Macrophages Versus Escherichia coli: A Decisive Fight in Crohn’s Disease

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Abstract: The pathophysiology of Crohn’s disease (CD), a chronic inflammatory bowel disease, remains imperfectly elucidated. Consequently, the therapeutic armamentarium remains limited and has not changed the natural history of CD hitherto. Accordingly, physicians need to identify new therapeutic targets to be able to alter the intestinal damage. The most recent hypothesis considered CD as resulting from an abnormal interaction between microbiota and host immune system influenced by genetics and environmental factors. Several experimental and genetic evidence point out intestinal macrophages in CD etiology. An increase of macrophages number and the presence of granulomas are especially observed in the intestinal mucosa of patients with CD. These macrophages could be defective and particularly in responses to infectious agents like CD-associated Escherichia coli. This review focuses on, what is currently known regarding the role of macrophages, macrophages/E. coli interaction, and the impact of CD therapies on macrophages in CD. We also speculate that macrophages modulation could lead to important translational implications in CD with the end goal of promoting gut health.

Key Words: E. coli, Macrophages, Crohn’s disease

The intestine is the largest reservoir of macrophages in the whole body, and macrophages are the most abundant mononuclear phagocytes in the gut lamina propria (LP).1 Gut macrophages are strategically located in the subepithelial area of intestinal LP and in the gut-associated lymphoid tissue and the luminal microenvironment. These locations allow intestinal macrophages to be very close to luminal bacteria and antigens.2 Functions of intestinal macrophages include the maintenance of homeostasis due to the tolerance of the commensal flora, the protection of the mucosa from pathogens, the elimination of cellular debris, and the regulation of inflammatory responses against luminal bacteria.

Crohn’s disease (CD) is a chronic disorder that causes inflammation of the gastrointestinal tract and affects as many as 2 million patients worldwide.3 This disease can be particularly debilitating in affecting the entire digestive tract and can alter significantly patients’ quality of life. The therapeutic armamentarium remains limited (steroids, immunosuppressants, anti-TNF agents, and anti-integrins therapies) and noncurative in CD, hence the necessity to better understand the disease to identify the best targets. Although the complex etiology of CD has not been yet fully elucidated, several experimental and genetic evidence point out defective innate immunity in CD. Thus, several studies have shown that the number of intestinal macrophages is increased in patients with CD4–7 and that CD is characterized by the frequency of aggregates of epithelioid macrophages referred to as granulomas, the histopathological hallmark of CD.8,9 In addition, macrophages from patients with CD present disorders of their cytokine secretion profiles and particularly in response to infectious agents.10–13 Taken together, these defects could result in impaired detection and clearance of bacteria and inappropriate inflammatory response to infection. Gut bacterial dysbiosis associated with CD should also play a causal role in chronic inflammation. Patients with CD, compared with healthy controls, have fewer bacteria with anti-inflammatory properties such as Faecalibacterium prausnitzii in the Firmicutes phylum, and more bacteria with proinflammatory properties such as Escherichia coli, in the Proteobacteria phylum, and more particularly adherent-invasive E. coli a.k.a. AIEC.14

Here, we report the synthesis of the current knowledge and recent advances on macrophages in CD pathogenesis. This review consists of three main parts. In the first part, we present intestinal macrophages and describe abnormalities related to macrophages that have been observed in CD. The second part is dedicated to interactions between CD-associated E. coli and macrophages. In the last part, we discuss the impact of CD therapy on macrophages and their activities.
**INTESTINAL MACROPHAGES**

**Intestinal Macrophages and Their Functions**

Primitive macrophages rising from yolk sac–blood islands spread into embryos on the establishment of the blood circulatory system and colonize the whole embryo. In parallel, the hematopoiesis occurring in the fetal liver generates monocytes that will gain access to the blood and reach tissues, except the brain, a few days after yolk sac–derived macrophages. Fetal liver–derived monocytes will thereafter proliferate and differentiate into macrophages. After birth, these embryonic precursor cells are replaced in the intestine around the time of weaning by cells derived from blood monocytes which express the glycoprotein lymphocyte antigen 6C (Ly6C). These cells are continuously recruited in the intestine to renew or enrich the pool of resident macrophages. Factors influencing monocytes recruitment in the intestine include expression of C-C chemokine receptor type 2 (CCR2), cytokines (transforming growth factor-β [TGF-beta], interleukin-8 [IL-8]), and the microbiota.

