cells prevent or antagonize inflammation and/or promote type 2/Th2 immune responses. Whenever we refer specifically to macrophages generated in vitro, we will indicate the activator in parentheses [e.g., M1(LPS)], as recommended by Murray and colleagues (66), and add the 1 or the 2 to facilitate classification into one of the two broad categories of macrophage polarization.

Colonic Macrophages

Of the many immune cells present in the colon, the macrophage is one of the most abundant (76, 105). In fact, the colon is one of the most macrophage-dense organs (49). During development and shortly after birth, embryonic-derived macrophages populate the colon; however, monocyte-derived macrophages (MDMs) gradually replace these macrophages of embryonic origin and become the predominant population in the adult (6). Several studies agree that, in homeostasis and inflammation, colonic macrophages originate from circulating monocytes (7, 82, 96, 105). In mice, colonic macrophages can be identified by the following marker expression profile: CX3CR1lo/hi, CD64lo/hi, CD11b+CD11c+TGFβ/80°Ly6C−/hi, MHCIIdown, CD117+CD103+SiglecFCCR7− (Fig. 2) (7, 62, 96). The most useful of these markers to differentiate macrophages from dendritic cells in the colon are CD45, which macrophages express at high levels, but dendritic cells do not express or express only at low levels; CCR7, which macrophages do not express, and dendritic cells do; CD103, which macrophages do not express, and most colonic dendritic cells do; and, to a lesser extent, CD11c, which macrophages do not express or express at low to intermediate levels, whereas dendritic cells express at high levels. SiglecF is also useful for discriminating between macrophages, which are negative for this marker, and eosinophils, which are positive for this marker. Human colonic macrophages can be identified as CD68+CD14+HLA-DR+ CD64+CD45+CD163+ (86, 96, 97). The marker expression profile for colonic macrophages in the rat is not as well defined as in mice or humans. Rat colonic macrophages are thought to be immunophenotypically defined as CD11b+CD68+MHCIIdown, CD163+OX62(rat αβ2-integrin)− (8, 11, 18). Compared with their human and mouse counterparts, rat macrophages suffer from a paucity of identifying markers and a lack of antibodies targeting these markers (8, 40).

The Origins of the Colonic Macrophage: Development of Monocytes

Our understanding of the origin of monocytes has advanced significantly over the past few decades. Monocytes were classically thought to originate from the monoblast-promonocyte-monocyte sequence. However, several findings over the past decade and a half have revealed that the development of monocytes is more complex. Our current understanding is that monocytes derive from a common monocyte precursor, which in turn is derived from the macrophage dendritic cell progenitor (MDP) (35). The MDP originates from the granulocyte-macrophage progenitor (5). Granulocyte-macrophage progenitors arise from the common myeloid progenitor that, in turn, derives from hematopoietic stem cells (1). This differentiation process is highly dependent on the transcription factor PU.1 (17) and on macrophage colony-stimulating factor (M-CSF)-M-CSF receptor signaling (85). Recently, Mossadegh-Keller and colleagues (63) have shown, using single-cell analysis, that M-CSF increases PU.1 activation, thus inducing a myeloid lineage in hematopoietic stem cells. M-CSF deficiency leads to marked reduction in gut macrophages, most likely via depletion of monocytes (85). Although monocytes have long been
thought to give rise to dendritic cells in addition to macrophages, it is now thought that dendritic cells originate from the MDP-derived common dendritic cell progenitor rather than from monocytes (7, 75, 96).

The Origins of the Colonic Macrophage: Differentiation of Monocytes into Macrophages

From studies in the mouse, we now know that MDMs begin to emerge in the colon at ~2–3 wk after birth, becoming the predominant macrophage population in adulthood (~9 wk of age), at which point embryonic-derived macrophages are practically absent from the colon (6). Two hallmark studies have recently revealed the developmental series by which monocytes differentiate into macrophages within the colonic lamina propria (7, 96). According to these studies, monocytes are revascularized in a CCR2-dependent manner to the colon, where they differentiate into macrophages within the colonic lamina propria. Two hallmark studies have recently revealed the developmental series by which monocytes differentiate into macrophages within the colonic lamina propria (7, 96). According to these studies, monocytes are revascularized in a CCR2-dependent manner to the colon, where they differentiate into macrophages within the colonic lamina propria (7, 96).

Life span of the Colonic Macrophage

Macrophages are classically thought as having lifespans ranging from months to years. Notwithstanding, it is unclear whether colonic macrophages exhibit such a prolonged half-life. Data from recent experiments suggest a lifespan of at least 1–2 wk: Bain and colleagues found that adoptively transferred monocytes differentiated into macrophages in the colon of CCR2 knockout mice, in which colonic macrophages are nearly nonexistent, and were still present 1 wk posttransfer (7). Rivollier and colleagues (82) reported the presence of adoptively transferred MDMs at 2 wk after transfer in the colons of C11c diphtheria toxin receptor-expressing (CD11c-DTR) bone marrow chimeric mice depleted of macrophages by treatment with diphtheria toxin.

Homeostasis: Anti-inflammatory Phenotype and Macrophage-Microflora Interactions

In the steady state, colonic macrophages exhibit an anti-inflammatory, protolerogenic, M2-like phenotype (Fig. 3A). The M2 phenotype for colonic macrophages is supported by their expression of CD206 and CD163, production of IL-10, driving of epithelial cell regeneration and proliferation, and promotion of regulatory helper T-cell (Treg) proliferation (7, 33, 62, 78, 82).

The mechanism by which colonic macrophages signal expansion of Tregs has recently been described by Mortha and colleagues (62). They found that sensing of the microflora by TLRs on macrophages sets in motion a chain of events: first, activation of MyD88 leads to macrophage secretion of IL-1β; second, IL-1β activates MyD88 on granulocyte-macrophage colony stimulating factor (GM-CSF)-producing RORγt+ innate lymphoid cells (ILC3) via the IL-1R, resulting in secretion of GM-CSF (CSF-2); third, GM-CSF from the ILC3s signals macrophages and dendritic cells to produce IL-10 and retinoic acid; and fourth, IL-10 and retinoic acid promote the conversion of helper T cells to Tregs and their subsequent expansion. Curiously, NLRC4-triggered secretion of IL-1β by colonic mononuclear phagocytes has been reported as a mechanism for distinguishing between commensal and pathogenic...
bacteria, resulting in recruitment of neutrophils to fend off pathogenic bacteria (25). Perhaps lower levels of IL-1β are able to stimulate ILC3 and subsequently promote Treg expansion, whereas higher levels signal neutrophils to extravasate into the colon to attack bacterial pathogens. Further studies are required to elucidate how secretion of IL-1β can lead to such seemingly opposite responses.

