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Perturbations of mucosal homeostasis through interactions of intestinal microbes with myeloid cells

Regina Schey¹, Claudia Danzer¹, and Jochen Mattner^{1,2}

¹ Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, D-91054 Erlangen, Germany

² Division of Immunobiology, Cincinnati Children's Hospital, Cincinnati, OH 45229, USA

Abstract

Mucosal surfaces represent the largest areas of interactions of the host with its environment. Subsequently, the mucosal immune system has evolved complex strategies to maintain the integrity of the host by inducing protective immune responses against pathogenic and tolerance against dietary and commensal microbial antigens within the broad range of molecules the intestinal epithelium is exposed to. Among many other specialized cell subsets, myeloid cell populations - due to their strategic location in the subepithelial lamina propria - are the first ones to scavenge and process these intestinal antigens and to send consecutive signals to other immune and non-immune cell subsets. Thus, myeloid cell populations represent attractive targets for clinical intervention in chronic inflammatory bowel diseases (IBDs) such as ulcerative colitis (UC) and Crohn's disease (CD) as they initiate and modulate inflammatory or regulatory immune response and shape the intestinal T cell pool. Here, we discuss the interactions of the intestinal microbiota with dendritic cell and macrophage populations and review in this context the literature on four promising candidate molecules that are critical for the induction and maintenance of intestinal homeostasis on the one hand, but also for the initiation and propagation of chronic intestinal inflammation on the other.

Keywords

Inflammatory bowel disease; intestinal microbiota; mucosal tolerance; myeloid cells; surface molecules

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Correspondence: Regina Schey, Jochen Mattner; Mikrobiologisches Institut-Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, Wasserturmstr. 3/5, D-91054 Erlangen, Germany; Regina.Schey@uk-erlangen.de. Jochen.Mattner@uk-erlangen.de; Phone: +49 (0) 9131-8523640; Fax: +49 (0) 9131-85-1001.

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Introduction

The intestine is continuously exposed to various different antigens, which originate from dietary and commensal microbial sources. While the mucosal immune system has to act tolerogenic against those, it needs to protect the host against invading pathogens. Thus, the mechanisms underlying the balance between pro- and anti-inflammatory processes in the gut require distinct cellular and soluble mediators. The distinct cell populations that contribute to the maintenance of the integrity of the mucosal barrier include goblet cells, paneth cells, M cells, enteroendocrine cells, enterocytes and various different specialized immune cells (Garrett, et al., 2010) including innate and adaptive lymphocytes (Pearson, et al., 2012), macrophages, conventional CD11c^{hi} dendritic cells (DCs) and plasmacytoid DCs (pDCs) (Iwasaki, 2007, Smith, et al., 2005) with the latter three ones being the main focus of this review. These different immune cell populations reside within distinct locations in the gut associated lymphoid tissue (GALT) and the subepithelial lamina propria (Varol, et al., 2010) and express a broad range of immune receptors in order to immediately activate and shape the immune response. Among those are toll-like receptors (TLRs), Scavenger receptors (SR) and C-type lectin receptors (Gordon, 2002), but also T and B cell receptors. Upon activation of these receptors, potent immune modulatory functions are unleashed, such as the release of a broad range of different soluble and membrane-bound mediators. These include, for example, cytokines such as IL-17 and IL-22 released by Th17 cells (Harrington, et al., 2005) or group 3 innate lymphoid cells (ILC3) (Cella, et al., 2009, Cupedo, et al., 2009, Luci, et al., 2009, Sanos, et al., 2009, Satoh-Takayama, et al., 2008, Takatori, et al., 2009), defensins secreted by epithelial cells (Zhao, et al., 1996) and membrane-bound TNF-like cytokines such as the B cell activating factor BAFF expressed on T cells (Schneider, et al., 1999), monocytes, macrophages and DCs (Nardelli, et al., 2001, Shu, et al., 1999) or the proliferation inducing ligand APRIL, highly expressed on peripheral blood leukocytes (Kelly, et al., 2000) (Shu, et al., 1999).