During intestinal macrophages differentiation and through different steps, cells acquire several surface markers such as major histocompatibility complex II (MHC II), glycoprotein F4/80, clusters of differentiation (CD) 64, 11c, 11b, 40, 68, 80, 86, and 14, CCR2, toll-like receptors (TLRs) 2 and 4, and CX3C chemokine receptor 1 (CX3CR1). A large heterogeneity of resident macrophages exists in the intestine, and molecular signaling of their differentiation is not yet fully understood. This may reflect exposure of monocytes to local signals such as epithelial or stromal secretions (thymic stromal lymphopoietin, TGF-β, prostaglandin E2 [PGE2], semaphorin 7A, and colony stimulating factors 1 and 2), and exposure to specific cellular microenvironment such as abundance of innate and adaptive lymphocytes (type 2 innate lymphoid cell [ILC2], natural killer [NK] and natural killer T [NKT] cells, B cells, zδ and γδ T lymphocytes), mast cells, and other myeloid cells. Macrophage differentiation is also under the influence of the microbiota. Mortha et al. showed that intestinal microbiota modulates the production of the colony stimulating factor 2 and, as a consequence, the number of colonic macrophages.

Alongside dendritic cells, intestinal macrophages ensure the role of guardians of the gut. They maintain intestinal homeostasis, epithelial integrity, wound healing, and are key actors in intestinal inflammatory responses. They do not mediate strong proinflammatory responses after bacterial recognition and thereby maintain symbiotic relationships with the intestinal microbiota. On injury or infection, the intestinal inflammatory response is related to the recruitment of new monocytes from the blood circulation with proinflammatory properties that will differentiate into proinflammatory macrophages. These cells have a TLR-responsive CX3CR1<sup>+</sup> Ly6C<sup>+</sup> profile and produce several inflammatory mediators (inducible nitric oxide synthase, IL-1β, tumor necrosis factor [TNF-α], IL-12 and IL-6) that favor the amplification of immune responses and tissue injury. Intestinal macrophages contribute especially to the polarization of intestinal T helper cells (Th1/Th2 and Th17 cells), CD4<sup>+</sup> T cells subsets, within inflamed tissues, which are implicated in the pathogenesis of chronic inflammatory bowel diseases. In a normal situation, macrophages contribute to return to the tissue homeostasis by eliminating the causal agent of inflammation and the products resulting from the degradation of damaged tissue. They express arginase-1 (Arg-1), mannose receptor, and release anti-inflammatory cytokines (IL-4, IL-13, IL-10, and TGF-β1) to suppress all processes initiated during the inflammatory response and promote tissue repair.

**Macrophages in CD Mucosa**

Several studies have shown that the number of intestinal macrophages is increased in patients with CD. More specifically, macrophage aggregations, focal subepithelial dense accumulations, and infiltration throughout the mucosa have been reported in patients with CD. In addition, the colonic LP of patients with CD contains an increased number of macrophages that express cysteine-rich scavenger receptors such as CD68 and CD163. These proteins are implicated in monocytes/macrophages maturation and play a role in the development of the immune system and the regulation of the immune response. In inflamed mucosa of patients with CD, the presence of CD68<sup>hi</sup> and CD163<sup>hi</sup> macrophages is associated with an increase of IL-17 and TNF-α expression. These data suggest a role for CD68<sup>hi</sup> and CD163<sup>hi</sup> macrophages in the amplification and perpetuation of the ongoing mucosal inflammation in CD and subsequently with CD activity. The presence of a large number of CD14<sup>+</sup> macrophages was also observed in patients with CD compared with healthy subjects. These cells secreted high amounts of proinflammatory cytokines such as IL-23 and TNF-α and could favor Th17-related inflammatory pathways and innate pathways implicated in CD pathogenesis. The triggering receptor expressed on myeloid cells 1 (TREM1) is also overexpressed by intestinal macrophages of patients with CD compared with control subjects. TREM1<sup>+</sup> macrophages contribute to the intestinal inflammation by secreting proinflammatory mediators such as TNF-α, IL-6, IL-8, monocyte chemotactic protein-1, and IL-1β.

The hallmark of CD is the presence of epithelioid granulomas, a collection of macrophages and other inflammatory cells which are among the most uniformly observed and specific microscopic features of CD. However, the significance of epithelioid granulomas in CD is unclear. Several studies have attempted to identify an etiological agent in granulomas. The occasional finding of microorganisms-specific DNA may represent either a bystander phenomenon or the cause of the disease. It is conceivable that particulate matter from dead organisms or very slow growing organisms can trigger the granulomatous reaction. There are various lines of evidence suggesting that *E. coli* is involved in the formation of granulomas: (1) *E. coli* antigens are present in CD granulomas; (2) *E. coli* DNA is detected in 80% of microdissected granulomas from patients with CD; and (3) *E. coli* is able to induce the aggregation of infected human macrophages and that these aggregates recruit surrounding lymphocytes. A multivariate analysis of the studies focusing on the...
presence of epithelioid granulomas in CD revealed that their frequency may indicate a more aggressive disease process. This could be, in part, explained by the leakage of activated macrophages from granulomas. These cells could then secrete proinflammatory cytokines such as TNF-α, reactive oxygen species such as H2O2, O2•−, O2•*, nitrogen derivatives and proteolytic enzymes that damage the neighboring tissue. In addition, granulomas were frequently identified in biopsies with PPs,36 the immune sensors of the intestine. PPs are also composed of activated macrophages and supposed origin of the earliest mucosal lesions in ileal CD.37,38 Thus, the implementation of studies to investigate the role of intestinal macrophages during CD could bring major elements in understanding CD pathogenesis and in the identification of new therapeutic targets.

