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Review

Monocytes and their pathophysiological role in Crohn's disease

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Abstract. Our immune system shows a stringent dichotomy, on the one hand displaying tolerance towards commensal bacteria, but on the other hand vigorously combating pathogens. Under normal conditions the balance between flora tolerance and active immunity is maintained via a plethora of dynamic feedback mechanisms. If, however, the balancing act goes faulty, an inappropriate immune reaction towards an otherwise harmless intestinal flora causes disease, Crohn's disease for example. Recent developments in the immunology and genetics of mucosal diseases suggest that monocytes and their derivative cells play an important role in the pathophysiology of Crohn's disease. In our review, we summarize the recent studies to discuss the dual function of monocytes - on the one hand the impaired monocyte function initiating Crohn's disease, and on the other hand the overactivation of monocytes and adaptive immunity maintaining the disease. With a view to developing new therapies, both aspects of monocyte functions need to be taken into account.

Keywords. Crohn's disease, monocytes, immune balance, susceptibility genes.

Introduction

Crohn's disease is a relapsing, transmural inflammatory disease of human gastrointestinal mucosa. This disease and a similar condition known as ulcerative colitis are collectively called Inflammatory Bowel Diseases (IBD). Crohn's disease primarily occurs in adolescents and young adults. The quality of life of patients with Crohn's disease dramatically decreases due to symptoms caused by intestinal ulcerations and complications including strictures, abscesses, or fistulas. In addition to gastrointestinal lesions, extraintestinal organ involvement, which occurs in about 25 % of patients with Crohn's disease, is an important clinical characteristic, including uveitis, arthritis, erythema nodosum, etc. The most specific histological characteristic of Crohn's disease, found in about 40 % of all patients, is granulomas in intestinal ulcerative lesions. This is an aggregation of epithelioid macrophages surrounded by a lymphocyte cuff [1, 2].

Our immune system shows a stringent dichotomy. Under normal conditions, the balance between flora tolerance and active immunity is maintained via a plethora of dynamic feedback mechanisms involving all the components of the immune system (e. g. immune cells, cytokines and chemokines). If, however, the balancing act involved goes faulty, an inappropriate immune reaction towards an otherwise harmless intestinal flora causes debilitating and sometimes

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even lethal disease. Recent developments in the immunology and genetics of mucosal disease suggest that Crohn's disease originates from a lack of innate immunity. In particular, dysfunctional intracellular destruction of pathogens or deficient phagocyte recruitment gives rise to chronic mucosal inflammation. On the other hand, once disease is established, chronic overactivation of the immune system, including overactivation of the monocyte compartment and T cells compartment, constitutes an important element for maintaining disease activity. This is demonstrated by the efficacy of therapies that target the overactivated immune activity. Hence, the role of innate immunity in Crohn's disease seems dichotomal and the design of novel rational therapeutic strategies for dealing with Crohn's disease should take the dual nature of the role of the monocyte compartment into account. In this context, it is attractive to compare the role of the monocyte to the yin and yang concept of Chinese philosophy, which describes two opposing and, at the same time, complementary aspects of any one phenomenon, in this case both underactivation and overactivation of the monocyte perpetuating mucosal disease. In our review, we illustrate the monocyte dual function from the viewpoint of Crohn's disease.

Monocytes in non-pathogenic gastrointestinal mucosal immunity

Monocytes derive from myelomonocytic stem cells in bone marrow, emigrating into blood vessels under chemokine signal stimulation, such as CCR2 [3]. Using an adoptive precursor cell transfer strategy into mononuclear-phagocyte-depleted mice, Geissmann and his colleagues [4] recently demonstrated that macrophage/dendritic cell-restricted bone marrow precursors give rise to bone marrow and blood monocytes. In mice, blood monocytes consist of two functional subsets: a short-lived $CX_3CR1^{lo}CCR2^+Gr1^+$ subset that is actively recruited to inflamed tissues and a $CX_3CR1^{hi}CCR2^-Gr1^-$ subset characterized by CX_3CR1-dependent recruitment to non-inflamed tissues. In humans, the level of CX_3CR1 defines the two major human monocyte subsets, the $CD14^{hi}CD16^-$ and $CD14^+CD16^+$ monocytes, which share phenotype and homing potential with the mouse subsets. An interesting question is whether the balance between these subgroups is disturbed in Crohn's disease, as $CD14^{hi}CD16^-$ monocytes may well have sentinel functions and counteract disease, whereas the inflammatory $CD14^+CD16^+$ monocytes may be actively involved in maintaining the chronic nature of the disease.