The exact combination of factors that lead colonic macrophages to acquire their characteristic M2/anti-inflammatory phenotype is currently unknown and likely results from a combination of TLR-signaling regulation, IL-10 signaling, and interactions with both intestinal epithelial cells (IECs) and the gut microflora. The hyporesponsiveness and anti-inflammatory response signature to bacterial products enacted by intestinal macrophages was originally thought to result from a lack of TLR expression. Subsequent studies have shown that intestinal and colonic macrophages indeed express TLRs (7, 92), and that this lack of an inflammatory response is likely due to regulation of the signaling pathways downstream of the TLR (92). Nevertheless, other factors may also be involved. This is supported by the fact that macrophages from IL-10−/− mice, but not macrophages from IL-10−/− mice with a macrophage-specific loss of MyD88 (Ly5.1-Mx1ΔMyD88/IL-10 knockout), are hyporesponsive to bacterial products. These macrophages express higher levels of proinflammatory cytokines (IL-12p40, IL-1β, IL-6, TNF-α) and promote Th1 and Th17 responses (36). Furthermore, macrophages from germ-free mice, but not MyD88−/− mice, produce less IL-10 than their wild-type counterparts (82). Therefore, both IL-10 and microbial antigens and/or products are implicated as possible factors regulating colonic macrophage phenotype.

IL-10 has long been known to play a critical role in the maintenance of intestinal homeostasis. Mice deficient in IL-10 develop spontaneous colitis in a microbiota-dependent manner, evidenced by the absence of colitis in IL-10-deficient mice kept in physiological conditions, monocytes extravasate into the colonic lamina propria and differentiate into macrophages, which initially develop a proinflammatory/M1 phenotype, but later acquire the characteristic anti-inflammatory/M2 phenotype. The signals that cause the colonic macrophage to replace the initial proinflammatory phenotype with an anti-inflammatory and protelogenic profile are incompletely understood, but can include IL-10 secreted from intestinal epithelial cells (IECs) and regulatory T (Treg) cells, granulocyte-macrophage colony stimulating factor (GM-CSF) secreted from type 3 innate lymphoid cells (ILC3), and bacterial products from the colonic microflora. The colonic macrophage contributes to homeostasis by clearing bacteria and bacterial products that translocate into the lamina propria without mounting an immune response and by interacting with other mucosal cells. In Crohn’s disease, monocytes recruited to sites of inflammation differentiate into macrophages that maintain their proinflammatory/M1 phenotype instead of transitioning to the characteristic anti-inflammatory/M2 phenotype of colonic macrophages through mechanisms that remain poorly understood but that likely involve the proinflammatory milieu. Increased monocyte recruitment is thought to result from increased secretion of CCR2-ligating chemokines (i.e., CCL2, CCL7, CCL8), possibly resulting from IEC sensing of Gram-positive bacteria or activation of CD169+ macrophages at the base of the colonic crypts. In response to the epithelial damage and bacterial translocation resulting from the disease, the M1 macrophages secrete several proinflammatory mediators, some of which can promote the expansion of type 1 helper T (Th1) cells and type 17 helper (Th17) cells. Th1 cells reinforce the proinflammatory phenotype of the M1 macrophages, whereas Th17 cells promote neutrophil infiltration, possibly contributing to crypt abscess formation. C: in ulcerative colitis, as with Crohn’s disease, monocytes differentiate into proinflammatory/M1 macrophages. However, atypical M2 macrophages [possibly M2b/M2c(1c)] produce inflammation-promoting factors, recruit eosinophils through CCL11 secretion, and signal type 2 helper T (Th2) cells to expand via IL-4 and IL-13 secretion. Th2 cells promote B-cell differentiation into plasma cells, and immunoglobulins (Ig) secreted by these plasma cells form immune complexes that can further reinforce the M2b/M2c(1c) macrophages. Expansion of Th17 cells induced by M1 macrophage-derived IL-23 leads to neutrophilic infiltration as seen in Crohn’s disease (B).

Fig. 3. Colonic macrophage differentiation and phenotype in homeostasis and inflammatory bowel disease (IBD). The colonic macrophage is a monocyte-derived tissue macrophage. A: in physiological conditions, monocytes extravasate into the colonic lamina propria and differentiate into macrophages, which initially develop a proinflammatory/M1 phenotype, but later acquire the characteristic anti-inflammatory/M2 phenotype. The signals that cause the colonic macrophage to replace the initial proinflammatory phenotype with an anti-inflammatory and protelogenic profile are incompletely understood, but can include IL-10 secreted from intestinal epithelial cells (IECs) and regulatory T (Treg) cells, granulocyte-macrophage colony stimulating factor (GM-CSF) secreted from type 3 innate lymphoid cells (ILC3), and bacterial products from the colonic microflora. The colonic macrophage contributes to homeostasis by clearing bacteria and bacterial products that translocate into the lamina propria without mounting an immune response and by interacting with other mucosal cells. B: in Crohn’s disease, monocytes recruited to sites of inflammation differentiate into macrophages that maintain their proinflammatory/M1 phenotype instead of transitioning to the characteristic anti-inflammatory/M2 phenotype of colonic macrophages through mechanisms that remain poorly understood but that likely involve the proinflammatory milieu. Increased monocyte recruitment is thought to result from increased secretion of CCR2-ligating chemokines (i.e., CCL2, CCL7, CCL8), possibly resulting from IEC sensing of Gram-positive bacteria or activation of CD169+ macrophages at the base of the colonic crypts. In response to the epithelial damage and bacterial translocation resulting from the disease, the M1 macrophages secrete several proinflammatory mediators, some of which can promote the expansion of type 1 helper T (Th1) cells and type 17 helper (Th17) cells. Th1 cells reinforce the proinflammatory phenotype of the M1 macrophages, whereas Th17 cells promote neutrophil infiltration, possibly contributing to crypt abscess formation. C: in ulcerative colitis, as with Crohn’s disease, monocytes differentiate into proinflammatory/M1 macrophages. However, atypical M2 macrophages [possibly M2b/M2c(1c)] produce inflammation-promoting factors, recruit eosinophils through CCL11 secretion, and signal type 2 helper T (Th2) cells to expand via IL-4 and IL-13 secretion. Th2 cells promote B-cell differentiation into plasma cells, and immunoglobulins (Ig) secreted by these plasma cells form immune complexes that can further reinforce the M2b/M2c(1c) macrophages. Expansion of Th17 cells induced by M1 macrophage-derived IL-23 leads to neutrophilic infiltration as seen in Crohn’s disease (B).
under germ-free conditions. Patients with IL-10 receptor (IL-10R) deficiency develop severe pediatric IBD (30). Li and colleagues (52) were the first to demonstrate that macrophages are responsible for mediating the anti-inflammatory effects of IL-10 during murine dextran sodium sulfate (DSS) colitis, given that mice bearing a macrophage-specific deficiency in the α-subunit of the IL-10R (Lysocre IL-10R<sup>fl/fl</sup>) phenocopied disease progression seen for IL-10<sup>−/−</sup> and IL-10R<sup>-/-</sup> mice. They also demonstrated that disease progression was similar in CD4<sup>+</sup>IL-10R<sup>−/−</sup>, CD11<sup>+</sup>Lysocre IL-10R<sup>−/−</sup>, or CD19<sup>+</sup>IL-10R<sup>−/−</sup> mice compared with their IL-10R<sup>−/−</sup> littersmates; that neutrophils were not involved in the effect observed for Lysocre IL-10R<sup>−/−</sup> as neutrophil depletion before DSS administration did not alter the disease course for these mice; and that Lysocre IL-10R<sup>-/-</sup> mice did not develop spontaneous colitis (mice were housed under Helicobacter spp.-free conditions). Interestingly, Zigmond and colleagues (104) did observe spontaneous colitis in mice with macrophage-specific deficiency of IL-10Rα (C3CR1<sup>-/-</sup>IL10R<sup>−/−</sup>) but not IL-10 (C3CR1<sup>-/-</sup>IL10<sup>−/−</sup>). Of note, these C3CR1<sup>-/-</sup>IL10R<sup>−/−</sup> and C3CR1<sup>-/-</sup>IL10<sup>-/-</sup> mice were positive for Helicobacter, which could explain the seemingly discordant results obtained by Li et al. (52) and Zigmond et al. (104), as Helicobacter bacteria have been linked to colitis development in IL-10<sup>-/-</sup> mice. Nevertheless, the fact that macrophage-specific IL-10Rα deficiency, but not IL-10 deficiency per se, led to spontaneous colitis indicates that IL-10 signaling in macrophages is more important than IL-10 production by macrophages, and, therefore, colonic macrophage secretion of IL-10 does not appear to be essential for homeostasis in the colon of these mice. Shouval and colleagues (90) have shown that IL-10Rβ deficiency in mice leads to spontaneous colitis, decreased anti-inflammatory colonic macrophages, and increased proinflammatory colonic macrophages. They also showed that M1(LPS+IFN-γ) bone marrow-derived macrophages (BMDM) from IL-10Rβ-deficient mice and GM-CSF-generated MDMs from patients with IL-10R deficiency exhibited enhanced proinflammatory properties (90). Furthermore, M2(IL-10 or IL-4+TGF-β+IL-10) BMDM from IL-10Rβ-deficient mice and M2(IL-4) MDM from IL-10R-deficient patients manifested a diminished anti-inflammatory phenotype and an augmented proinflammatory potential (90).