**CD-Associated Polymorphisms: Impact on Macrophage Functions**

Many genetic variants have been identified as CD susceptibility factors.39 The successful genome-wide association studies have provided a rational framework for novel mechanistic insights and directions regarding research in CD. Overall, 30 loci were specifically associated with CD. They are involved in defective intracellular bacteria killing and innate immunity (CARD15/nucleotide oligomerization domain 2 [NOD2], immunity-related GTPase family M [IRGM], IL23R, LRRK2, and ATG16L1) and deregulated adaptive immune responses (IL-23 and Th17 cell pathway: IL23R, IL12B, STAT3, JAK2, and TYK2).40,41

Here, we report CD loci that may affect directly the function of macrophages. The first locus identified as a risk factor for CD was NOD2.52,43 NOD2 is a member of the NOD-like receptor family, which encompass intracellular sensors of pathogen/microbe-associated molecular patterns playing crucial roles in the innate immunity.44 NOD2 recognizes muramyl dipeptide, a component of the peptidoglycan present in the bacterial cell wall and has several functions which have been highlighted to provide a potential link to its association with CD: (1) the maintenance of the intestinal epithelial barrier integrity,45–48 (2) the regulation of immune homeostasis in the gut,49–51 and (3) the regulation of the microbiota.52,53 Three mutations in NOD2 have been documented to be strongly associated with CD onset. They are located within the leucine-rich repeat domain of the NOD2 protein, which corresponds to the microbe-associated molecular pattern recognition region.54 These mutations result in a truncated NOD2 protein (frameshift mutation, L1007fsinsC) or in amino acid substitutions (R702W and G908R).52,45,55 NOD2 mutations lead to decreased muramyl dipeptide–induced cytokine responses which are critical in the control of commensal microbiota in the lumen of the gut.56 NOD2 mutations have also important functions in relation to autophagy, a complex intracellular protein degradation mechanism by which the cell forms double-membrane vacuoles that ultimately fuse with lysosomes whose functions include the elimination of proteins arising from cellular stress responses or ingested pathogenic bacteria, and the processing of antigens for their presentation in immune responses.57,58 The most prevalent NOD2 mutation in patients with CD (L1007fsinsC) result in a frameshift mutation that generates a truncated NOD2 protein and had a profound effect on the autophagy response triggered by intracellular bacteria.59

Besides NOD2, other genes involved in autophagy are associated with CD. Indeed, several genome-wide association studies indicate a highly significant and replicated association between CD and three variants of autophagy genes, i.e., ATG16L1 (Autophagy-related like 1),60,61 IRGM,52–64 and ULK1 (unc-51 like autophagy activating kinase).65 Other CD genetic associations were also defined for genes encoding several proteins implicated in autophagy regulating pathways such as PTPN2 (protein tyrosine phosphatase nonreceptor type 2),66,67 LRRK2 (leucine-rich repeat kinase 2),41,68 CYLD (cylindromatosis),69 and XBP-1 (X-box binding protein 1).70 Macrophages expressing autophagy risk variants (ATG16L1, NOD2, and IRGM) harbor defects in autophagy induction, bacterial trafficking, and antigen presentation.71–73 Autophagy-deficient macrophages fail to kill intracellular bacteria and to present antigen by MHC class II, leading to inappropriate activation of the adaptive immune system and to a high production of IL-1β and IL-18. This mechanism induces a severe inflammation and participate to the maintenance of chronic inflammatory status.74

**Phagocytic Capability of CD Macrophages and Cytokine Secretion Profiles**

Data regarding monocyte (the precursor cell of macrophages) phagocytosis in CD are conflicting, some report phagocytic dysfunction,75–77 whereas some do not.78,79 In the same way, results regarding cytokine secretion profiles of macrophages in CD are also conflicting (Table 1) but most of them highlight large defects of cytokine secretion profiles of macrophages from patients with CD. This could favor chronic inflammatory status and merit further investigations to bring major elements in the understanding of CD pathogenesis and in the identification of new therapeutic targets. For that, it will be important to take into account the complexity of the cytokine response and of the phenotype of macrophages from patients with CD, but also a wide range of factors which could deeply influence the results such as (1) the stimulating signal considered (e.g., TLRs ligand versus bacteria), (2) the macrophage origin (e.g., monocyte-derived macrophages [MDMs] versus intestinal macrophages), (3) the cell preparation, (4) the disease status (e.g., active versus quiescent), (5) treatment of the patients, (6) genetic factors, and others.