Recognition of intestinal bacteria

The release of these different compounds is initiated upon ligation of different innate and adaptive immune receptors. Dependent on the receptor(s) engaged, inflammatory or tolerogenic immune responses can be generated. This complex network is required for the regulation of the intestinal immune homeostasis on a molecular and cellular level that needs to mount inflammatory immune responses against pathogens, but also is required for the maintenance of tolerance against the physiological intestinal microbial flora. Thus, in order to maintain intestinal homeostasis, microbial signals are continuously sensed by intestinal epithelial cells (Nenci, et al., 2007, Zaph, et al., 2007). Despite serving as a physiological barrier, the disruption of the intestinal epithelial layer can lead to the translocation of intestinal microorganisms and the activation of different receptors on subepithelial immune cell subsets. Distinct, highly-conserved pathogen-associated molecular patterns (PAMPs) of microbes as diverse as bacteria, fungi or viruses, for example, are thereby recognized by relatively few TLRs (Rakoff-Nahoum, et al., 2004) (Blasius and Beutler, 2010). However, single receptors within the TLR family are able to distinguish between different formations of the same microbial ligand. Gram-negative bacteria, for example, differ in the structure of their lipopolysaccharides (LPS) in the outer membrane, namely the lipid A structure, the

core sugars, the O-polysaccharide chain and the O-antigen repeats (Raetz, 1990). The divergent recognition of these different, species-specific LPS structures leads to alterations in TLR4-activation, and subsequently to changes in the recruitment of signaling components, in the release of cytokines and/or chemokines and in the expression of costimulatory and/or adhesion molecules (Beutler, 2000, Freudenberg, et al., 2008, Jerala, 2007). However, bacterial ligands such as the zwitterionic polysaccharide A (PSA) from *Bacteroides fragilis* exhibit even dual functions without apparent formation changes: on the one hand PSA serves as major pathogenic factor for *Bacteroides fragilis* as PSA is critical for bacterial growth and the formation of intra-abdominal abscesses (Surana and Kasper, 2012), which is at least partially dependent on TLR 2 (Wang, et al., 2006). PSA-dependent TLR2-engagement induces also the production of TNF- α and iNOS as well as the expression of MHC II. The subsequent presentation of PSA by MHC II on antigen presenting cells activates CD4⁺ T cells to secrete IFN- γ (Wang, et al., 2006). On the other hand, PSA directs potent anti-inflammatory responses through the engagement of TLR2, mainly through the subsequent production of IL-10, which exhibits potent macrophage-deactivating and anti-inflammatory effects (Bogdan, et al., 1991) and regulates the growth, function and/or differentiation of multiple cell populations (Moore, et al., 2001) including Tregs (Murai, et al., 2009). Allelic variations within the *il-10* or the *il-10r* gene are closely associated with susceptibility to IBD (Kaser, et al., 2010) and mice that are deficient in either IL-10 or the IL-10R2 develop severe enterocolitis (Berg, et al., 1995, Kuhn, et al., 1993, Spencer, et al., 1998). A recent study highlighted thereby the critical protective role of IL-10R expression on macrophages (Zigmond, et al., 2014). Thus, IL-10 plays a substantial role in the maintenance of intestinal homeostasis and the PSA induced T cell-dependent IL-10-production under steady state conditions in the intestinal tract might reflect a protective probiotic effect in this context (Round, et al., 2011). Furthermore, peritoneal macrophages can release IL-10 upon exposure to *Bacteroides fragilis* (Cohen-Poradosu, et al., 2011).

Thus, on the one hand it has been well established that bacterial compounds can shape the extent and phenotype of an ongoing immune response. On the other hand, however, it has not been investigated in great detail so far whether polymorphisms within the genes encoding the different innate immune receptors influence the microbial composition of the gut and subsequently the balance between inflammation and mucosal tolerance.

Intestinal microbiota

The identification of different microbial species that densely populate the intestine and the characterization of distinct bacterial colonization clusters have evolved as central research focus within recent years. Although bacterial species themselves vary extensively among individuals, the intestinal bacterial composition exhibits similarities within the respective intestine-populating bacterial families (Turnbaugh, et al., 2009). The main bacterial phyla present in the gut flora are *Firmicutes* (95% *Clostridia*) and *Bacteroides* (Eckburg, et al., 2005, Suau, et al., 1999). In contrast, the phyla *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* are scarce (Eckburg, et al., 2005).