In addition to IL-10-producing T cells, IECs are a potential source for IL-10 in the human colon and could also contribute to the development of an anti-inflammatory phenotype in colonic macrophages. Spöttl and colleagues (94) were the first to show that human MDMs could acquire properties of colonic macrophages via coculture with human secondary colonic epithelial cell (HT-29) spheroids. These macrophages gradually downregulated CD14 expression and produced less IL-1β transcripts than monocyte/macrophages cultured alone or with noncolonic epithelial cell spheroids. Kristek and colleagues (44) demonstrated that mouse secondary MDMs (J774.A.1) conditioned with media from mouse secondary colonic epithelial cells (CMT-93) acquired certain characteristics of colonic macrophages, such as increased phagocytic ability and attenuated proinflammatory cytokine secretion and reactive oxygen and nitrogen species production in response to LPS. Although IL-10 secretion was not augmented by conditioning with medium from IECs, this could be due to a lack of bidirectional interactions between the macrophages and IECs. Hyun et al. (38) have recently shown that coculturing human secondary colonic IECs (SW840, Caco-2 cell lines) with mouse peritoneal macrophages in the presence of the TLR-4 ligand LPS leads to increased IEC secretion of IL-10 (38). The cellular and molecular mechanisms for this phenomenon were described as follows: TLR-4 ligation on IECs triggers initial IL-10 release and inhibits peroxisome proliferator-activated receptor-γ (PPAR-γ) degradation, and on macrophages activates p38 and ERK mitogen-activated protein kinases. This results in increased expression of Cox-2 and subsequent production of 15-deoxy-Δ<sub>12,14</sub> prostaglandin J<sub>2</sub> (15d-PGJ2). Macrophage-derived 15d-PGJ2 then causes nuclear accumulation and activation of PPAR-γ in IECs, resulting in an augmented second induction of IL-10 (38).

Furthermore, the anti-inflammatory, or M2, properties of colonic macrophages may be a result of and/or enhanced by interaction with bacterial products. Butyrate, a short-chain fatty acid that can be found at high concentrations in the colon and is secreted mainly by Bacteroidetes and Firmicutes phyla bacteria, has been found to downregulate colonic macrophage transcripts for the proinflammatory factors IL-6, IL-12, and NOS2 in vitro and in vivo (12). From in vitro experiments using BMDMs, it has been suggested that the regulatory function of butyrate is a result of it functioning as a histone deacetylase inhibitor, rather than through activation of G-protein-coupled receptors (12).

It is of paramount importance for the maintenance of homeostasis that an immune response is not mounted against antigens derived from food and commensal microorganisms, and that oral and mucosal tolerance is maintained. The gastrointestinal tract (GI), in addition to being one of the largest macrophage reservoirs, is also one of the most microbe-rich organs, containing several trillion microbes (51). Notably, most of these microbes reside in the colon (51). Gut microflora normally penetrate the epithelial barrier and reach the lamina propria, where they are phagocytosed and cleared by macrophages in the colon, as reported for the small intestine (53). The uptake of penetrating bacteria by colonic macrophages is supported by ex vivo data showing that colonic macrophages are highly phagocytic (82, 96) and avidly phagocyteyce fluorescently labeled Escherichia coli (6, 7).

Although the mechanisms and pathways by which colonic macrophages sample luminal antigens, either in the lumen or lamina propria, remain poorly understood, they are thought to be similar to those of the macrophages in the small intestine (64). As such, the main route by which intestinal macrophages sample luminal contents is thought to be by extension of transepithelial dendrites (TEDs) into the lumen of the gut (69, 81). Rescigno and colleagues (81) first described TEDs both in an in vitro model, in which epithelial monolayers were cocultured with dendritic cells, and in vivo, documenting TEDs emanating from CD11c<sup>+</sup> cells. Subsequently, Niess and colleagues demonstrated that TEDs were found on CX3CRI<sup>+</sup> CD11b<sup>+</sup>CD11c<sup>+</sup>MHC II<sup>+</sup> cells, and their expression was dependent on the presence of CX3CR1 (69). In light of recent findings (7, 96), the TED-expressing cells are now thought to be macrophages, rather than dendritic cells (64). Although macrophages normally do not migrate to mesenteric lymph nodes, they may contribute to oral tolerance by using gap junctions to transfer the antigens that they have uptaken to...
dendritic cells, which can then migrate to lymph nodes and induce Treg cells (59).

Although much of the preceding discussion has focused mainly on macrophages found in the mucosa, macrophages located in the muscularis propria, or main muscle layers of the colon, are also involved in maintaining homeostasis in the large intestine. These muscularis macrophages have been shown to play an important role in regulating colonic motility via cross talk with enteric neurons (65). Muller and colleagues (65) demonstrated that these macrophages regulate motility by producing bone morphogenetic protein 2, which signals bone morphogenetic protein receptor-positive enteric neurons; that these neurons in turn secrete M-CSF, which is required for the development of muscularis macrophages; and that this cross talk is regulated by the intestinal microflora.