**E. coli AND MACROPHAGES IN CD**

**Dysbiosis in CD: Abnormal Colonization by E. Coli**

Chronic inflammation in CD results from the activation of mucosal immune system by the gut microbiota. Over the last 15 years, genome-wide association studies identified links between disease and genes involved in detection, microbial signaling, and
### TABLE 1. Cytokine Secretion Profiles of Macrophages in CD Reported

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<thead>
<tr>
<th>Macrophage origin</th>
<th>Cytokines</th>
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<td>Increase</td>
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**Marks et al**<sup>79a</sup>  
Quiescent CD  
Peripheral venous blood  
CD versus HC  
MDP  
Wound fluid  
C5a  
TNF-α  
IL-8  
IL-1β  
IL-10  
IL-8  
IL-10

**Sewell et al**<sup>11</sup>  
Quiescent CD  
Peripheral venous blood  
CD versus HC  
Pam3  
Flagellin  
LPS  
LPS  
TNF-α

**Smith et al**<sup>12</sup>  
Quiescent CD  
Peripheral venous blood  
CD versus HC  
Heat-killed *E. coli* clone NCTC  
10418  
TNF-α  
INF-γ

**Campos et al**<sup>13</sup>  
CD (undefined status)  
Peripheral venous blood  
CD  
*E. faecalis*  
IL-12  
TNF-α  
IL-10  
IL-23  
IL-12  
IL-10  
IL-12  
IL-10  
IL-23  
IL-10  
IL-12  
IL-10  
IL-23  
IL-10  
IL-12  
IL-10

*E. Coli*  
IL-12  
TNF-α  
IL-10  
IL-23

*M. avium subsp. paratuberculosis*  
TNF-α  
IL-10  
IL-23

*M. avium subsp. avium*  
TNF-α  
IL-10  
IL-23

HC  
*E. faecalis*  
TNF-α  
IL-12  
IL-10  
IL-23  
IL-10  
IL-12  
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IL-23  
IL-10

*E. Coli*  
TNF-α  
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<th>Decrease</th>
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<td>Unstimulated</td>
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<td>IL-23</td>
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<td>Vazeille et al</td>
<td>Quiescent and active CD</td>
<td>CD AIEC LF82</td>
<td>IL-8</td>
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<td>IL-6</td>
<td>TNF-α</td>
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<td></td>
<td>HC AIEC LF82</td>
<td>IL-8</td>
<td>TNF-α</td>
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<td>Nonpathogenic E. coli K-12</td>
<td>IL-8</td>
<td>TNF-α</td>
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<td>CD versus HC</td>
<td>Unstimulated</td>
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<td>AIEC LF82</td>
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<td>TNF-α</td>
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<td>Elliott et al</td>
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<td>IL-8</td>
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CD, Crohn’s disease; HC, healthy controls; LPS, lipopolysaccharides; MDP, muramyl dipeptide; UC, ulcerative colitis.
clearance of bacteria, shedding in light of the role of gut microbiota in CD pathogenesis. As both genetic mutation and inflammation induced remodeling of the gut microbiota, it is not surprising to observe dysbiosis in patients with CD. Reduced diversity in microbiota of patients with CD has been reported, especially within Firmicutes and Bacteroidetes phyla. At the species rank, imbalance was also observed with reduction of bacteria with anti-inflammatory properties such as Faecalibacterium prausnitzii and increased amount of bacteria with proinflammatory potential such as E. coli.

Particular efforts have been performed to identify specific infectious agents involved in CD pathogenesis over the past 2 decades and still continue. As disorders observed in CD resemble those of intestinal tuberculosis and Johne’s disease, mycobacteria have been suspected to play a role in CD. In addition, associations of CD with measles virus or intestinal pathogens such as Listeria or Yersinia were reported but not confirmed.

Besides involvement of pathogens, the hypothesis is that remodeling of the gut microbiota that occurs in patients with CD may favor emergence and/or expansion of pathobionts. Abnormal colonization of ileum and colon mucosa of patients with CD by E. coli strains with adhesive and invasive properties has been convincingly reported by independent groups. Association of AIEC with terminal ileum mucosa should be related, at least in part, to increased ileal expression of carcinoembryonic antigen–related cell adhesion molecule 6 (CEACAM6), which act as a receptor for AIEC bacteria. Other important pathogenicity mechanisms of AIEC have been identified and are nicely described in reviews.

The next section will focus on CD-associated E. coli and macrophage interactions.

The Presence of E. Coli in CD Macrophages

Macrophages are, with dendritic cells, key cellular vehicles that enable bacterial trafficking to mesenteric lymph nodes (MLNs). By using culture-dependent techniques, independent groups showed that bacteria are more frequently isolated from MLN in patients with CD than in controls. E. coli has been immunodetected in MLN of patients with CD and among bacteria isolated and cultured from MLN. E. coli was the dominant species. Recently, O’Brien et al have characterized the microbial communities of resected nodes from patients with CD and controls by high-throughput sequencing analysis. They found that E. coli was more abundant in nodes from patients with CD than from controls and that patients with terminal ileal CD had a greater proportion of E. coli reads in their nodes than patients with colonic or ileocolonic disease.