The majority of intestinal microbes consists of commensal bacteria that provide benefits for the host, including the degradation of nutrients and various defense mechanisms against pathogenic organisms (reviewed in Shanahan, 2002). One of those, the gut flora-mediated colonization resistance, however, can be disrupted during antibiotic treatment allowing the outgrowth of diarrhea-causing agents such as *Salmonella species* or *Clostridium difficile* (Spencer, 1998). *Vice versa*, fecal transplants reconstituting the physiological microbial flora in the gut proved to be effective for the treatment of - otherwise even refractory - *Clostridium difficile* infections (van Nood, et al., 2013). In particular, *Clostridium scindens*, a bile-acid 7 α -dehydroxylating intestinal bacterium, has been recently associated with resistance to *Clostridium difficile* infections (Buffie, et al., 2014). Furthermore, immune-mediated disorders causing inflammation within intestinal mucosal tissues such as chronic inflammatory bowel diseases (IBD) are also frequently accompanied by alterations in the composition of the intestinal bacterial flora. Crohn's disease (CD), for example, is associated with a reduced diversity of the bacterial phylum *Firmicutes* (Frank, et al., 2007, Manichanh, et al., 2006) and an amplified population by *Enterobacteriaceae* such as *Escherichia coli* (Willing, et al., 2009). Patients suffering from ulcerative colitis (UC) reveal diminished numbers of bacteria considered as commensals such as *Lactobacillus salivarius*, *Lactobacillus manihotiborans* and *Pedococcus acidilactici* in faecal samples during active colitis periods compared to phases under remission (Bullock, et al., 2004). Furthermore, different numbers of the anaerobic bacterium *Clostridium leptum* are recovered when ulcerated with non-ulcerated intestinal tissue samples within the same group of patients are compared (Zhang, et al., 2007).

Chronic inflammatory diseases

As described above, a disturbed intestinal homeostasis either reflected or induced by changes in the intestinal microbial composition is associated with inflammatory bowel diseases (IBD) (Maloy and Powrie, 2011). Ulcerative colitis (UC) and Crohn's disease (CD) represent the two major forms of these devastating gut disorders (Podolsky, 2002) which occur after the second and third decades of life (Xavier and Podolsky, 2007). Although the exact etiology is still unknown, a combination of deregulated immune mechanisms, genetic factors and environmental triggers is suspected as causative (Hollander, 2003). While Th1 cells and the related cytokines IL-12 and IFN- γ dominate in CD, more IL-5 and IL-13, two Th2-associated cytokines, are detectable in UC (Xavier and Podolsky, 2007). Both, CD as well as UC patients, however, exhibit heavy infiltrations of the gut mucosa by Th17 cells (Fujino, et al., 2003). Besides these alterations in the lymphoid cell compartment, the homeostasis of other cell populations is disrupted as well. Particularly, myeloid cell populations are detected in different locations under inflammatory compared to physiologic conditions. For example, scavenger macrophages characterized by the expression of acid phosphatase (Seldenrijk, et al., 1989) reside under steady state conditions usually underneath the enterocytes. In contrast, these scavenger macrophages are detected in higher numbers in the gut mucosa of patients suffering from CD or UC (Seldenrijk, et al., 1989). Furthermore, DCs are mainly present underneath the muscularis mucosae and in the lymph follicles of healthy individuals, whereas they co-localize in the gut of IBD patients with scavenger

macrophages, in so-called band-like zones at the bottom of the intestinal ulcerations (Seldenrijk, et al., 1989).

Myeloid cell populations

It can be very difficult to distinguish macrophages and DCs because they share phenotypical and functional characteristics. Nonetheless, there exist several markers to classify these two cell types and their respective subpopulations. Whereas plasmacytoid DCs (pDCs) in mice are characterized as CD11c^{int}, B220⁺ and CD11b⁻ cells (Asselin-Paturel, et al., 2001), high CD11c expression is considered as typical for conventional DCs. The utilization of CD11b and CD8α as markers allows thereby the specification of three subtypes of conventional CD11c^{hi} DCs: CD11c^{hi}CD11b⁺CD8α⁻, CD11c^{hi}CD11b⁻CD8α⁺ and CD11c^{hi}CD11b⁻CD8α⁻ (Iwasaki and Kelsall, 2001). However, CD11c is not DC-specific as it can be also expressed by mononuclear phagocytes (Varol, et al., 2010). Therefore additional markers are used to characterize mononuclear cells in the intestine: the chemokine receptor CX₃CR1 (Niess, et al., 2005) and the integrins CD103 (Schulz, et al., 2009) and CD11b (Varol, et al., 2010). Intestinal resident macrophages are characterized as CD11c⁻CD64⁺F4/80⁺CD11b⁺CD103⁻CX₃CR1^{-/+} cells (Bain and Mowat, 2014, Cerovic, et al., 2014). The intestinal macrophage pool is thereby continuously replenished by circulating Ly6C^{high} monocytes in a CCR2-dependent manner under steady state conditions (Bain and Mowat, 2014) (Figure 1). DCs are characterized as CD11c⁺MHCII⁺F4/80⁻CD64⁻ cells (Cerovic, et al., 2014). The respective expression patterns of these markers allow the identification of four subsets of intestinal mononuclear phagocytes in the murine lamina propria: (1) CD103⁺CD11b⁺, (2) CD103⁻CD11b⁺ CX₃CR1^{int}, (3) CD103⁺CD11b⁻CD8α⁺ (4) CD103⁻CD11b⁻ (Cerovic, et al., 2014) (Figure 1).