Nevertheless, the exact dynamics of monocyte recruitment to the intestinal mucosa in general remain only partly understood. Smythies and colleagues recently reported that intestinal macrophages and blood monocytes do not proliferate as detected by proliferation-specific autoradiography of *in vitro* cultures of macrophages isolated from fresh jejunum sections or peripheral blood monocytes of normal human donors [5]. Using the green fluorescent protein reporter system driven at the locus of the *Cx$_3$cr1* gene in mice, $CX_3CR1^+CD117^+Lin^-$ cells specifically differentiated into macrophages and dendritic cells *in vitro* and *in vivo* [6, 7]. Furthermore, grafted $Gr1^+$ "inflammatory" blood monocytes shuttle back to the bone marrow in the absence of inflammation, convert into Gr^- monocytes, and contribute further to mononuclear phagocyte generation. The grafted monocytes replenish intestinal lamina propria and lung dendritic cells, whereas macrophage/dendritic cell-restricted bone marrow precursors replenish conventional $CD11c^{high}$ dendritic cells in the spleen [8]. Hence, the size of the monocyte compartment is under active and very dynamic control.

Combining the intravital confocal microscopy imaging technique, that allows *in vivo* observation of cells within capillaries and postcapillary vessels, and the green fluorescent protein reporter system, Geissmann and his colleagues again showed that the "resident" $Gr1^-$ monocytes patrol healthy tissues through long-range crawling on the resting endothelium. This patrolling behavior depends on the integrin LFA-1 and the chemokine receptor CX_3CR1 and is required for rapid tissue invasion at the site of an infection by this "resident" monocyte population, which initiates an early immune response and differentiates into macrophages [9]. The constant replenishment of monocytes from the peripheral blood probably makes this experimentally highly accessible compartment an excellent reservoir of cells with properties similar to that of the monocytes entering the inflamed intestinal mucosa.

The migration of myeloid cells from the vascular lumen to the tissues involves a series of sequential molecular interactions. The forward migration of monocytes through endothelial junctions is regulated sequentially by the platelet-endothelial cell adhesion molecule 1 (PECAM-1, also known as CD31) and CD99, which is a heavily O-glycosylated type 1 transmembrane protein expressed on both monocytes and endothelial cells [10]. Although both macrophages and monocytes express chemokine receptors, CXCR1 and CXCR2, TGF-ßRI and RII, the *N*-formylmethionyl-leucyl-phenylalanine receptor (fMLPR), and C5aR, only monocytes but not macrophages migrate towards chemoattractant ligands. This

suggests that blood monocytes are the exclusive source of macrophages in inflamed intestinal mucosa [5]. Furthermore, lamina propria matrix products IL-18 and TGF-ß, probably secreted by epithelial cells and mast cells, chemotax monocytes in the absence of inflammation [5]. CD23, a chemokine constitutively expressed by human intestinal epithelial cells apparently triggers upregulation of IL-8 and CCL20 at the mRNA and protein levels *in vitro* and induces migration of dendritic cells in a CCL20-dependent manner. Accordingly, CCL20 is constitutively expressed by epithelial cells [11]. The knowledge of the molecular mechanisms that govern monocyte entry into the inflamed compartment may ultimately prove exceedingly useful for designing novel rational treatment of Crohn's disease.

In healthy individuals, the microorganisms that are encountered daily are detected and destroyed within minutes or hours by innate immune systems that do not require a prolonged period of induction because they do not rely on the clonal expansion of antigen-specific lymphocytes. Approximately 2×10^{15} bacteria reside in the human gastrointestinal tract. The epithelial surface serves as an effective barrier for this microbiological onslaught, and is rapidly repaired if wounded. Recently, the tight junction genes were found associated with ulcerative colitis, suggesting pathogenic involvement of epithelial permeability [12]. In addition, the acid pH of the stomach, peristalsis movement of the gut, α-defensins made by Paneth cells and normal nonpathogenic bacteria that compete with pathogenic microorganisms for nutrients and for attachment contribute to the defense as well [13]. Direct testing of the anti-microbial activity of intestinal flood of patients and healthy controls suggest that the diminished presence of anti-microbial first-line of defense substances is a common phenomenon in Crohn's disease[14].