Comparison of microbial communities of mucosa and nodes from a same patient showed that they were similar. Whether or not, E. coli bacteria recovered in MLN of patients with CD are AIEC remains to be determined. In transgenic mice expressing human CEACAM6 (CEABAC10 mouse model), we recently showed that AIEC bacteria are able to disseminate to MLN. Abilities of AIEC to translocate across M cells lining PPs and to resist macrophage killing (see Handling of AIEC by Macrophages) are both required for bacteria to gain access to MLN. These properties might also explain the high proportion of E. coli observed in ileal MLNs of patients with CD.

The presence of E. coli within macrophages underneath ulcers, along fissures, and in the mucosal LP of patients with CD was also reported. Prevalence of LP macrophages that contain E. coli was compared in healthy controls and patients with CD and ulcerative colitis (UC). E. coli-containing macrophages were found in LP in 71% of patients with CD versus 11% of patients with UC and were not found in healthy controls.

Handling of AIEC by Macrophages

Comparison of bacteria uptake in MDMs obtained from healthy donors and patients with CD revealed that higher numbers of AIEC are internalized within MDM irrespectively of their origin compared with nonpathogenic E. coli, suggesting that AIEC bacteria may express specific factors or variants that confer them specific ability or best affinity to interact with macrophages. Classically, vacuoles that contain phagocytized material transit along the endocytic pathway and fuse sequentially with early and late endosome and finally with lysosome to form degradative organelles termed phagolysosomes. Although nonpathogenic bacteria are rapidly degraded in phagolysosomes, many pathogens have evolved strategies to escape early from the vacuole, to block the transit of the vacuole, to resist harsh environment of phagolysosomes, or even gain access to intracellular niches that support their intracellular replication. In 2001, Glasser et al showed for the first time that AIEC bacteria are able to survive and replicate within macrophages. In the absence of specific molecular marker of AIEC, this feature is used in combination with the ability of bacteria to adhere to and to invade intestinal epithelial cells, to phenotypically define whether an E. coli strain belongs to the AIEC pathovar. Recently, it has been shown that, in contrast to nonpathogenic bacteria that are efficiently killed by macrophages, AIEC bacteria are able to resist macrophage killing within MDM isolated from healthy controls, and patients with CD and UC. Intramacrophagic replication of AIEC bacteria was observed in MDM from patients with CD but not in MDM from UC and healthy controls, highlighting inability of CD macrophages to control intracellular AIEC. In vitro studies performed in murine and human macrophage cell lines demonstrated that most of the AIEC-containing vacuoles mature without diverting from the classical endocytic pathway and deliver AIEC to lysosomes. To survive in such harsh environment, bacteria should be equipped. Interestingly, AIEC take advantage of the acidic pH of phagolysosomes to replicate. Several bacterial factors involved in survival and/or replication of AIEC within macrophages have been identified and are described in the next section.

Part of AIEC bacteria entering macrophages are rapidly, at their site of entry in the cell, targeted by autophagy, which is a lysosomal degradative pathway involved in detection and elimination of intracellular pathogens. In contrast to bacteria that traffic along the “classical” endocytic pathway, targeting of...
AIEC within autophagosomes leads to their rapid degradation.\textsuperscript{72} Strong association of polymorphisms in several autophagy-related genes including ATG16L1, IRGM, LRRK2, and NOD2 and risk of CD have been identified.\textsuperscript{42,43,60,68} Decrease of ATG16L1 expression with specific siRNA impairs xenophagy against AIEC in the human macrophage cell line THP-1 and favors their intracellular persistence.\textsuperscript{72} Interestingly, pharmacological induction and physiological induction of autophagy enable to overcome defects in intracellular AIEC clearance.\textsuperscript{72} Independent groups reported that monocytes and MDM from patients with CD who are homozygous for the ATG16L1-T300A risk allele are defective in controlling intracellular AIEC.\textsuperscript{10,115} In addition, dendritic cells isolated from donors with CD-associated Nod2 variants and peri toneal macrophages isolated from Nod2 knockout mice exhibited also impaired autophagy against AIEC.\textsuperscript{72,116} These results suggest that AIEC persistence in CD macrophages could be favored by genetic polymorphisms in autophagy-related genes. However, it has been also reported that ATG16L1, NOD2, IRGM, and II-23R genotypes have no influence on the handling of AIEC bacteria by MDM.\textsuperscript{108,117}

Beside genetic conditions, environmental conditions could impact on AIEC–macrophage interactions. Anti–Saccharomyces cerevisiae antibodies (ASCAs) are detectable in the serum of patients with CD.\textsuperscript{118} Epitope for these antibodies is a mannan (mannose \(\alpha1–3\) mannos) that is present in the yeast cell wall and transmembrane glycoproteins of some Mycobacterium species.\textsuperscript{119–122} In vitro studies showed that exposition of human adherent monocytes and MDMs to mannans from microbial origins impaired bacterial activity of these cells and support intramacrophagic persistence of CD-associated \textit{E. coli}.\textsuperscript{122} However, the cellular mechanism involved remains to be identified. To note, other environmental conditions such as iron and production of cellulose by AIEC might also impact on phagocytosis of these bacteria by macrophages and modulate the inflammatory response.\textsuperscript{123}

