Review article

Wnt signaling in intestinal inflammation

Lavanya Moparthi*a,b, Stefan Kocha,b,*

Abstract

Chronic inflammatory bowel diseases, including Crohn’s disease and ulcerative colitis, are a major health burden worldwide. Numerous conserved signaling pathways control tissue injury and repair during colitis, but owing to the complexity of the inflammatory process, their individual contribution remains poorly understood. A key regulatory pathway in the intestinal mucosa is Wnt/β-catenin signaling, which acts as the central organizer of epithelial stem cell identity and maintenance. Apart from this core function, there is mounting evidence that the Wnt pathway is highly interconnected with numerous other signaling cascades, and that combinatorial signaling events shape epithelial homeostasis and tissue regeneration. Here we provide an updated view of how Wnt signaling interacts with major inflammatory pathways, with a particular focus on intestinal inflammation. Elucidating the reciprocal actions of Wnt ligands and cytokines has the potential to reveal new treatment options for chronic colitis and other inflammatory disorders.

1. Epithelial barrier function in inflammatory bowel diseases

Inflammatory bowel diseases (IBD) are debilitating disorders of the gastrointestinal tract. The two major IBDs – Crohn’s disease and ulcerative colitis – affect millions of people worldwide, with rapidly rising incidence rates particularly in newly industrialized countries (Ng et al., 2018). The hallmark of IBD is chronic recurring inflammation of the intestinal mucosa, which causes bloody diarrhea and severe abdominal pain, among other symptoms. The ebb and flow of tissue injury and repair eventually leads to loss of function of the mucosa, and can trigger colitis-associated cancers (Ullman and Itzkowitz, 2011).

Despite decades of intensive investigation, the etiology of IBD remains incompletely understood. Although intrinsic (such as host genetics) and extrinsic factors (such as the intestinal microbiome) are alternatingly being favored as new research tools become available, it is most likely that IBDs are multi-factorial, and require at least two independent triggers (Kaser et al., 2010). Indeed, experimental evidence from mice suggests that genetic susceptibility is common but insufficient to drive chronic colitis, which is then initiated by e.g. dysbiosis or acute epithelial colitis (Cadwell et al., 2010; Khounlotham et al., 2012). Regardless of the cause, the key event at the onset of colitis is a breakdown of the intestinal epithelial barrier, which separates gut contents from the mucosal immune system and thus assures intestinal homeostasis (Atreya and Neurath, 2015; Koch et al., 2013). Loss of barrier function leads to an influx of luminal pathogens and subsequent massive immune response, which causes tissue damage and ultimately clinical symptoms characteristic for IBD. Because of this, resealing of the epithelial barrier is of primary importance and indispensable for the regeneration of the injured mucosa.

To maintain barrier integrity in the challenging environment of the gut, the single-layered intestinal epithelium undergoes constant renewal driven by a small number of highly proliferative stem cells at the base of the intestinal crypts (Barker, 2014). As cells migrate along the crypt-surface axis, they differentiate into functionally distinct epithelial cell populations, and are ultimately shed into the intestinal lumen. In this way, most epithelial cells are rapidly replaced to avoid attrition by mechanical injury or pathogen exposure, with an estimated turnover rate of the human colorectal epithelium of four days (Darwich et al., 2014). Under normal homeostatic conditions, this rate remains constant throughout life, with minor variations related to age and tissue. However, during epithelial restitution following injury, epithelial cells at the wound margin dynamically increase their proliferation rate to cover the denuded area (Iizuka and Konno, 2011; Seno et al., 2009). Importantly, this behavior can also be observed in IBD and animal models thereof. In

Abbreviations: AKT, Protein kinase B; DKK, Dickkopf-related protein; DSS, Dextran sulfate sodium; EGF, Epidermal growth factor; ERK, Extracellular signal-regulated kinase; GSK, Glycogen synthase kinase; IBD, Inflammatory bowel diseases; IFN, Interferon; IKK, Inhibitor of κB; IL, Interleukin; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LRP, Low-density lipoprotein receptor-related protein; MAPK, Mitogen Activated Protein Kinase; NF-κB, Nuclear factor kappa B; STAT, Signal transducer and activator of transcription; TCF, Transcription factor; TFF, Tumor necrosis factor; WNT, Wingless/Int1

a Wallenberg Centre for Molecular Medicine (WCMM), Linköping University, S-581 85 Linköping, Sweden
b Department of Clinical and Experimental Medicine (IKE), Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden

* Correspondence to: Linköping University, IKE/MII – Plan 12, S-581 85 Linköping, Sweden.
E-mail address: stefan.koch@liu.se (S. Koch).

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ulcerative colitis, for example, the epithelial turnover rate is strongly increased during active disease, but lower during remission (Serafini et al., 1981). Similarly, we observed that in the dextran sulfate sodium (DSS) colitis model in mice, epithelial cell proliferation increases specifically at the margin of mucosal ulcers (Nava et al., 2010). In addition, mature epithelial cells adjacent to the wound undergo a differentiation process that is distinct from normal enterocyte differentiation (Iizuka and Konno, 2011; Miyoshi et al., 2017). Injury-associated epithelia rapidly migrate across the ulcer to seal the wound, before they are replaced by regular intestinal epithelial cells. Thus, injury and inflammation trigger a regenerative program that transiently alters intestinal epithelial cell proliferation and differentiation to restore the epithelial barrier.