Normal wear and tear of the intestine allows bacteria to pass the barrier fairly frequently. Following such translocation and possible replication in the submucosal layer of the microbe involved, macrophages serve as sentinels, recognizing, ingesting, and destroying the pathogens involved in these routine translocation events. Macrophages recognize pathogens by means of their cell-surface receptors. Recognition of pathogens is mediated by germ-line encoded pattern recognition receptors (PRRs). Several groups can be distinguished including (C-type) lectins, mannose receptors, complement receptors, scavenger receptors, Nod proteins and Toll-like Receptors (TLRs). These receptors have evolved by natural selection and are specific for a limited number of highly conserved microbial motifs (pathogen associated molecular patterns or PAMP)[15, 16]. Toll-like receptors belong to the cytokine receptor family and so far more than ten different TLRs have been described. Their extracellular domains contain leucine-rich repeats that participate in ligand recognition; the intracellular domains contain regions that are highly similar to the intracellular domain of the IL-1 receptor. After activation, all TLRs finally activate NF-κB but important differences remain. TLR4 is activated when the LPS-LPS Binding Protein-complex is transferred from the CD14 receptor[17, 18]. Binding of lipopolysaccharides (LPS) to a TLR-4 heterodimer-myeloid differentiation 2 (MD2) complex results in the activation of different domains of the intracellular region. Roughly, an early myeloid differentiation protein 88 (MyD88)-dependent and a late MyD88-independent pathway can be distinguished [19]. Both seem important for effective innate responses, which include phagocytosis of the pathogen, followed by its death inside the phagocyte via auto/allophagia. In addition to triggering phagocytosis, binding of pathogens to TLRs can also trigger the cell-surface displaying co-stimulatory molecules that eventually lead to the induction of adaptive immunity. The importance of TLR4-dependent recognition of pathogens for maintaining gut homeostasis is emphasized by the linkage of TLR4 polymorphisms to Crohn's disease [20]. After engulfment of pathogens, the nascent phagosomes fuse with lysosomes to generate phagolysosomes, the mature compartment where killing and degradation of pathogens occur.

Cytokines and chemokines secreted by macrophages induce the next phase of the innate immune response. In this phase, an inflammatory response recruits neutrophil granulocytes to the site of infection as well as complement system proteins that cooperate with the phagocytes in the engulfment of the pathogens. Neutrophils are the most abundant leukocytes in human peripheral blood, killing many microbes by "respiratory burst" and dying soon after they have accomplished a round of phagocytosis [21]. This is a phenomenon more associated with acute inflammation than with the chronic inflammation that characterizes the inflammatory bowel diseases, in which the monocyte compartment is much more important.

If the stimulation of microorganisms cannot be quickly cleared by intestinal innate immune reactions, adaptive immune reactions are induced via antigen presentation and cytokine secretion of dendritic cells and macrophages. Peptides generated in phagosomes can be loaded onto major histocompatibility complex (MHC) molecules and presented at the cell surface. Upon the recognition of MHC-associated peptides by T cell receptor (TCR) molecules, the T-cell compartment is activated for clonal expansion [22]. Monocytes and their derivative cells present antigens to

CD4+ T cells by antigen-MHC class II complexes and to CD8+ T cells by antigen-MHC class I complexes, the latter being a so-called antigen cross-presentation. The common view has long been that exogenous proteins, internalized by endocytosis or phagocytosis, are presented by the MHC class II pathway. In contrast, the MHC class I presentation pathway was considered restricted to endogenously synthesized proteins, including self proteins and those resulting from viral infection [23]. A large body of evidence indicates that the capacity of pathogens to prevent phagosome maturation is likely to affect antigen processing and presentation. For instance, in phagosomes containing *Mycobacterium tuberculosis*, the formation of bacterial antigen-MHC class II complexes is decreased when live bacteria, rather than heat-killed bacteria, are phagocytosed [24]. Antigens from pathogens, including mycobacteria, *Salmonella, Brucella*, and *Leishmania,* elicit an MHC class I-dependent response promoting the proliferation of CD8+ cytotoxic T cells through endoplasmic reticulum (ER)-mediated phagocytosis and the delivery of ER proteins to phagosomes, involving the retrotranslocation of exogenous peptides from the phagosome lumen to the cytosol [25–27]. Taken together, monocytes and their derivative cells are the central mediators for both innate and adaptive immunity. In most people the monocyte compartment is effective and prevents excessive inflammation in the mucosa. As we shall discuss below, however, genetic mutations may render this compartment less effective, causing chronic inflammatory reactions and Crohn's disease.