**Macrophage Phenotype in IBD**

IBD is a chronic inflammatory condition of the intestines that affects ~1.4 million people in the United States (Centers for Disease Control) (11a). The etiology of IBD is unknown, yet the leading hypothesis consists of an interaction between genetic, environmental, immune, and microbial factors. Ulcerative colitis (UC) and Crohn’s disease (CD) are the two main forms of IBD. It is traditionally believed that UC results from a TH2 helper T-cell autoimmune response, and that CD arises from a TH1 helper T-cell autoimmune response. Several studies have demonstrated that TH17 helper T cells are involved in both UC and CD. UC is characterized by inflammation of the colonic mucosal and superficial submucosal layers, whereas CD is typified by granulomatous, transmural inflammation mainly in the terminal ileum and colon, although it can affect any part of the GI tract. The inflammation seen in UC often commences in the rectum and spreads proximally in a continuous manner. In contrast, the inflammation found in CD affects distant portions of the GI tract in a discontinuous fashion. Proinflammatory, or M1, macrophages have been detected in the colon of animals from models of IBD and of patients with IBD. These macrophages have been linked to disease severity and progression and have been postulated as targets for therapeutic intervention. In the next section we will first review what has been learned in terms of macrophage phenotype and function from animal models of IBD and will then proceed to describe what is currently known in humans.

**Proinflammatory macrophages accumulate in colon during inflammation in models of IBD.** Several studies in mouse models of IBD conclude that M1, or proinflammatory, macrophages accumulate in the large intestine during colitis. Taoutounour and colleagues demonstrated that, after T-cell transfer in a mouse model of T-cell-mediated colitis, proinflammatory macrophages became the predominant colonic macrophage population as early as 12 h posttransfer and remained elevated even 3 wk after transfer, when they contributed more than one-half of all colonic macrophages (96). These macrophages were immunophenotypically consistent with the P1 and P2 populations described in the normal development of colonic macrophages above. Expression of the proinflammatory cytokines and reactive nitrogen species-producing iNOS was mostly evident in the M1-like P2 subset of colonic macrophages, although these cells began to express this enzyme in detectable quantities several weeks after colitis was initiated by T-cell transfer. In the DSS model of colitis, administration of DSS in the drinking water compromises the integrity of the epithelial barrier, presumably by toxic effects on IECs, and leads to the development of colitis. Using the DSS model, Bain and colleagues observed a marked increase in proinflammatory macrophages, mainly of the P1 and P2 subsets of colonic macrophages, within 24 h after commencing the DSS regimen, and this increase was still present 5 days later (7). Through ex vivo analyses, they were able to show that, despite IL-10 production, the majority of these cells produced TNF-α. From the developmental series described above for the colon in homeostasis, one can conclude that there is a degree of plasticity in these colonic macrophages, in which M1 macrophages gradually become M2 macrophages. What is less clear is whether the mature colonic macrophage that has acquired a vast array of M2 properties can turn into an M1 macrophage in response to tissue damage and encroachment by luminal bacteria. Zigmond and colleagues (105) interestingly demonstrated that daily administration, commencing 2 days after colitis induction with DSS, of a depleting antibody for CCR2 (which is essential for monocyte recruitment to the colon) reduces monocyte-derived cell infiltration in the colon, colonic proinflammatory cytokine levels, weight loss, and colonic damage in mice. Although these infiltrating, proinflammatory cells were initially considered dendritic cells, their Ly6C$^+$CX3CR1$^+$ and antigen-presenting capacity are more consistent with M1 macrophages (P1/P2). This proinflammatory program might be regulated in part by the serine/threonine kinase Akt-1, given that Akt-1-deficient mice develop macrophages with an M1 phenotype and are more susceptible to DSS colitis (3a).

The exact signals that trigger the influx of monocytes and accumulation of M1 macrophages are incompletely understood. It has recently been suggested by Nakashima and colleagues (68) that Gram-positive commensal bacteria in the colon, likely by promoting IEC secretion of CCR2-ligating chemokines, are responsible for the recruitment of the proinflammatory monocytes and macrophages that propagate colitis. Compared with DSS-treated mice that did not receive antibiotic treatment or whose Gram-negative bacteria were depleted with the antibiotic colistin, vancomycin-mediated depletion of commensal Gram-positive bacteria in mice undergoing DSS colitis reduced colonic monocyte and macrophage numbers, weight loss, colon shortening, colonic TNF-α and IL-6 levels, histological damage, and IEC transcript levels of CCR2-ligating chemokines (CCL2, CCL7, and CCL8). Depletion of Gram-positive and Gram-negative bacteria with a combination of ampicillin, metronidazole, neomycin, and vancomycin produced results similar to those of vancomycin-mediated depletion of Gram-positive bacteria. Another recent study by Asano and colleagues (4) has shown that a subset of mature colonic macrophages, characterized by CD169 expression on Ly6C$^+$ CD64$^+$CX3CR1$^+$ macrophages and usually present at the base of the mucosa under homeostatic conditions, is responsible for recruiting proinflammatory monocytes to the colon of mice undergoing DSS colitis. Ablation of these cells with diphtheria toxin in CD169-DTR mice reduced DSS-induced weight loss, colonic hemorrhage, histological damage, and proinflammatory monocyte and macrophage infiltration in the colon (4). Furthermore, the authors demonstrated that CD169$^+$ macrophages signaled monocyte recruitment via CCL8, as levels of this chemokine were significantly reduced in mice in which
CD169⁺ macrophages were ablated. Targeting of CCL8 with a neutralizing antibody reduced DSS-induced weight loss, colonic shortening, histological damage, and colonic IL-17A transcript levels in these mice (4). Signal-transducing adapter protein-2 (STAP-2) has been shown by Fujita and colleagues (27) to also regulate monocyte/macrophage recruitment to the colon in DSS-treated mice. First, STAP-2-deficient mice were protected from DSS colitis compared with wild-type mice with regards to weight loss, disease activity, histological damage, and macrophage infiltration. Second, loss of STAP-2 in the bone marrow compartment, achieved by transferring STAP-2-deficient cells to lethally irradiated wild-type mice, reduced DSS-induced weight loss and hemorrhage compared with wild-type mice receiving wild-type bone-marrow cells. Third, colonic IEC STAP-2 deficiency, achieved by transferring wild-type bone-marrow cells into lethally irradiated STAP-2-deficient mice, also reduced the effects of DSS on weight loss, hemorrhaging, and colonic proinflammatory cytokine transcript levels. Fourth, STAP-2-deficient MDMs displayed decreased migratory potential in vitro (27). Of note, both Asano and colleagues (4), and Fujita and colleagues (27) did not obtain similar results in Th1-mediated models of colitis, suggesting that CD169⁺ macrophages and STAP-2 signaling play a more important role in non-Th1-mediated colitis, such as that seen in UC.