Furthermore, according to their localization and migration behavior DCs could be alternatively divided into two distinct types: (a) tissue DCs that reside in the mucosa, skin or internal organs and (b) blood DCs detected in the blood and lymphoid tissues (Itano and Jenkins, 2003). Tissue DCs, also called migratory DCs, phagocyte antigens while scavenging their local tissue sites and migrate upon antigen encounter to the respective draining lymph nodes. The blood DCs or lymphoid tissue-resident DCs migrate as precursors from the blood into the lymph nodes and develop there into mature DCs (Naik, et al., 2006).

Apart from these different classification categories, further distinct population entities based on the expression of surface markers that have been associated with IBD might be identified as well. The characterization of these cell populations and the analysis of the molecular mechanisms driving their differentiation might not only contribute to an improved understanding of the pathogenesis of IBD, but might also allow the development of novel therapeutic strategies that are urgently needed for the treatment of these devastating diseases. Thus, we will focus on four molecules, that are expressed on intestinal myeloid cells and have been involved in the pathogenesis of IBD, among many other markers described in this context (Table 1).

Association of distinct surface molecules on myeloid cells with the pathogenesis of IBD

CX₃CR1

CX₃CR1 is a seven transmembrane G protein-linked chemokine receptor for the CX₃C chemokine fractalkine (FKN), also called neurotactin (NTN) or NKAF (Imai, et al., 1997). This novel chemokine is characterized by a three amino acid spacing between the N-terminal cysteines (Bazan, et al., 1997) and a unique membrane-bound structure from which it gets proteolytically cleaved into its soluble form. The cell-surface-bound form is expressed on activated endothelial cells and attracts leukocytes, whereas the soluble form attracts T cells and monocytes (Bazan, et al., 1997). The receptor CX₃CR1 is expressed on natural killer cells, T cells and monocytes (Imai, et al., 1997), intestinal epithelial cells (Brand, et al., 2002) and microglial cells in the brain (Raport, et al., 1995). However, CX₃CR1-expressing antigen presenting cells (APCs) still consist of a rather heterogeneous population of DCs and macrophages (Maloy and Powrie, 2011), which might contribute to the discrepant role attributed to CX₃CR1 in mouse models (Kostadinova, et al., 2010, Medina-Contreras, et al., 2011).

CX₃CR1-positive DCs are abundantly found in the lamina propria of the small intestine and the colon (Niess, et al., 2005). They develop from Ly6C^{hi} monocytes in a GM-CSF-dependent manner (Varol, et al., 2009). The dendrites of the CX₃CR1⁺ DCs sample antigens from the gut lumen (Niess, et al., 2005) suggesting them as important sensor for harmless and pathogenic compounds. Those compounds trigger specific innate receptors, such as TLRs, and thus, might change the expression of CX₃CR1, but might also influence the availability of the CX₃CR1 ligand. CX₃CR1 exhibits a DRY motif, *i.e.* the amino acids aspartic acid-arginine-tyrosine, which is important for the interaction with G_{ai} proteins leading after binding to FKN to cell adhesion and chemotaxis (Imai, et al., 1997).

In the dextran sodium sulphate- (DSS-) induced mouse model of colitis there were increased amounts of CX₃CR1-positive cells recovered from the colon. Furthermore, CX₃CR1-deficient mice exhibit an attenuated intestinal inflammation after DSS-application, caused by a reduction of intracolonic iNOS-producing macrophages and a reduced secretion of the proinflammatory cytokines IL-6, TNF- α and IFN- γ (Kostadinova, et al., 2010). A subsequent study, however, reported opposite results suggesting a critical role of CX₃CR1 in the maintenance of macrophage homeostasis in the lamina propria, inhibition of bacterial translocation to mesenteric lymph nodes (mLNs) and the limitation of Th17 responses (Medina-Contreras, et al., 2011). Furthermore, the deficiency of CX₃CR1-expressing macrophages in the same study enhanced the severity of colitis, an effect that was rescued by the transfer of CX₃CR1⁺ macrophages. Next to mouse-strain dependent differences, the expression of CX₃CR1 on different cell subsets and subsequent altered deletion strategies might contribute to the observed discrepant results.