Factors Involved in AIEC Survival and Replication Within Macrophages

Ability of AIEC bacteria to survive and replicate within the harsh environment of phagolysosomes may be supported by expression of bacterial factors. Expression of almost all factors identified so far to be essential for the intramacrophagic survival and/or replication of AIEC is induced on stress encountered in the phagolysosomes (e.g., acidic pH or nutritional and oxidative stress). However, it is important to notice that no virulence factor specific of all AIEC strains and involved in bacteria resistance to macrophage killing has been identified to date. Hypothesis is that it is rather expression of a set of factors involved in AIEC resistance to stress that may account to their ability to survive intracellularly rather than a specific virulence factor.

Hfq is a central mediator of sRNA-based gene regulation in bacteria that controls virulence and fitness of several intracellular bacteria.\textsuperscript{124} Hfq is required for AIEC to survive and replicate in vitro within the mouse macrophage cell line J774.\textsuperscript{125} Disability of AIEC Hfq–negative mutant to resist macrophage killing could be attributed to the important role of Hfq in promoting bacterial adaptation to stress encountered in the intravacuolar milieu such as acidic pH or reactive oxygen and nitrogen species.\textsuperscript{125}

Screening of a transposon mutant library constructed in the paradigm AIEC LF82 strain points at five bacterial genes (\textit{htrA}, \textit{dsbA}, \textit{yfgL}, \textit{slvB}, and \textit{yraP}) involved in the ability of bacteria to resist macrophage killing.\textsuperscript{126} Among these genes, particular attention has been paid in studying the role of HtrA, a serine protease and chaperone in the periplasmic space, and DsbA, a periplasmic oxidoreductase, in intramacrophagic behavior of AIEC bacteria. \textit{In vitro} experiments showed that expression of \textit{htrA} and \textit{dsbA} genes is both induced by stressful conditions of the intracellular environment and required for intracellular bacteria replication (HtrA) and survival (DsbA).\textsuperscript{126,127} Given that \textit{htrA} and \textit{dsbA} are not virulence factors per se since they are also expressed by commensal and nonpathogenic \textit{E. coli}, one could speculate that this is rather regulation of their expression that may account for their role in AIEC intracellular survival and replication.

Recently, involvement of the GipA (growth in PPs) factor in the ability of AIEC bacteria to disseminate to MLN and to replicate within macrophages has been shed on light.\textsuperscript{107} Except for the probiotic \textit{E. coli} Nissle 1917 strain, none of the sequenced commensal and nonpathogenic \textit{E. coli} strains harbors the \textit{gipA} gene. Interestingly, a higher number of \textit{E. coli} harboring the \textit{gipA} gene were observed in patients with CD (27.3\%) than in controls (17.2\%).\textsuperscript{107} Expression of \textit{gipA} is induced by reactive oxygen species, a stress to which bacteria are particularly exposed in the intracellular environment.\textsuperscript{107} In addition to its role in PPs colonization by AIEC, GipA is also involved in the ability of AIEC to resist oxidative stress and to replicate within macrophages, and it has been shown in a mouse model that deletion of \textit{gipA} impaired translocation of the AIEC to MLN.\textsuperscript{107} Besides, Cieza et al\textsuperscript{128} have also reported a role for IbeA (invasion of the brain endothelium protein A) in the ability of AIEC to invade and translocate across M cells and to survive within macrophage. As for GipA, IbeA might favor intramacrophagic persistence of AIEC by supporting resistance to oxidative stress because deletion of \textit{ibeA} in APEC (avian pathogenic \textit{E. coli}) makes bacteria more sensitive to hydrogen peroxide.\textsuperscript{129}

Other bacterial factors are suspected to support intracellular survival/replication of AIEC within macrophages. Comparison of genomes of AIEC strains with non-AIEC strains and study of their interaction with macrophages identified that growth of AIEC required iron and that the presence of \textit{chuA} gene, which encodes a heme transporter, correlated with persistence in macrophages.\textsuperscript{130}

It cannot also be excluded that, as already described for other pathogens such as \textit{Salmonella typhimurium}, phenotypic heterogeneity among intravacuolar AIEC exists with bacteria that replicate and others that switch on vacuolar conditions toward a nonreplicative phenotype of persisters.\textsuperscript{131} Persisters are bacterial cells that are multidrug tolerant and are suspected to play a role in disease relapse.\textsuperscript{131} Identification of such AIEC bacterial populations and involved factors need to be investigated.
Macrophages, Cytokines, and AIEC