A key regulator of epithelial proliferation is the homeostatic Wingless/Int1 (Wnt) pathway. In-depth reviews on the molecular mechanisms of Wnt signaling can be found elsewhere (Niehrs, 2012; Nusse and Clevers, 2017). Briefly, in the canonical Wnt signaling branch, Wnt and R-spondin family ligands induce the stabilization of the transcription co-factor β-catenin via inhibition of a multi-protein destruction complex. β-catenin, together with Transcription factor / Lymphoid enhancer-binding factor (TCF/LEF) type transcription factors, drives the expression of target genes that promote stem cell identity and cell cycle progression (Fig. 1). Thus, Wnt/R-spondin signaling is a central determinant of stem cell maintenance and proliferation in various organs. In the intestine, in particular, active Wnt signaling is essential to maintain epithelial homeostasis, and pathway inhibition results in crypt loss and tissue degeneration (Kühnert et al., 2004). Recent studies using β-catenin/TCF reporter animals demonstrate Wnt pathway activation also during tissue repair. Among others, extensive Wnt reporter activity has been observed in the regenerating skin, heart, and bone (Whyte et al., 2012), all of which exhibit low cell turnover under normal homeostatic conditions. Collectively, these and other studies suggest that Wnt signaling is an evolutionarily conserved tissue (re)generation program, which can be transiently activated for wound repair.

2. Intersection of WNT and inflammatory signaling pathways

Inflammation triggers tissue regeneration as well, but to what extent Wnt signaling contributes to inflammatory injury repair remains to be resolved. Numerous external cues, such as growth factors and cytokines, bacterial antigens, and extracellular matrix properties, control the life and death of epithelia during colitis. On the molecular level, these cues are transduced by various highly conserved cell signaling pathways (Koch and Nusrat, 2012). Classic inflammatory signaling pathways include Nuclear Factor kappa B (NF-κB), Mitogen Activated Protein Kinase (MAPK), Protein Kinase B (PKB/AKT), and Signal Transducer and Activator of Transcription (STAT) signaling. Collectively, these pathways integrate the vast majority of inflammatory signals to guide host defense and tissue repair during intestinal inflammation (Koch and Nusrat, 2012; Schmitz et al., 2011). Although

![Integration of Wnt/β-catenin signaling with inflammatory pathways. Schematic illustration of the core Wnt/β-catenin pathway components, and possible connections that link the homeostatic Wnt pathway to inflammation-associated signaling events. Non-Wnt pathway components are depicted in orange. Detailed explanations can be found in the manuscript body, and in supplemental table 1.](image-url)
Wnt signaling is not generally considered an inflammatory pathway, there is mounting evidence that it can moonlight as a driver of injury repair (Karim and Clevers, 2016). Indeed, on the molecular level, there is a high degree of interaction between the Wnt pathway and inflammatory signaling cascades (Fig. 1, and Supplemental Table 1), which we review below. As a caveat lector, we note that many of the following studies have not explored signal cross-talk in intestinal inflammation, and so the relevance of these findings to mucosal wound repair in colitis remains to be confirmed.

2.1. NF-κB signaling

Considerable cooperation has been described between the Wnt and NF-κB signaling pathways, comprehensively reviewed in (Ma and Hottiger, 2016). NF-κB controls inflammation mainly through the transcriptional regulation of genes involved in immune activation and cell survival, including cytokines, chemokines, and growth factors. Wnt signaling intersects with NF-κB on multiple levels, leading to extensive reciprocal regulation of these pathways in a cell type and stimulus-dependent manner. In the context of intestinal pathobiology, the majority of studies indicate that Wnt activation inhibits NF-κB signaling primarily through direct interaction of β-catenin with NF-κB (Ma and Hottiger, 2016). β-catenin physically associates with NF-κB p65 and p50, and inhibits their DNA binding and target gene expression in colonic epithelial cells (Deng et al., 2002; Du et al., 2009; Liu et al., 2013). Consistently, β-catenin stabilization reduces Salmonella-induced NF-κB activation and cytokine expression in intestinal epithelia, both in vitro and in mice (Duan et al., 2007; Sun et al., 2005). It should be noted, however, that Schön and colleagues observed that TNFRSF19, a β-catenin/TCF target, activates NF-κB signaling in intestinal epithelial cells in a possible feedback loop (Schon et al., 2014). Thus, at least in cases of sustained β-catenin stabilization such as colorectal cancers, Wnt signaling may have a biphasic effect on NF-κB activity (Schwitalia et al., 2013).