Furthermore, the reduced numbers of CX₃CR1⁺ macrophages in the lamina propria cause a decreased secretion of IL-10, which is critical for the generation of antigen-specific FoxP3⁺ regulatory T cells (Hadis, et al., 2011). The accumulation of CX₃CR1⁺ macrophages and

DCs itself depends thereby on the intestinal flora, as both cell populations were reduced in germ free mice (Niess and Adler, 2010). In addition, CX₃CR1-deficiency attenuates the intestinal pathology induced through the adoptive transfer of T cells correlating with reduced numbers of DCs in the mLNs and reduced serum IFN- γ - and IL-17-levels (Niess and Adler, 2010). Thus, in summary, although CX₃CR might preferentially contribute to the induction of intestinal pathology, these results should not be generalized as opposite results have been reported, reflecting the effects of CX₃CR-deletion on heterogenous myeloid cell subsets.

CD103

CD103 is an α_E integrin, a subunit of a cell adhesion molecule mediating cell-cell contacts or cell-matrix contacts (Hynes, 1987), that is expressed on T cells (Andrew, et al., 1996) and DCs (Kilshaw, 1993). CD103 binds together with the β_7 chain, the second subunit of the integrin molecule (Hadley, et al., 1997), to E-cadherin (Cepek, et al., 1994) which mediates the adhesion of T lymphocytes to epithelial cells (Cepek, et al., 1993). Next to intestinal epithelial cells (Hermiston and Gordon, 1995), E-Cadherin is also expressed on keratinocytes (Wheelock and Jensen, 1992) and Langerhans cells (Tang, et al., 1993). Most of the T cells in the gut and a subset of intestinal DCs express CD103 (Annacker, et al., 2005), whereas CD103 is only sparsely expressed on T cells in the blood, spleen and lymph nodes (Cerf-Bensussan, et al., 1987, Kilshaw and Murant, 1990). CD103⁺ DCs in the gut develop from macrophage-DC precursors (MDPs) *via* nonmonocytic DC-committed intermediates (pre-cDCs) under the control of the receptor-type tyrosine-protein kinase 3 ligand, Flt3L (Varol, et al., 2009). The mature DCs migrate from the intestine into the mLNs in a CCR7-dependent manner (Jang, et al., 2006) and present antigens to naïve T cells (Bogunovic, et al., 2009). However, until to date, the mechanisms how CD103⁺ DCs acquire antigens have remained unknown. It has been suggested that CX₃CR1⁺ macrophages take up antigens from the intestinal lumen (see above) (Niess, et al., 2005) and transfer them to CD103⁺ DCs (Scott, et al., 2011). CD4⁺ T cells co-cultured *in vitro* with CD103-positive DCs obtained *ex vivo* from the lamina propria and mLNs produce retinoic acid (Coombes and Powrie, 2008) and up-regulate the gut-homing chemokine receptors CCR9 (Annacker, et al., 2005) and $\alpha_4\beta_7$ mediating the homing of T cells in the intestine. Accordingly, CD103-deficient mice exhibit reduced numbers of intraepithelial lymphocytes (IELs) and T cells in the lamina propria (Schon, et al., 1999). CD103-positive DCs from the mLNs and the lamina propria mediate under homeostatic conditions the conversion of naïve CD4⁺ T cells into FoxP3⁺ T cells through the secretion of TGF- β and retinoic acid and thus, contribute to the maintenance of intestinal tolerance (Coombes, et al., 2007). However, CD103⁺ DCs lose their tolerogenic properties and ability to induce FoxP3⁺ T cells under inflammatory conditions as they express significantly reduced amounts of TGF- β and ALDH1, an enzyme, that catalyzes the oxidation of retinal, the aldehyde form, to retinoic acid (Laffont, et al., 2010). Furthermore, in the absence of CD103, FoxP3⁺ T cells are unable to suppress colitis induced by the adoptive transfer of naïve T cells (Annacker, et al., 2005). In contrast, CD103⁺ DCs can even support the generation of IFN- γ -producing T cells under inflammatory conditions (Laffont, et al., 2010).