AIEC induce strong inflammation in mouse models.\textsuperscript{132–134} Invasive CD-associated \textit{E. coli} strains, particularly those isolated from inflamed CD tissues, induced secretion of high levels of TNF-\textit{z} by macrophages.\textsuperscript{97,112} Secretions of cytokines by MDM obtained from healthy donors and patients with CD or UC in response to infection with AIEC, non-AIEC or laboratory \textit{E. coli} strains have been compared in two recent studies.\textsuperscript{10,108} In the study by Elliott et al\textsuperscript{108}, decreased secretions of TNF-\textit{z} and IL-23 and increased secretion of IL-10 by MDM obtained from patients with CD compared with MDM obtained from healthy controls were observed in response to infection by AIEC, non-AIEC, and laboratory \textit{E. coli} strains. No modification of IL-8 secretion was observed between MDM obtained from CD and healthy controls infected with different \textit{E. coli} strains.\textsuperscript{108} In another study, Vazeille et al\textsuperscript{10} reported that AIEC-infected MDM obtained from patients with CD secrete higher amount of IL-6 than AIEC-infected MDM obtained from patients with UC and healthy controls. Increased secretions of IL-6 and TNF-\textit{z} were observed in MDM obtained from patients with CD infected with AIEC compared with nonpathogenic \textit{E. coli}\.\textsuperscript{10} In addition, the levels of IL-8 secreted by MDM obtained from patients with CD infected with AIEC and nonpathogenic \textit{E. coli} were decreased compared with infected MDM obtained from patients with UC or healthy controls.\textsuperscript{10} Interestingly, a correlation between the number of intramacrophagic AIEC and the amount of secreted TNF-\textit{z} has been reported in the murine macrophage cell line J774-A1 and in MDM from patients with CD.\textsuperscript{10,135} Conversely, data obtained with J774-A1 macrophages suggest that replication of AIEC within macrophages could be dependent of TNF-\textit{z} secretion.\textsuperscript{135} However, the cellular mechanism driving TNF-\textit{z} effect on AIEC replication within macrophages has not been yet elucidated.

Autophagy plays a crucial role in the regulation of inflammation.\textsuperscript{136,137} Elliott et al\textsuperscript{108} showed that polymorphisms in \textit{ATG16L1}, \textit{NOD2}, \textit{IRGM}, and \textit{ILC}-23R genes do not modulate the level of TNF-\textit{z} secreted by AIEC-infected MDM from patients with CD. However, these results might be re-evaluated in a larger cohort. Defects in autophagy induced by altered expression of \textit{ATG16L1} and \textit{IRGM} due to siRNA treatment in human THP-1 macrophages or by deletion of \textit{Nod2} in mice result in exacerbated inflammatory responses in \textit{AIEC}-infected macrophages.\textsuperscript{72} Hypothesis is that increased secretion of proinflammatory cytokines in macrophages with impaired autophagy is related to uncontrolled intracellular persistence of AIEC. In patients with CD, polymorphisms in \textit{ATG16L1}, \textit{IRGM}, or \textit{NOD2} could favor persistence of intracellular such as AIEC by impairing autophagy and as a consequence profoundly tip the balance toward inflammation.

EFFECTS OF CD THERAPY ON MACROPHAGES AND THEIR ACTIVITY

The therapeutic armamentarium remains limited in CD encompassing steroids, immunosuppressant therapies (thiopurines, methotrexate), and anti-TNF agents. Recently, a new class of treatment has been approved for CD targeting the intestinal T-cells homing (anti-integrins therapies).\textsuperscript{138} Here, we review the effect of the main treatments used in CD on macrophages and their activity. Owing to available data, we focused on steroids, immunosuppressant therapies, and anti-TNF agents.

Effect of Corticosteroids and Immunosuppressant Therapies on Macrophages in CD

For many years, corticosteroids have been used effectively in clinical practice to achieve clinical remission in CD.\textsuperscript{139} Despite their wide use, their exact mechanism of action is still elusive. In fact, although the interaction between corticosteroids and macrophages is known for many years, data interesting in this relationship in CD remain very sparse. Particular effect of corticosteroids is their ability to downregulate the NF-\textit{kB} pathway\textsuperscript{140–143} which is markedly induced in patients with CD and strongly influences the course of mucosal inflammation.\textsuperscript{144} This effect could be in part explained by the ability of corticosteroids to induce the expression and the production of the glucocorticoid-induced leucine zipper, a protein constitutively produced by macrophages to inhibit the production of RANTES (regulated on activation normal T cell expressed and secreted), macrophage inflammatory protein 1z (MIPz also known as CCL3),\textsuperscript{145–148} and TLR-2.\textsuperscript{145} In addition to their effect on the NF-\textit{kB} pathway, corticosteroids could have an impact on macrophage phenotype,\textsuperscript{149,150} TH2-type immune responses initiation and amplification,\textsuperscript{151–153} NLRP3 inflammasome induction and subsequently on the production on IL-6, TNF-\textit{z}, and IL-1\beta.\textsuperscript{154} All of these processes have been put forward directly or indirectly in CD pathogenesis.\textsuperscript{105,155,156}