The origin of Crohn's disease pathogenesis: microbial invasion, adaptive immune disorders, or innate immune disorders?

Microbial invasion and mucosal ulceration

Crohn's disease was first described as a clinical entity in 1932 by American gastroenterologist Burrill B. Crohn [28]. After three-quarters of a century of investigation, the hypothetical pathogenic causes that directly result in ulceration of intestinal mucosa can be categorized into three aspects: microbial invasion, adaptive immune disorders, and innate immune disorders. Microbial pathogens have long been postulated to cause Crohn's disease, particularly *Mycobacterium avium* subspecies *paratuberculosis* (MAP) which causes chronic enteritis affecting humans (intestinal tuberculosis), cattle (Johne's disease) and other species [29, 30]. Human intestinal tuberculosis extremely resembles Crohn's disease, including the location and appearance of ulceration, granuloma formation, and systemic involvement, and is rarely seen in developing countries nowadays [31]. In agreement with a mycobacterial cause for Crohn's disease are the observations of Naser and colleagues who reported the culturing of viable MAP from the blood of 14/28 (50%) patients with Crohn's disease, 2/9 (22%) with ulcerative colitis, and 0/15 individuals without inflammatory bowel disease. They also reported detecting MAP DNA by nested PCR from uncultured blood of 13/28 (46%) patients with Crohn's disease, 4/9 (44%) with ulcerative colitis and 3/15 (20%) individuals without inflammatory bowel disease [32]. The authors concluded that the use of immunosuppressive medication did not correlate with a positive MAP finding among patients with Crohn's disease. However, the authors are not able to exclude that positive MAP was the consequence of the use of immunosuppressive medication that increases the risk of opportunistic infection, when compared with the control group comprised of individuals not using immunosuppressive medication. Recently, the Australian Crohn's disease study group reported that their two-year prospective, parallel, placebo-controlled, double-blind, randomized trial did not find a sustained benefit of antibiotic treatment against MAP in patients with Crohn's disease [33]. Hence it is fair to say that for now little evidence supports the direct mycobacteriogenic origin of this disease, although it may play a role in further pathogenesis. Most investigators have given up on the possibility that a specific microbiological entity – analogous to *H. pylori* in gastric ulceration – is causative in Crohn's disease. Tantalizingly, the difficulty that a healthy immune system has in eliminating MAP as well as the clinical similarity between MAP infection and Crohn's disease may indicate that an initial failure to eliminate mucosal invading bacteria could underlie Crohn's disease. Indeed, Crohn's disease clearly involves interaction of the mucosal immune system with the resident flora. Genetically manipulated mice developing chronic intestinal inflammation in a conventional environment do not do so, or develop attenuated disease, in a germ-free environment [34, 35]. Hence, it is now generally assumed that the role of enteric microorganisms in Crohn's disease pathogenesis is most likely to provide the antigens that stimulate the intestinal immune system.

Adaptive immune disorders were categorized into IL-12/Th1 and IL-23/Th17 pathways

The immune disorders of patients with Crohn's disease were first observed in T cell compartments characterized by exaggerated proliferation and activation of T cells. The importance of this finding is emphasized by the effectiveness of T cell apoptosis in inducing remission in Crohn's disease [36] or in the

combat of experimental colitis in experimental rodents [37–39]. Recent research suggests that at adaptive immunity level, T helper 1 (Th1) and T helper 17 (Th17) lymphocytes are master regulators that directly attribute to the intestinal mucosal ulceration. Kontoyiannis and colleagues showed that a mouse, by deletion in the 3′ AU-rich elements (AREs) of tumor necrosis factor (TNF) mRNA ($Tnf^{\Delta ARE}$ mouse), overproduced TNF and developed a unique Crohn's disease phenotype with remarkable histopathological similarity, including patchy transmural inflammation, lymphoid aggregation and granulomata restricted to the terminal ileum at 4–8 weeks of age. The intestinal pathology in this model depended on Th1-like cytokines such as interferon-γ (IFN-γ) and interleukin-12 (IL-12) and required the function of $CD8^+$ T lymphocytes. $CD8^+$ T cells, but not $CD4^+$ T cells, expressed activated/memory markers CD44, CD69, and CD25, and enhanced lysis of epithelial cells *in vitro*, due to increased cytotoxic activity. By crossing $Tnf^{\Delta ARE}$ mice into CD4, β2-microglobulin (CD8), μMT(B cell), and TcRδ (γδTCR T cells) deficient backgrounds respectively, the authors confirmed that $CD8^+$ T cells, mediated by $CD4^+$ T cells, play an essential role in the development of chronic progressive inflammation. B cells and γδTCR T depletion did not change the disease's severity in this animal model [40].