Taken together, these studies suggest an important role of CD103-positive DCs in the maintenance and even reconstitution of intestinal tolerance. Thus, the application of factors that induce the tolerogenic properties of CD103⁺ DCs might reflect a promising therapeutic option. Those factors include next to specific cytokines such as TGF- β or IL-10, neurotransmitters such as the vasoactive intestinal peptide VIP, intestinal bacteria such as *Bacteroides* and *Bifidobacteria* strains and dietary components such as vitamin A, that condition CD103⁺ DCs (Scott, et al., 2011). Thus, dietary supplements might support conventional therapeutic approaches.

TNF-like factor 1A (TL1A)

TL1A, also called TNFSF15, is a member of the tumor necrosis factor (TNF) superfamily and binds to the death domain receptor 3 (DR3) (Migone, et al., 2002) expressed particularly on activated lymphocytes (Screaton, et al., 1997). DR3 belongs to the TNF-receptor superfamily and contains a death domain (DD), a globular structure of 60 amino acids. Historically, DDs were considered as cell death inducers through the activation of caspases (Migone, et al., 2002). However, recent work provided evidence that death domain containing receptors provide also costimulatory signals for T cell activation (Siegel, et al., 2000). TL1A can induce both signaling pathways (Migone, et al., 2002). The induction of one or the other depends thereby on the microenvironment and the cells on which TL1A acts (Migone, et al., 2002).

The expression of TL1A was first described on endothelial cells (Migone, et al., 2002). Later on, it was shown that lamina propria lymphocytes, tissue macrophages and plasma cells in the gut mucosa (Bamias, et al., 2003) as well as DCs in the lamina propria (Bamias, et al., 2006) of IBD patients also express TL1A.

The engagement of DR3 by TL1A activates NF- κ B mediated signaling pathways and induces greater IL-2-responsiveness of T cells and the production of pro-inflammatory cytokines, in particular within a combined response together with IL-12 and IL-18 (Migone, et al., 2002). This selective Th1 activation explains the predominant role of TL1A in CD (Bamias, et al., 2003). However, also an increased IL-17 production after TL1A stimulation in mice treated with DSS has been reported (Takedatsu, et al., 2008).

In intestinal tissue biopsies of CD and UC patients, TL1A protein expression is up-regulated on lymphocytes and tissue macrophages correlating with the severity of disease. The amount of TL1A-mRNA, however, was only increased in patients with CD suggesting post-translational modification mechanisms in UC. Intestinal biopsies from IBD patients also revealed increased expression of DR3 correlating with the severity of inflammation (Bamias, et al., 2003). The observations in patient samples suggesting a pathogenic role of TL1A in the progression of disease were supported by the examination of two mouse models for chronic ileitis, namely the SAMP1/YitFc and the TNF^{ARE} mice (Bamias, et al., 2006). The constitutive expression of TL1A upon induction of colitis due to the application of DSS or the adoptive transfer of T cells promoted increased fibrosis and subsequent fibrostenosis in the gut of the mice. Consistent with these observations in mice CD patients with higher TL1A expression suffered more frequently from intestinal strictures (Barrett, et

al., 2012). A genome-wide association study in Japanese and UK patients with CD revealed a strong disease association by SNPs discovered within the TL1A gene *TNFSF15* (Kakuta, et al., 2006, Yamazaki, et al., 2005). *TNFSF15* haplotypes are also associated with increased susceptibility to CD and UC in non-Jewish Caucasian IBD patients. While one of the three haplotypes, the haplotype B is associated with the severity of disease in Jewish CD patients, there was no association in Jewish UC patients (Picornell, et al., 2007). Bacteria can also induce species-dependent TL1A expression in monocytes and monocyte-derived DCs *via* p38 and NF- κ B. Thereby living bacteria exhibited a more potent ability to induce TL1A expression in antigen presenting cells (APCs) than dead bacteria (Shih, et al., 2009).

These data suggest that the expression of TL1A is associated with more severe intestinal inflammation in mouse models and IBD patients. As TNF- α is a potent mediator of IBD (Breese, et al., 1994, Nicholls, et al., 1993), the production of therapeutic molecules targeting additional pathways involving the TNF superfamily such as TL1A next to the already successful anti-TNF- α antibodies might open novel routes for clinical intervention (Feagan, et al., 2005, Schreiber, et al., 2005, Schreiber, et al., 2007, Stack, et al., 1997).