Colonic M2 macrophages antagonize inflammation and promote healing in models of IBD. Although the colonic M1 macrophage population expands during colitis, M2 macrophages are still present and likely antagonize or regulate the inflammatory reaction. A subpopulation of mature colonic macrophages secretes IL-10, but not TNF-α, even in a DSS model of colitis (7). Qualls and colleagues (79) have shown that depletion of colonic mononuclear phagocytes before colitis induction results in a more severe colitis compared with mice that were not depleted of macrophages, as evidenced by greater weight loss, disease activity scores, colon shortening, and microscopic damage. Interestingly, this study found that colonic transcript levels of IL-10 were significantly decreased by mononuclear phagocyte depletion, whereas transcript levels for proinflammatory cytokines such as IFN-γ and TNF-α were not significantly affected. This suggests that mononuclear phagocyte depletion led to a reduction in the M2 mature colonic macrophages, thus rendering mice more susceptible to DSS colitis. On the other hand, several studies have shown that increasing the proportion of colonic M2 macrophages ameliorates colitis. Hunter and colleagues (37) found that injecting M2(IL-4+IL-13) macrophages but not M1(IFN-γ) macrophages intraperitoneally 2 days before colitis induction reduced disease activity, histological damage, myeloperoxidase activity, and colon shortening in a mouse model of colitis induced with dinitrobenzene sulfonic acid (DNBS), a haptenating agent that produces colitis when administered intracolonomically. Fluorescently labeled M1(IFN-γ) and M2(IL-4+IL-13) macrophages were documented in the colon of recipient animals, suggesting that intraperitoneally administered macrophages could enter into circulation and extravasate into the colon. Administering M2(IL-4+IL-13) macrophages 6 h after colitis induction had similar effects on disease parameters (37). Leung and colleagues (50) obtained similar results using bone marrow-derived M2(IL-4+IL-13) macrophages, rather than peritoneal macrophages; mice undergoing M2(IL-4+IL-13) macrophage adoptive transfer 2 days before, or 6 h after, DNBS treatment showed less disease activity, colon shortening, and histological damage compared with mice treated with DNBS only. This effect appeared to be partially due to IL-10 secretion by these macrophages, as mice receiving M2(IL-4+IL-13) macrophages generated from the bone marrow of IL-10⁻/⁻ mice fared worse than mice receiving BMDMs from wild-type mice. Additionally, the authors showed M2(IL-4+IL-13) macrophage adoptive transfer had a consistent effect when administered 6 h after DNBS treatment in three consecutive rounds, each round spaced 2 wk apart. Enderlin Vaz da Silva and colleagues (22) showed that intravenous administration of bone-marrow derived M0(unpolarized) or M2(IL-4) but not M1(IFN-γ) macrophages on the 3rd and 4th day after beginning DSS administration increased ulcer repair, despite the fact that all three types of macrophages reached the inflamed colon in comparable amounts. Lastly, it has recently been shown that Akt-2 deficiency protects mice from DSS colitis, and that Akt-2-deficient macrophages develop an M2 phenotype in vitro (3a). In this DSS colitis model, adoptive transfer of the Akt-2-deficient macrophages into macrophage-deficient wild-type or Akt2⁻/⁻ mice led to decreased weight loss, colon shortening, and histopathology (3a).

In addition to their anti-inflammatory effects, colonic macrophages also promote healing by driving epithelial cell regeneration and proliferation. Pull and colleagues (78) demonstrated that macrophages were required for colonic epithelial progenitor cell proliferation in response to DSS colitis. One mechanism by which colonic macrophages may promote healing is by activating the WNT-β-catenin signaling pathway in epithelial/stem cells. Cosín-Roger et al. (15) have shown that, compared with unpolarized and M1(LPS+IFN-γ) macrophages, M2(IL-4) macrophages expressed higher transcript levels of WNT1 and WNT3A in human monocyte- and U937-derived macrophages. They also demonstrated that coculturing U937-derived M2(IL-4) macrophages with Caco-2 colonic epithelial cells increased nuclear β-catenin protein levels in epithelial cells, which was dependent in part on macrophage WNT1 expression. Recently, they found that signal transducer and activator of transcription (STAT) 6 signaling in M2(IL-4) macrophages not only contributes to the acquisition of an anti-inflammatory phenotype in these cells, but is also important for WNT-β-catenin pathway activation in epithelial cells (16). M2(IL-4)-polarized peritoneal macrophages from STAT6-sufficient, but not STAT6-deficient, mice increased nuclear β-catenin levels in cocultured Caco-2 cells. Furthermore, adoptive transfer of STAT6-sufficient peritoneal M2(IL-4) macrophages into STAT6-deficient mice undergoing trinitrobenzene sulfonic acid (TNBS)-induced colitis improved disease parameters and increased nuclear accumulation of β-catenin in the colon compared with colitic STAT6-deficient mice receiving STAT6-deficient M2(IL-4) macrophages.

Macrophage phenotype in CD. Colonic tissue from patients with CD contains marked macrophage infiltration, and these macrophages have been shown to be proinflammatory in nature (Fig. 3B). Thiesen and colleagues (97) have shown a marked increase in CD14⁺HLA-DRdim macrophages in inflamed intestinal tissue from CD patients compared with non-inflamed tissue from CD and control patients, and these macrophages were similar to proinflammatory monocytes. Magnusson and colleagues (54) have recently confirmed these results, also
finding a significant increase in CD14\(^+\)HLA-DR\(^{\text{dim}}\) macrophages in inflamed colonic tissue from CD compared with noninflamed colon from these same patients or noninflamed colon from non-IBD controls. The similarity between these macrophages and proinflammatory monocytes is reminiscent of the P1 and P2 proinflammatory macrophage subsets from the developmental series described for colonic macrophages in the mouse (7, 96). Consistent with the proinflammatory phenotype of P1 and P2 mouse colonic macrophages, analyses by Kamada and colleagues (42) of CD14\(^+\)CD33\(^+\) macrophages from CD patients have revealed a proinflammatory phenotype akin to that of M1 macrophages. Upon stimulation with commensal *E. coli* or *Enterococcus faecalis*, these CD14\(^+\)CD33\(^+\) macrophages secreted higher levels of the proinflammatory cytokines IL-23 and TNF-\(\alpha\) than CD14\(^-\)CD33\(^+\) macrophages from the same patients and CD14\(^+\)CD33\(^+\) macrophages from normal patients (42). These cells also exhibited an augmented capacity to induce IFN-\(\gamma\)-producing (Th1) T cells in response to the aforementioned commensals (42). Ogino and colleagues (70) observed CD14\(^+\)CD163\(^{\text{lo}}\) macrophages with Th1-inducing ability and noted that this subset could also induce IL-17-producing (Th17) T cells. It is intriguing that they also found, upon comparing CD14\(^+\)HLA-DR\(^{\text{dim}}\) macrophages from inflamed CD mucosa with CD14\(^+\)CD163\(^{\text{lo}}\) macrophages, that the former contained higher transcript levels of the proinflammatory cytokines IL-6 and IL-23 (p19), and TNF-\(\alpha\), as well as the anti-inflammatory cytokines IL-10 and TGF-\(\beta\). This suggests that these two subgroups of cells are not entirely identical, despite similar functional properties. Nevertheless, M1 macrophages seem to be the predominant macrophage population in the inflamed colon of patients with CD (Table 1). The study by Kamada et al. (42) found that approximately one-half of CD33\(^+\) macrophages in inflamed intestinal tissue from CD patients were the CD14\(^+\) proinflammatory macrophages discussed above. Using double immunohistochemical staining for CD68 or CD163 as macrophage markers in conjunction with pSTAT1, which is the activated form of STAT1 that results from interferon signaling, or RBP-J, a mediator of Notch signaling, Barros and colleagues (9) have found that one-half or more of macrophages in the CD colon are of the M1 phenotype. They also found that M2 macrophages make up only about one-third to one-fifth of colonic macrophages in CD, as determined by staining for c-Maf, an important transcription factor for mediating IL-10 gene expression in macrophages, in CD63- or CD68-positive colonic macrophages, respectively. M2 macrophages, as determined by positivity for the M2 marker CD206, have been shown by Hunter et al. (37) and Vos et al. (99) to contribute to less than one-fifth of colonic macrophages stained with CD68 by immunofluorescence and immunohistochemistry, respectively, in tissue from patients with CD (37, 99).