In 1986, Mosmann and colleagues described the presence of two types of $CD4^+$ T helper cell clones that had distinct profiles of cytokine production –Th1/Th2 polarization profiles [41]. Based on this concept, Crohn's disease is defined as a IL-12 driven Th1 cell mediated process including increased levels of IFN-γ, granzyme, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), Fas ligand (FasL), and chemokine (C-C motif) ligand 5 (CCL5) [42]. Recently, an IL-23 driven Th17 cell compartment has been defined that is clearly distinct from Th1 cells and that might play an important role in Crohn's disease pathogenesis. In IL-10 deficient mice, the development of spontaneous IBD was completely prevented by crossing these mice to IL-23p19-deficient mice [43]. A plausible interpretation is that IL-12/Th1 and IL-23/Th17 cells work together to control intestinal microbial infection. Whereas the IL-12/Th1 pathway predominantly induces cytotoxic factors important for the direct killing of microbes or infected cells, the IL-23/Th17 pathway characterized by increased levels of IL-17, IL-6, IL-22, TNF, CXCL1, and α3 integrin, is associated with local tissue inflammation that produces swelling, heat, and pain, and sets up an environment with heightened immune responses [44].

The pathways that lead to hyperactivated adaptive immune responses in Crohn's disease can roughly be divided into related processes: i) antigen presentation of luminal components to the adaptive immune system by components of the innate immune system and ii) genetic mutation of components of adaptive immunity. As discussed later, currently identified IBD susceptibility genes and their functional studies suggest that the first pathway predominates. The data on how innate immune cells mediate Th1 and Th17 cells in patients with Crohn's disease are still very limited. We now know that both IL-12 and IL-23 are produced by dendritic cells and macrophages, suggesting the causal role of innate immunity in exaggerated T cells responses in Crohn's disease. It is also known that IL-23 driven $CD4^+$ memory T cells activation and IL-8, CXCL1, TNF and G-CSF cytokine secretion are responsible for locally promoting rapid neutrophil recruitment, which is important for the control of acute infection [44]. Taken together, monocytes and their derived dendritic cells and macrophages are most likely the "starting" point of pathogenesis of Crohn's disease, the inadequacy of their responses inducing disease. The subsequent recruitment of more inflammatory types of phagocytes, in conjunction with the adaptive immune system, subsequently ensures its propagation.

Innate immune disorders and their implications

The high frequency of extra-intestinal organ involvement in patients with Crohn's disease and the effectiveness of immunosuppressive rather than antibiotic treatment of extra-intestinal symptoms including uveitis, arthritis, erythema nodosum etc, indicates that fundamental defects in the immune system function play an essential role in the pathogenesis of Crohn's disease. Indeed, two clinical trials suggested impaired neutrophil function in patients with Crohn's disease. Marks and colleagues investigated inflammatory responses in patients and controls by quantifying neutrophil recruitment and cytokine production after acute trauma. In patients with Crohn's disease, trauma to rectum, ileum, or skin led to abnormally low neutrophil accumulation (differences from healthy individuals of 79 %/n=8, 57 %/n=3, 50 %/n=13, respectively) and lower production of proinflammatory interleukin 8 (63 %/n=7, 63 %/n=3, 45 %/n=8) and interleukin 1β. Interleukin 8 secretion by cultured macrophages was reduced after exposure to acute wound fluid. Also, the local inflammatory reaction to inoculation with *E. coli* was attenuated in patients, as quantified by changes in blood flow [45]. The most straightforward interpretation of these data is that patients with Crohn's disease have abnormally weak innate responses[46]. Furthermore, the United States' sargramostim study group reported that in their placebo-controlled, randomized trial, sargramostim,