Among compounds used in immunosuppressant therapies, azathioprine is mostly known for its mechanism of action related to decreased purine synthesis and subsequent apoptosis of fast proliferating cells such as CD4\textsuperscript{+} T-cells.\textsuperscript{145} This cytotoxic effect was, for a long time, the only proposed mechanism for azathioprine-induced immunosuppression.\textsuperscript{151} However, other studies have shown that thiopurines could also affect macrophage inflammatory and proliferative processes\textsuperscript{157,158} and functions such as phagocytosis and chemotactic responses.\textsuperscript{159,160} Azathioprine could act on macrophage proliferation, polarization, and activation through inhibition of inducible nitric oxide synthase\textsuperscript{157,158,161,162} whose activity is increased in CD,\textsuperscript{163} and through downregulation of CD163 expression, a specific macrophage activation marker whose expression is increased in mucosa of active CD patients.\textsuperscript{5,164} Besides, 6-mercaptopurine, another immunosuppressant molecule, has been described to robustly suppress mRNA macrophage expression of monocyte chemotactic protein-1,\textsuperscript{165} one of the key chemokines that regulate migration and infiltration of monocytes/macrophages and which are upregulated in CD.\textsuperscript{166,167} Finally, in vitro data show that methotrexate induces apoptosis in primary murine macrophages at low concentrations in vitro\textsuperscript{168} and suppresses NF-\textit{kB} activity.\textsuperscript{169} Proinflammatory effects of methotrexate has also been suggested,\textsuperscript{170,171} but the mechanisms involved remain to be explored.
Anti-TNF, Macrophages, and CD

Effect of Anti-TNF on Macrophages in CD

Although, anti-TNF agents are, to date, the most effective therapeutic class in CD, their mechanisms of action are not fully understood and depend on their nature. Several hypothesis have been advanced to explain their efficacy such as induction of apoptosis of immune cells by receptor signaling through transmembrane TNF, neutralization of soluble and transmembrane TNF, alteration of cytokine secretion, as detected in serum and in cultures of LP biopsies, with decreased interferon-γ production, and, more recently induction of immunoregulatory macrophages.

It has been suggested that active CD is associated with increased macrophage activation. In this line, Dige et al have assessed macrophage activation before and after starting anti-TNF treatment using the soluble form of hemoglobin–haptoglobin scavenger receptor CD163, a specific biomarker of macrophage activation. They reported that anti-TNF therapy using infliximab and adalimumab rapidly decreased the levels of soluble CD163 in patients with CD supporting the hypothesis that anti-TNF agents may directly target macrophage activation in CD, as suggested by previous in vitro data and observations in peripheral blood. However, macrophage activation did not reach the basal level observed in healthy controls. It could mean that anti-TNF therapy was not able to fully annihilate macrophage hyperactivation, even in patients in remission, if the level of soluble CD163 was not correlated to the disease activity.

Among the drugs targeting TNF-α, some of them have different functional properties which could explain their different efficacy in inflammatory bowel diseases. Data from Vos and colleagues showed that an anti-TNF needs to bind to membranous TNF, and on macrophage cytokine production in response to bacterial infection. They showed that infliximab induce elevated TNF-α macrophage secretion regardless the type of bacteria used for the infection. They concluded that infliximab treatment increased the production of CD macrophage–induced TNF-α in response to bacteria, which seemed to depend on enrichment of CD16+ circulating monocytes. Therefore, they hypothesized that infliximab therapy was associated with the presence of CD macrophages that were more able to induce a dormant MAP phenotype, suggesting that the predominant induction of this form may explain the lack of MAP reactivation during anti-TNF therapy in CD.

Finally, to date, regarding AIEC bacteria and anti-TNF therapies, the capability of macrophages from patients with CD treated with anti-TNF to control intramacrophagic AIEC infection remains to be explored since few data are available and depend on the model used.

CONCLUSION

An altered macrophage response to microorganisms is observed in CD. Nazareth and colleagues have investigated the impact of infliximab treatment on circulating monocyte subsets and on macrophage cytokine production in response to bacterial infection. They showed that infliximab treatment increased the production of CD macrophage–induced TNF-α in response to bacteria, which seemed to depend on enrichment of CD16+ circulating monocytes. Therefore, they hypothesized that infliximab therapy was associated with the presence of CD macrophages that were more able to induce a dormant MAP phenotype, suggesting that the predominant induction of this form may explain the lack of MAP reactivation during anti-TNF therapy in CD.

Finally, to date, regarding AIEC bacteria and anti-TNF therapies, the capability of macrophages from patients with CD treated with anti-TNF to control intramacrophagic AIEC infection remains to be explored since few data are available and depend on the model used.

REFERENCES