**Macrophage phenotype in UC.** Macrophage infiltration has also been observed in tissue from patients with UC, but the phenotype of these cells is less clear (Fig. 3C). Diseased areas of human UC colon biopsies contain elevated levels of CD14\(^+\)HLA-DR\(^{+}\)CD64\(^+\) macrophages (96). Magnusson and colleagues (54) found that CD14\(^+\)HLA-DR\(^{\text{dim}}\) colonic macrophages in inflamed UC tissue were increased to a similar extent as seen in inflamed CD tissue compared with control tissues. The CD14\(^+\)CD33\(^+\) macrophages previously shown to infiltrate inflamed tissue also infiltrate inflamed UC tissue, but their phenotype in UC is less clear. Studies by Kamada and colleagues (42) reveal that CD14\(^+\) macrophages compose approximately one-fourth of CD33\(^+\) colonic macrophages in inflamed UC tissue, and that these CD14\(^+\)CD33\(^+\) cells respond to the commensals *E. coli* and *E. faecalis* in a manner more similar to CD14\(^+\)CD33\(^+\) cells from normal colon tissue than to CD14\(^+\)CD33\(^+\) cells from CD tissue. The main distinguishing feature between normal and UC CD14\(^+\)CD33\(^+\) macrophages in these studies was that cells from UC tissue secreted higher levels of IL-6. Lampinen and colleagues (47) report that CD14\(^+\)CD33\(^+\) macrophages can account for nearly two-thirds

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**Table 1. Prevalence of M1 and M2 macrophages in Crohn’s disease and ulcerative colitis**

<table>
<thead>
<tr>
<th>IBD Subtype</th>
<th>Macrophage Phenotype</th>
<th>Marker(s) Used</th>
<th>Contribution to Colonic Macrophages, %</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease</td>
<td>M1</td>
<td>CD14(^+)CD33(^+)</td>
<td>40–50</td>
<td>Kamada et al. (42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD163(^+)pSTAT1(^+)</td>
<td>48.551</td>
<td>Barros et al. (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD163(^+)RBP-J(^+)</td>
<td>61.594</td>
<td>Barros et al. (9)</td>
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<tr>
<td></td>
<td></td>
<td>CD68(^+)pSTAT1(^+)</td>
<td>80.734</td>
<td>Barros et al. (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD68(^+)RBP-J(^+)</td>
<td>72.477</td>
<td>Barros et al. (9)</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>CD68(^+)CD206(^+)</td>
<td>~15</td>
<td>Hunter et al. (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD68(^+)CD206(^+)</td>
<td>14.133</td>
<td>Vos et al. (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD163(^+)CMAF(^+)</td>
<td>35.507</td>
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</tr>
<tr>
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<td>CD68(^+)CMAF(^+)</td>
<td>18.349</td>
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</tr>
<tr>
<td></td>
<td>Uncertain</td>
<td>CD14(^+)HLA-DR(^{\text{dim}})</td>
<td>~40</td>
<td>Thiesen et al. (97)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD14(^+)HLA-DR(^{\text{dim}})</td>
<td>~70</td>
<td>Magnusson et al. (54)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD14(^+)HLA-DR(^{\text{hi}})</td>
<td>~30</td>
<td>Magnusson et al. (54)*</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>M1</td>
<td>CD68(^+)CD86(^+)</td>
<td>~20–40</td>
<td>Cosín-Roger et al. (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CLEC5A(^+)</td>
<td>41</td>
<td>González-Dominguez et al. (31)</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>CD68(^+)CD206(^+)</td>
<td>28.625</td>
<td>Vos et al. (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD68(^+)CD206(^+)</td>
<td>~50–70</td>
<td>Cosín-Roger et al. (15)</td>
</tr>
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<td></td>
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<td>CD163L1(^+)CLEC5A(^+)</td>
<td>59</td>
<td>González-Dominguez et al. (31)</td>
</tr>
<tr>
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<td>Uncertain</td>
<td>CD14(^+)CD33(^+)</td>
<td>~25</td>
<td>Kamada et al. (42)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD14(^+)CD33(^+)</td>
<td>~55–60</td>
<td>Lampinen et al. (47)*</td>
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<td>CD14(^+)HLA-DR(^{\text{dim}})</td>
<td>~78</td>
<td>Magnusson et al. (54)*</td>
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<td></td>
<td></td>
<td>CD14(^+)HLA-DR(^{\text{hi}})</td>
<td>~22</td>
<td>Magnusson et al. (54)*</td>
</tr>
</tbody>
</table>

*No functional assays or cytokine/marker analysis performed. †These cells were less proinflammatory than those from CD. ‡At least a subset of these cells produce the Th2 chemokine CCL11, and macrophage number correlated both with eosinophil infiltration and CCL11 mRNA expression.

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of CD33⁺ rectal macrophages in UC tissue, in contrast to the findings by Kamada et al. (42) that CD14⁺CD33⁺ macrophage infiltration correlated with rectal eosinophil numbers and levels of CCL11 and that at least some of these CD14⁺ macrophages express CCL11, as demonstrated by double immunofluorescence. This population of CD14⁺CD33⁺ colonic macrophages has been shown by Uo and colleagues (98) to secrete TNF-α in response to immunoglobulin G ICs, which are thought to play a role in UC pathogenesis. As yet, it is difficult to determine whether these CD14⁺CD33⁺ cells consist of a single macrophage population, or whether they consist of both pro- and anti-inflammatory macrophages. Immunohistochemical analyses of tissue from UC patients seem to indicate that, although M1 macrophages are present, the predominant macrophage population exhibits an M2 phenotype (Table 1). Cosín-Roger and colleagues (15) have demonstrated that M1 macrophages, defined as CD68-positive macrophages that stain for CD86, compose about two-fifths of CD68-positive macrophages and one-fifth of CD68-positive macrophages in colonic tissue from newly diagnosed and chronic UC, respectively. Using CLEC5A as a marker for M1 macrophages, González-Domínguez and colleagues (31) found that 41% of colonic macrophages from UC patient tissue were of the M1 phenotype. Although Vos et al. (99) have reported CD206⁺ two-thirds of CD68⁺ macrophages in newly diagnosed UC and about one-half of CD68⁺ macrophages in chronic UC. Lastly, González-Domínguez and colleagues (31) demonstrated that M2 macrophages, defined as CD163L1⁺CLEC5A⁻ cells, account for nearly two-thirds of total colonic macrophages in UC tissue.