a granulocyte-macrophage colony-stimulating factor which stimulates cells of the intestinal innate immune system, significantly improved patients' condition, when compared to the placebo group, as judged by the reaching of a clinical response (which was defined by a decrease from the baseline of at least 100 points in the Crohn's disease Activity Index (CDAI) score on day 57) and of remission (defined by a Crohn's disease AI score of 150 points or less on day 57). The sargramostim group also had significant improvements in the quality of life [47]. Thus, weak innate immune responses are associated with Crohn's disease and stimulating these responses seems beneficial. In apparent agreement, genetic aberrancies of innate– and in particular granulocyte immune function (e.g. NADPH oxidase deficiency or Wiskott Aldrich Syndrome) cause pathology highly reminiscent of Crohn's disease and may actually be misdiagnosed as Crohn's disease.

Since neutrophil recruitment is mediated by cytokines and chemokines secreted by the monocyte compartment, manipulating monocyte function is a potential therapeutic strategy for the impaired neutrophil recruitment in patients with Crohn's disease. Recent studies performed in mice show that IL-23, secreted by macrophages and dendritic cells in response to microbial products and inflammatory cytokines, regulates granulopoiesis in a neutrostat regulatory feedback loop through IL-17A-producing neutrophil regulatory (Tn) cells [48]. At the site of infection, IL-23 was induced rapidly after *E. coli* infection in a TLR4 signaling-dependent manner. In response to IL-23, IL-17 produced by γδT cells facilitated the influx of neutrophils. Accordingly, neutralization of IL-17 resulted in a reduced infiltration of neutrophils and an impaired bacterial clearance [49]. The prediction would be that reduced activity of this system, and thus reduced capacity of the mucosa for bacterial clearance, would be the predisposition for Crohn's disease and as discussed below, this might be true.

Susceptibility genes indicate the pathophysiological role of monocytes in Crohn's disease

Crohn's disease is considered the poster child with respect to the elucidation of the susceptibility genes underlying a complex inherited disease, and the number of alleles associated with increased susceptibility increase almost every week. Unfortunately, these alleles do not show remarkable correlation with the success of particular treatment modalities, and the relative risks of the alleles involved tend to be low (typical lod scores ranging from 1.5 to 5). Nevertheless, studying the functions of these genes is an efficient way to understand the pathogenesis of Crohn's disease. Among the more than 30 distinct susceptibility loci for Crohn's disease, NOD2, IL-23R, and autophagy genes are most strongly associated [50], and all indicate the pathophysiological role of monocytes in Crohn's disease.

NOD2, an intracellular receptor of bacterial cell wall muramyldipeptide (MDP), is mainly expressed on antigen presenting cells [51] and three single nucleotide polymorphisms have been identified that confer increased risk for Crohn's disease [52, 53]. A variety of immunological mechanisms linking Crohn's disease to these polymorphisms has been proposed, most of them involving a reduced activity of a component of the innate immune system (e.g. diminished anti-microbial alpha-defensin activity of the Paneth cell [54]). In the monocyte compartment, NOD2 plays a negative role mediating the IL-12/Th1 cell inflammation pathway. NOD2 signaling inhibited Toll-like receptor 2-driven activation of NF-kappa B, and thus, in NOD2 knockout mice, NOD2 deficiency or the presence of a Crohn's disease-like Card15 mutation increased the Th1 cytokine profile character following Toll-like receptor 2 stimulation [55]. In contrast, NOD2 overexpressing transgenic mice were resistant to the induction of peptidoglycan (PGN) colitis. Antigen presenting cells from these mice exhibited diminished PGN-driven IL-12 response [56]. As we discussed earlier, adaptive immune disorders in Crohn's disease were categorized into IL-12/Th1 and IL-23/Th17 pathways. Interestingly, in a recent study, NOD2 was shown to have a positive regulatory effect on the IL-23/Th17 inflammatory pathway. Upon NOD2 stimulation by MDP, dendritic cells enhanced Toll-like receptor agonist-dependent induction of IL-23 and IL-1 production, which in turn promoted IL-17 expression in Th17 memory cells but not naive Th cells [57]. Taken together, NOD2 protein in the monocyte compartment plays a dual function in response to microbial products, on the one hand negatively regulating the Th1 inflammation pathway, on the other hand positively regulating the Th17 inflammation pathway which again points to fundamental flaws in the action of the phagocyte system as the fundamental cause of the disease.