Triggering receptor expressed on myeloid cells-1 (TREM-1)

TREM-1 is a 26kDa glycoprotein belonging to the immunoglobulin superfamily (Ig-SF) (Bouchon, et al., 2000). It contains an extracellular hydrophobic signal peptide followed by a type-V Ig SF domain, a transmembrane domain and a cytoplasmic domain (Bouchon, et al., 2000). The ligand for TREM-1, however, has remained unknown so far (Weber, et al., 2014).

Membrane-bound TREM-1 is not detected on the surface of intestinal macrophages of healthy individuals and thus, cannot induce inflammatory reactions against bacterial commensals. However, the expression of TREM-1 can be induced on neutrophils and monocytes after stimulation with bacteria and fungi, but also defined bacterial ligands such as lipoteichoic acid (LTA), LPS and flagellin in a TLR2-, TLR4- or TLR5-dependent manner (Knapp, et al., 2004). In contrast, CpG and nonbacterial ligands such as poly dI-dC and imidazoquinoline, are unable to promote TREM-1-expression (Knapp, et al., 2004) as TLR3, -7 and -9 are expressed on DCs and B cells, but not monocytes and neutrophils (Hornung, et al., 2002, Jiang, et al., 2003, Muzio, et al., 2000).

The soluble form of TREM-1 (sTREM-1) is either a splice variant or a shedded version of the membrane-bound form (Oku, et al., 2013). Crosslinking of TREM-1 with a monoclonal antibody triggers the release of IL-8 and myeloperoxidase (MPO) by neutrophils and of IL-8, chemo attractant protein-1 (MCP-1) and TNF- α by monocytes. Activation of TREM-1 also induces an up-regulation of adhesion molecules leading to the enhanced migration of neutrophils and monocytes to the site of inflammation via tyrosine phosphorylation of phospholipase C (PLC)- γ and extracellular-signal-regulated kinases 1/2 (ERK1/2) followed by an influx of calcium. The activation of this signaling cascade is mediated through an intracellular adapter molecule of TREM-1, DAP12 (Bouchon, et al., 2000). In contrast, TREM-1 expression is down-regulated in the gut in the presence of IL-10 and TGF- β (Schenk, et al., 2005).

The intestinal lamina propria of IBD patients exhibits increased numbers of TREM-1-expressing macrophages compared to healthy intestinal biopsies correlating with the severity of disease. These macrophages also express CD14, a co-receptor with TLR4 for LPS and receptor for other pathogen-associated molecular patterns (PAMPs), such as LTA (Jersmann, 2005) and CD89, the receptor for the Fc fragment of IgA (FCAR). In contrast, these two molecules are absent from macrophages in gut biopsies without intestinal inflammation (Schenk, et al., 2007). Furthermore, the concentration of the soluble form of TREM-1 (sTREM-1) is significantly higher in the serum of patients with severe CD (Park, et al., 2009). The expression of sTREM-1 correlates also with the serum levels of TNF- α and the severity of disease in patients suffering from UC (Tzivras, et al., 2006). The same study reported also a correlation between the serum levels of sTREM-1, the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP). Therefore the authors suggested sTREM-1 as a clinical marker for the activity of UC (Park, et al., 2009), but not of CD (Jung, et al., 2012). The pathogenic role of TREM-1 under inflammatory conditions could be also confirmed in two different mouse models (Schenk, et al., 2007). *Trem1*^{-/-} mice exhibited a less severe DSS-induced colitis and *Trem1*^{-/-} $\times$ *Rag2*^{-/-} mice suffered from significantly reduced intestinal inflammation after the adoptive transfer of naïve T cells (Weber, et al., 2014).

In summary, the expression of TREM-1 on intestinal macrophages as well as the serum level of sTREM-1 correlate with the severity of IBD suggesting TREM-1 as an adequate clinical marker for disease activity. Furthermore, TREM-1 might serve as target for clinical intervention as the administration of antagonistic TREM-1 peptides rescued mice from DSS colitis (Schenk, et al., 2007) (Zhou, et al., 2013).