Macrophage Phenotype in CRC

The macrophage is an important component of the tumor microenvironment (91). Tumor-associated macrophages (TAMs) have been identified in several different types of tumors and for the most part are thought to be of the M2 phenotype (93). The anti-inflammatory, prohealing properties of these TAMs prevent immune responses against tumor cells and promote the growth and dissemination of these tumors, or “wounds that never heal” (93). TAMs have classically been considered MDMs recruited to the tumor by the tumor cells. Interestingly, a recent study in a mouse model of mammary tumors has shown that, although TAMs are monocyte derived, they have a higher proliferative capacity and less of an M2-like transcriptional profile than mammary macrophages (26). Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells present in tumor-bearing hosts and can be subdivided into monocytic MDSCs (M-MDSCs) and granulocytic MDSCs (G-MDSCs) (58). Within tumors, M-MDSCs, but not G-MDSCs, rapidly differentiate into TAMs (14, 45, 46). Therefore, TAMs may originate from monocytes, by local proliferation, and from M-MDSCs. The mechanistic details on how TAMs acquire an M2 phenotype are incompletely understood. A recent study by Colegio and colleagues (13) shows that lactic acid resulting from tumor cell glycolysis is one of the possible signals that leads TAMs to acquire an M2 phenotype by inducing VEGF and arginase-I expression in these cells via hypoxia-inducible factor-1α. Pello and colleagues (73) suggest that the transcription factor c-Myc may also be involved in promoting the M2 phenotype of TAMs. Nevertheless, it is worth mentioning that, while M2 TAMs promote tumor growth and metastasis, M1 macrophages also play important roles in tumorigenesis and cancer. They can contribute to the induction of this oncogenic process by the prolonged secretion of proinflammatory mediators in settings of chronic inflammation, yet they can antagonize the growth of established tumors via stimulation of an antitumor immune response and direct tumoricidal activities (91).

In the United States, CRC is the third most common form of cancer in men and in women, according to the American Cancer Society (2). CRC is also the second deadliest form of cancer after lung cancer for the general population. Sporadic CRC is the most common modality of CRC and results from the adenoma-carcinoma sequence. In this sequence, genetic and epigenetic alterations in the colonic epithelium lead to the development of an adenoma, a benign tumor also known as an adenomatous polyp, that continues to accumulate additional alterations and, by doing so, progresses to carcinoma (Fig. 4) (77, 101).

Several in vitro studies support an M2 phenotype for CRC TAMs and an antitumor role for M1 macrophages in CRC. Pello and colleagues (73) demonstrated that treating human MDMs with conditioned medium from SW480 human CRC cells induces c-MYC expression to levels comparable to those of M2(IL-4) macrophages. Similarly, Edin and colleagues (21) have shown that in vitro exposure of human MDMs to conditioned media from three colon cancer cell lines (RKO, SW480, Caco-2) induced an M2-like phenotype, and that conditioned media from cancer cell lines affected the phenotype of M1(LPS+IFN-γ), but not M2(IL-4 or IL-10) macrophages. Engström and colleagues (23) found that treating Caco-2 and HT-29 cancer cells with conditioned medium from M1(LPS+IFN-γ) macrophages, but not M2(IL-4+IL-13) or M0 macrophages, reduced colon cancer cell line numbers by inhibiting cell cycling. The NF-κB pathway in both cancer cells and macrophages appears to be important for the induction of an M2 phenotype in macrophages conditioned with media from cancer cells. Inhibition of the NF-κB pathway with small interfering RNA targeting the p50 subunit of NF-κB in mouse peritoneal macrophages reduces the M2 phenotype induced in...
these cells by conditioned media from CT26 mouse colon cancer cells (43). Conditioned media from CT26 cancer cells were also found by Ryan and colleagues (84) to induce an M2-like phenotype in mouse macrophages from the RAW cell line; however, conditioned media from CT26 cancer cells transfected to express a degradation-resistant version of the NF-κB inhibitor IkB (therefore rendered NF-κB-deficient), induced an M1-like phenotype in RAW macrophages, which was characterized by increased IL-12p40 and NO2 secretion. Ryan et al. (84) also demonstrated that tumors generated from NF-κB-deficient CT26 cells were smaller with more mononuclear cells and iNOS+ mononuclear cell infiltration, less angiogenesis, and more apoptotic tumor cells in the center of the tumor. Taken together, these in vitro studies indicate that TAMs in CRC manifest an M2 phenotype and suggest that inducing an M1 phenotype in CRC TAMs could serve as a therapeutic approach for treating this malignancy.

Conflicting reports have indicated that the presence of TAMs in CRC can be associated with either a better or worse prognosis (24). However, those studies evaluated only the presence of TAMs, disregarding their phenotype. Few studies have investigated the phenotype of TAMs in tissue from patients with CRC. Pander et al. (72) reported that the M2 phenotype predominated in the tumors of 10 patients with untreated stage III CRC, as determined by immunohistochemical staining for CD163. Ong et al. (71) found that the percentage of proinflammatory macrophages (M1), measured by double immunofluorescence for CD68 and IFN-γ, in tumors from five patients with CRC were 6.6, 8.3, 16, 31, and 50% (71). The most extensive study was recently performed by Edin et al. (20), where colorectal tumors from 485 patients were evaluated for the presence of iNOS, an M1 marker, and CD163, an M2 marker. This study found that the M2 phenotype predominated in the invasive tumor front, and that increased numbers of M1 macrophages correlated with a better prognosis. Interestingly, McLean et al. (60) found that colonic adenomatous polyps contained increased macrophages compared with surrounding normal areas, that the number of macrophages increased with the severity of the lesion (i.e. higher numbers in cancerous polyps and lower numbers in polyps with low-grade dysplasia, a premalignant neoplastic change), and that cancerous polyps had a higher proportion of M2 macrophages than those polyps with dysplasia alone. Interestingly, M1 macrophages appeared to predominate in dysplastic polyps, reportedly contributing 45 and 67% of total macrophages in polyps with low- and high-grade dysplasia, respectively, and only 34% in cancer-containing polyps. This M1 phenotype in polyp TAMs is likely a consequence of the inflammatory milieu generated in response to the influx of microbial products that results from barrier defects in adenomas reported by Grivennikov and colleagues (32). This increased permeability, however, could also contribute to tipping the balance in favor of M2 TAMs. CRC-associated E. coli, but not commensal E. coli, has been shown to survive within macrophages derived from the THP-1 human monocyte cell line and to induce Cox-2 expression in these cells (80). Cox-2 activity is important for the M2 phenotype of TAMs, as inhibition of this enzyme skews TAMs toward an M1 phenotype in tumors of ApcMin/+ mice (67). Based on these data, it can be concluded that M2 macrophages predominate in CRC TAMs (Fig. 4).