IL-23 is secreted by macrophages and dendritic cells. In Crohn's disease-induced mouse colitis, intestinal inflammation was associated with IL-23 mRNA-producing intestinal dendritic cells and depended on IL-23 secretion [58]. Our knowledge on the underlying mechanisms by which IL-23 participates in the intestinal inflammation is still very limited. The pathophysiological role of IL-23 in Crohn's disease is discussed in the section "The origin of Crohn's

disease pathogenesis: microbial invasion, adaptive immune disorders, or innate immune disorders?" Although more indirect, these data are best explained by deficient innate immune function being pivotal in the establishment of disease.

The protein encoded by ATG16L1 takes part in cellular autophagy and is broadly expressed in the intestinal epithelium, the antigen presenting cells and the lymphocytes. Autophagy delivers cytoplasmic constituents for lysosomal degradation, playing an important role in antigen presentation. Recent studies demonstrated that this pathway mediates resistance to pathogens and is targeted for immune evasion by viruses and bacteria. Bacteria and viruses have developed strategies to escape destruction via macroautophagy. After phagocytosis, successful microbial pathogens either leave the phagosome for the cytosol before fusion with lysosomes, as is the case for *Listeria monocytogenes*, or stop their maturation to acidic vesicles, as for *Mycobacterium tuberculosis*. Both cytosolic bacteria and endosome-enwrapped pathogens can then be targeted by macroautophagy. Upon the fusion of autophagosomes and lysosomes, lysosomal degradation products, including pathogenic determinants, are surveyed by the adaptive immune system to elicit antigen-specific T cell responses [59]. Hence again, disorders in the first line of mucosal defense against pathogens seem involved in establishing chronic disease. The adaptive immunity is further induced by antigen presentation and cytokine secretion. It is to be predicted that other risk alleles now being identified will also reveal genes involved in this defense and particularly in monocyte function.

The wax and wane of monocyte function: the immune disorder dualism of patients with Crohn's disease

The lamina propria of the gastrointestinal mucosa contains the largest population of mononuclear phagocytes in the body. Monocytes execute their function by a complex signaling pathway network system, not a linear pattern. Receptors of the cellular surface provide environmental cues which functionally direct monocytes into effect actions such as phagocytosis, antigen presentation, cytokine secretion, or apoptosis. The detailed description of these networks goes beyond the scope of this review, but we should realize that bacteria, as complex particles, always simultaneously activate multiple receptors and each receptor is able to initiate the responses of multiple kinases sequentially, resulting in multiple consequences. For example, *E. coli* are recognized by integrin receptors, Fcγ receptors, complement receptors, mannose receptors, TLR receptors, scavenger receptors etc, and through the activation of Rho family kinases, phospholipase C, PIP2 kinase, PI3K kinase or MAP kinase, resulting in monocyte phagocytosis, antigen presentation and cytokine secretion processes. Many of the details of the signaling pathways involved have now been elucidated and vast knowledge of directional control of kinase signaling pathways has been gained, highlighting e.g. the function of scaffold proteins that force signal transducers together, adding directionality to such pathways. These have now also begun to be clinically exploited, e.g. the clinical success of Raf inhibitors in Crohn's disease [60, 61]. In clinical practice, manipulating the activity of key kinases is an effective way to control the intensity of monocyte functional outcomes; although in practice it is difficult to make predictions about the outcome of therapy because of the non-linearity in signaling pathways and network effects that may produce unexpected effects. Thus, monocyte signal transduction in mucosal inflammation has multiple checks and balances. Under non-pathological conditions, an intricate web of feedback mechanisms ensures proper responses to non-dangerous organisms.

The pathophysiological role of monocytes in Crohn's disease shows a dual pattern. As discussed above, diminished innate function of monocytes seems a critical actor in establishing disease. An inadequate innate response to bacterial invasion allows the accumulation of potential immune inducers (commensal bacteria), and secondary lines of defence of the body (i.e. adaptive immunity) are mobilised to deal with these bacteria. Adaptive immunity, however, is by its nature much less precisely controlled as compared to innate responses, and this less regulated response in turn produces the intestinal inflammation typical of Crohn's disease. In patients with active Crohn's disease, monocyte subpopulations of CD16+ and CD56+ are expanded and $CD14^{low}CD16^{+}$ monocytes express higher levels of CX3CR1, a chemokine receptor which promotes monocyte trafficking, as compared to the healthy controls [62]. In general, intestinal macrophages lack the expression of the innate-immune receptor CD14. Tantalizingly, a subset of unique CD14+ intestinal macrophages was identified in intestine tissue of patients with Crohn's disease, which produced large amounts of IL-23 and TNF-α, compared with those in normal controls or patients with ulcerative colitis [4]. It is reasonable to propose that these unique CD14+ macrophages come from the increased CD14+CD16+ "inflamed" monocyte population, contributing to the setup of local lesions.