Discussion

There have been several susceptibility genes and environmental triggers implicated in the pathogenesis of IBD. However, the etiology of both, CD and UC has still remained largely unknown. Although far from being complete, we discussed here the impact of the intestinal microbial flora on the phenotypic presentation of myeloid cell populations within the gut mucosa and the regulation of four promising candidate molecules, CX₃CR1, CD103, TL1A and TREM-1 within this context. Subsequently, while the suppression of signaling pathways would represent a favorable approach for clinical intervention targeting TL1A and TREM-1, therapeutic approaches involving CD103 should enhance either its expression and/or function. The role of CX₃CR1, in contrast, is more controversial, likely due to its expression on rather heterogeneous cell populations. Although the application of distinct microbial ligands or bacterial species would be an elegant and attractive approach for clinical intervention, the expression of CD103 appeared not to be regulated upon TLR ligation or engagement of other innate immune receptors (Bogunovic, et al., 2009, Scott, et al., 2011). However, CD103 affects signaling pathways that are associated to other receptor families. Thus, CD103, for example, sensitizes DCs in the lamina propria for the production of defined cytokines such as IL-23 upon engagement of TLR5 (Kinnebrew, et al., 2012). Hence, not only the regulation of the expression of the target molecule itself, but also the target molecule-mediated sensitization for other innate receptors would be of great interest for clinical intervention. Furthermore, the analysis of negative or positive cells for a defined

marker might serve as an indicator for altered bacterial composition and thus, allow predictions about the progression, relapse or remission of disease. Based on those markers, the respective treatment strategies might be initiated, continued or interrupted.

In this context and with respect to the regulation of innate immune receptors, Ly6C^{hi} monocytes might reflect a promising cellular target for molecular intervention as they represent on the one hand the precursors for CX₃CR1^{hi} macrophages in the lamina propria of healthy individuals. CX₃CR1⁺ macrophages are a critical cell population for intestinal homeostasis, although discrepant results about their contribution to the pathogenesis of IBD have been published (Kostadinova, et al., 2010, Medina-Contreras, et al., 2011). On the other hand, Ly6C^{hi} monocytes differentiate into dominant pro-inflammatory effector cells in the inflamed gut due to the up-regulation of TLR2 and NOD2 (Zigmond, et al., 2012). Furthermore, CD14-expressing macrophages that are more sensitive to LPS (Lee, et al., 1992) are increased in the intestinal mucosa of IBD patients compared to healthy controls (Obana, et al., 2002) and might contribute to the initiation and maintenance of the inflammatory environment. Thus, not only the regulation of the respective target molecules itself, but also the modulation of receptors and signaling pathways influenced by their expression might represent promising targets for clinical intervention.

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Abbreviations

CD	Crohn's disease
DC	dendritic cell
IBD	inflammatory bowel disease
TLA1	TNF-like factor 1A
TREM-1	Triggering receptor expressed on myeloid cells-1
UC	ulcerative colitis

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Figure 1. The origin, localization and phenotype of intestinal DC and macrophage populations are displayed

DCs and dendritic cell precursors (pre-DCs) are components of isolated lymphoid follicles (ILFs). Upon antigen encounter and/or due to paracrine signals elicited by microbial or dietary compounds from the intestinal lumen, pre-DCs within the ILFs or the Lamina propria (L.p.) migrate in a CCR7-dependent manner into mesenteric lymph nodes (mLNs). Dependent on the antigen presented or the paracrine signals provided, these migratory DCs differentiate into four subpopulations that can be distinguished from each other as well as from mLN-resident DCs by the expression of CD11b, CD8 α , CX3CR1 and CD103. In contrast to the concept that macrophages residing within steady-state tissues are derived from embryonic precursors, recent observations suggested that the intestinal macrophage pool is constantly replenished by circulating monocytes. The morphologic and phenotypic changes together with the respective surface markers expressed during the different maturation steps are displayed.

The Figure is adapted from Cerovic et al., Trends in Immunology, 2014.

Table 1

The table summarizes molecules expressed on myeloid cell populations that have been associated with the pathogenesis of IBD.

Factor	cells	relevance in IBD	reference
CD14	MΦ	Polymorphisms in the CD14 gene are associated with CD	(Klein, et al., 2002)
CD83	DC	Increased number in the subepithelial dome of patients with CD	(Salim, et al., 2009)
DC-SIGN	DC	Increased number in colonic mucosa of patients with CD	(te Velde, et al., 2003)
E-cadherin	DC	Associated with the pathogenesis of intestinal inflammation	(Siddiqui, et al., 2010)
Langerin	immature DC	Increased number in IBD gut biopsies	(Kaser, et al., 2004)
Ly6C	Monocytes	Dominant mononuclear cell type in L.p. during acute colitis	(Zigmond, et al., 2012)
TLR2/TLR4	MΦ, DC	Increased expression in the gut of IBD patients	(Frolova, et al., 2008)