Colonic Macrophages as Therapeutic Targets and Probiotic Therapy

Given the key roles that this cell plays in homeostasis, inflammation, and cancer, the colonic macrophage is an intriguing therapeutic target. In chronic inflammatory conditions such as IBD, diminishing the proinflammatory effects of M1 macrophages and/or augmenting the anti-inflammatory activity of M2 macrophages could both treat the condition and reduce the risk of developing inflammation-induced comorbidities. In cancer, inhibiting the protumor functions of TAMs and/or inducing M1-like tumoricidal activities may lead to tumor shrinkage and decreased metastases (Fig. 5). Several approaches for targeting these cells are currently under investigation, including the use of adoptive transfer, biologics, micro- and nanoparticles, and probiotics. The beneficial effect of M2 macrophage adoptive transfer in models of colitis (3a, 37, 50) has led to proposing the use of M2 macrophages derived from autologous monocytes as a potential anti-inflammatory treatment for patients with IBD (37, 50).

Monoclonal antibodies, also known as biologics, targeting TNF-α are currently used as therapeutic agents for patients with IBD. Vos and colleagues (99, 100) have shown in vitro that these antibodies induce an M2-like phenotype in macrophages in an Fc fragment-dependent manner, and that the resulting macrophage secretes high levels of IL-10, antagonizes T-cell proliferation, and promotes wound healing. They also demonstrated that M2 macrophages (CD68+CD206+) were increased in the mucosa of patients that responded to infliximab (99). These findings suggest that biologics could be further modified to enhance their anti-inflammatory and prohealing effect on macrophages. However, it should be noted that, although treatment with anti-TNF-α antibodies reduces...
inflammation and granuloma numbers in CD patients, it also leads to submucosal fibrosis in these patients (87). Indeed, fibrosis is an unwanted consequence that can result when the M2 phenotype is unrestrained, and this should be kept in mind when developing therapies that enhance the numbers and/or activity of M2 macrophages.

Mannosylated bioreducible nanoparticles have been proposed as vehicles to deliver therapeutic agents to M2 macrophages, which express increased levels of the mannose receptor (CD206) (103). Ex vivo experiments show that these nanoparticles can be used to deliver small interfering RNA targeting TNF-α to macrophages and decrease levels of this cytokine (103). These mannosylated nanoparticles could, therefore, be an interesting vehicle to target macrophages in diseases associated with increased M2 macrophages, such as CRC and possibly UC. Getts and colleagues (29) have developed negatively charged immune-modifying microparticles that target circulating inflammatory monocytes for apoptosis in the spleen and have shown that these microparticles can reduce disease activity scores and mucosal and submucosal proinflammatory monocyte infiltration, resulting in increased epithelial proliferation and repair, in a model of DSS colitis.

In the liver, clodronate and gadolinium chloride are used to selectively eliminate M2 and M1 macrophages, respectively, in animal models (48). It seems that clodronate depletes M2 macrophages in the colon (79), yet it is unclear whether gadolinium chloride depletes colonic M1 macrophages. It has recently been shown that treatment with gadolinium chloride ameliorates colitis in mice treated with TNBS or DSS (19). This study did not find a significant reduction in macrophages in the colon of mice treated with gadolinium chloride, but this could be attributed to the use of F4/80 as the macrophage marker, as proinflammatory macrophages in the colon express low levels of F4/80 (7, 96).

The potential therapeutic effects of probiotics for patients with IBD and other conditions are actively being investigated. Probiotics, as defined by the World Health Organization and the United Nations Food and Agricultural Organization, are “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host” (24a). Patients with IBD often have reduced microbial diversity, increased levels of proteobacteria, and decreased levels of bacteroides and firmicutes (55). This dysbiosis, or detrimental alteration in the composition of the microflora, is thought to be a major contributing factor in IBD and CRC. Dysbiosis alone can induce colitis, as Garret and colleagues (28) demonstrated that rearing of healthy mice by colitic mice was sufficient to induce colitis in the previously healthy mice. Therefore, treatment with probiotics is a promising approach to correct dysbiosis and ameliorate or prevent its damaging effects.

A possible mechanism by which probiotics may promote homeostasis in the colon is by acting on colonic macrophages. As discussed above, these cells are located in close proximity to the epithelial barrier, they routinely sample luminal antigens that can penetrate this barrier, and they have avid anti-inflammatory capacity. A study by Hayashi and colleagues (34) demonstrated that administration of the probiotic Clostridium butyricum ameliorates DSS colitis in mice in an IL-10-dependent and a T-cell-independent manner. In this study, macrophage production of IL-10 and signaling through TLR-2 and MyD88 were deemed necessary for mediating the beneficial effects of C. butyricum.

The probiotic mixture VSL#3 has also been suggested to ameliorate colitis by acting on colonic macrophages. This probiotic formulation contains eight strains of Gram-positive bacteria: Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii subspecies bulgaricus, Lactobacillus casei, Lactobacillus plantarum, and Streptococcus salivarius subspecies thermophilus. Bassaganya-Riera and colleagues (10) reported that macrophage-specific depletion of the nuclear receptor PPAR-γ abrogates the effects of VSL#3 on histological damage in a mouse DSS model of acute colitis. Although the authors state that treatment with VSL#3 reduced colonic M1 macrophages, the data presented do not convey this finding for several reasons. First, they define M1 macrophages as F4/80+MCP-1+ cells. Monocyte chemotactic protein-1 (MCP-1) is not a defining chemokine for M1 macrophages in the mouse; in fact, this chemokine has been linked to Th2 responses (57), suggesting that it could actually indicate M2 rather than M1 macrophages. Additionally, and as discussed above, F4/80 positivity is more prevalent on the mature M2-like colonic macrophages than it is in M1 colonic macrophages. Third, the prevalence of these F4/80+MCP-1+ cells in the lamina propria of mice with different treatments do not correlate with increasing or decreasing pathology. Therefore, the in vivo effect of this probiotic formulation on colonic macrophages remains poorly understood, especially in terms of whether it alters M1 or M2 macrophage numbers or phenotype. In vitro data from our laboratory show that, while not greatly affecting the proinflammatory phenotype of M1(IL-4), and unpolarized macrophages produces a balanced phenotype in which secretion of anti-inflammatory and prohealing factors is increased alongside that of certain inflammatory cytokines (39). Regardless of our understanding of its effects on macrophages, VSL#3 appears to impart positive effects in patients with IBD (89) and in animal models of IBD-associated CRC (3).

Conclusion

In summary, colonic macrophages can both promote and antagonize homeostasis, depending on their phenotype. M1 colonic macrophages are involved in mediating colonic inflammation in models of colitis and in CD, yet might help battle tumors in CRC. M2 colonic macrophages normally promote homeostasis, but can also be subverted by tumors to promote their growth and metastasis. The roles of M1/M2 macrophages in UC remain poorly understood. Despite the great strides made in recent years regarding our understanding of the colonic macrophage, much remains to be known. Efforts should be made to expand our knowledge of colonic macrophages in humans, rats, and other species so as to facilitate the development of new therapies for patients with diseases of the colon, such as IBD and CRC. Future studies can take advantage of humanized mice models, specifically those engineered to foster development of human innate immune cells, such as that reported by Rongvaux et al. (83).

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DISCLAIMERS

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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