In addition to cytokine secretion, the monocyte compartment induces the activation of the T cell compartment by overexpressing its co-stimulatory factors. For instance, CD40-CD40 ligand interaction is

essential for the T lymphocyte-dependent immune response. In active Crohn's disease patients, CD40 expression on both circulation monocytes and intestinal CD68+ macrophages is significantly increased, compared with healthy donors and ulcerative colitis subjects [63]. In an *in vitro* experiment co-culturing mononuclear cells with epithelial cells, lamina propria mononuclear cells but not peripheral blood monocytes from patients with Crohn's disease resulted in the break down of the epithelial barrier function via the secretion TNF-α [64]. Hence, once disease is established, CD16 positive monocytes will be recruited and in conjunction with the T cell compartment play an active role in maintaining disease.

Current immunosuppressive therapeutic strategies for dealing with Crohn's disease are able to induce and maintain remission for most patients. These immunosuppressive drugs target T cell activation/apoptosis, possibly the over-activation of the monocyte compartment as well [65]. However, they may be of limited usefulness with respect to the events actually initiating Crohn's disease, which may explain the continuous recurrence of disease in patients. Azathioprine and its metabolite 6-mercaptopurine (6-MP) are the most frequently used immunosuppressive drugs for maintaining remission [66, 67]. Azathioprine and its metabolites induce apoptosis of T cells from patients with Crohn's disease and from control patients. Apoptosis induction requires co-stimulation with CD28 and is at least in part mediated by a specific blockade of Rac1 activation through binding of azathioprine-generated 6-thioguanine triphosphate (6-Thio-GTP) to Rac1 instead of GTP. In apparent agreement, the Rac1 targeting genes such as mitogen-activated protein kinase (MEK), NF-kappaB, and bcl-x(L) are suppressed by azathioprine and all these events may lead to a mitochondrial pathway of apoptosis. Azathioprine may well partially exert its action by converting a co-stimulatory signal into an apoptotic signal [65].

Treatment of Crohn's disease has been revolutionized by the introduction of anti-TNF medication, which is useful for both remission induction and maintenance. The two processes seem distinct on a molecular level, remission induction being dependent on apoptosis in the T cell compartment, whereas remission maintenance requires neutralization of TNF bioactivity *per se*. We have shown earlier that infliximab (an anti-TNF medication effective in Crohn's disease and rheumatoid arthritis) but not etanercept (an anti-TNF medication effective in rheumatoid arthritis) induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease [68]. It is well established that whereas infliximab is highly effective in inducing remission in Crohn's disease, etanercept absolutely does not have this capacity [69, 70]. Furthermore, mucosal apoptosis 24 hrs after an infusion of infliximab strongly correlates with the clinical success of infliximab with respect to inducing remission in patients with moderate to severe Crohn's disease [36]. On the other hand, certolizumab (another anti-TNF medication), at least in vitro, does not seem to induce apoptosis [71]. This is a significant observation as certolizumab, although apparently effective in maintenance therapy, is not very effective with respect to remission induction in Crohn's disease [72, 73]. Thus, these observations provide further strong support for the notion that anti-TNF medication is only beneficial for remission induction in Crohn's disease when it has the capacity to induce mucosal apoptosis. However, for long-term maintaining remission, the neutralization of TNF bioactivity which interferes with both adaptive and innate immunity seems important.

Clinical implications of a dichotomal role for monocytes in Crohn's disease

Current insights in Crohn's disease suggest that the monocyte compartment exerts such a dual function, on the one hand inadequacy in this compartment initiating disease, but subsequently its overactivity also maintaining the colitis. The current therapeutic strategy for Crohn's disease is immunosuppressive treatment, which can induce clinical remission in more than 60 percent of patients. However, a substantial percent of patients fail to respond to medication and more than 20 percent of patients show severe side effects to the treatment [74]. The life-long therapy causes socioeconomic problems as well. In order to achieve the final aim "a cure" we may well depend on the modulation of the monocyte compartment.

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