On the other hand, NF-κB signaling feeds back into the Wnt pathway, although the underlying mechanisms are less well understood. In intestinal epithelial cell lines, inhibitor of κB (IκB) Kinase (IKK)-α, a positive regulator of NF-κB signaling that is activated by inflammatory cytokines, interacts with and phosphorylates β-catenin to drive the expression of select TCF target genes (Albanese et al., 2003; Lamberti et al., 2001). Consistently, activation of NF-κB signaling by deletion of IκB enhances β-catenin/TCF-dependent gene transcription, and promotes the dedifferentiation of intestinal epithelial cells in vivo presumably by imposing a stem cell signature (Schwitalia et al., 2013). Of note, these authors also observed an expansion of stem cell markers in ulcerative colitis samples, suggesting that this NF-κB/β-catenin interaction also occurs in intestinal inflammation. Conversely, however, some earlier studies reported that NF-κB inhibits TCF-dependent transcription in model intestinal epithelial cell lines, which may involve the induction of the β-catenin interacting protein LATS2 (Cho et al., 2008, 2005). Given that NF-κB signaling a prototypical inflammatory pathway, it thus appears worthwhile to further study β-catenin/NF-κB interactions in IBD.

2.2. MAPK signaling

Similar to NF-κB, the c-Jun N-terminal kinase (JNK) MAPK pathway is an important mediator of inflammation and tissue remodeling, which controls the expression of major cytokines and chemokines. Wnt signaling extensively interacts with JNK/c-Jun on several levels. Wnt ligands induce JNK/c-Jun activation, and c-Jun is a Wnt/β-catenin target gene that is induced, e.g., in colon cancer cells (Mann et al., 1999). β-catenin/TCF forms a ternary complex with c-Jun to activate c-Jun target gene transcription, dependent on JNK activity (Nateri et al., 2005; Sancho et al., 2009). Conversely, this tripartite protein complex also drives the transcription of β-catenin/TCF targets and thereby the progression of Wnt-dependent tumors (Gan et al., 2008; Nateri et al., 2005). Interestingly, in the absence of c-Jun, JNK activation inhibits β-catenin signaling by controlling its stability or subcellular localization (Hu et al., 2008; Liao et al., 2006). β-catenin-independent Wnt signaling induced by non-canonical ligands such as WNT5A activates JNK, primarily to control cell polarity and migration (Niehrs, 2012). In the context of inflammation, however, WNT5A may thus additionally antagonize the canonical Wnt pathway, to switch cell fate from proliferation to differentiation via JNK.

Cross-talk is also been observed between Wnt signaling and other MAPK pathways. Ras, an oncogene that activates extracellular signal-regulated kinase (ERK) in the epidermal growth factor (EGF) pathway, is a target of the β-catenin destruction complex (Jeong et al., 2012). Consequently, Wnt ligands promote ERK signaling by stabilising Ras. Activating mutations in adenomatous polyposis coli (APC) or KRAS, which frequently co-occur in colorectal cancers, thus synergistically drive tumorigenesis through Wnt and ERK signaling, although whether this is also the case in inflammation remains to be determined. Reciprocally, it has been suggested that the activation of the Wnt/β-catenin pathway by EGF/ERK signaling contributes to epithelial regeneration and tumor cell migration (Georgopoulos et al., 2014; Ji et al., 2009). Mechanistically, ERK signaling increases β-catenin nuclear translocation and TCF-dependent transcription, possibly via Wnt receptor activation (Lemieux et al., 2015). Interestingly, Kabiri and colleagues recently reported that inhibition of Wnt secretion in the gut acutely activates ERK in epithelial stem cells, and increases cell proliferation in the small intestine (Kabiri et al., 2018). These findings suggest an additional layer of regulation between the Wnt and ERK pathways, which may increase systemic robustness of the stem cell niche.

Finally, Wnt ligands also activate p38 MAPK. Cooperation between Wnt signaling and p38 regulates cell proliferation via control of both β-catenin/TCF and Myocyte Enhancer Factor 2 (MEF2) target genes (Bikkavilli et al., 2008; Cervenka et al., 2011; Ehyai et al., 2016). However, the relevance of these observations for inflammatory diseases has not been investigated. It should be noted that Červenka and colleagues observed that all three MAP kinases discussed here, i.e., JNK, ERK, and p38, can phosphorylate LRP6 and thereby activate the Wnt pathway (Červenka et al., 2011). Thus, activation of Wnt receptors might be a common point of convergence by which growth factors and cytokines regulate β-catenin-dependent transcription.

2.3. AKT signaling

AKT is a critical regulator of several major signaling pathways, including NF-κB and mammalian target of rapamycin (mTOR) signaling. Although numerous studies have implicated AKT in epithelial regeneration during colitis, to what extent it participates in Wnt signaling remains highly controversial. The main point of contention concerns a key molecular target of AKT, Glycogen synthase kinase (GSK) 3. GSK3 exists in two discrete pools within the cell: a free cytosolic pool that controls a plethora of downstream targets, and a minor pool associated with the scaffold protein Axin as part of the β-catenin destruction complex. The current paradigm is that the activity of these GSK3 sub-pools is regulated by separate and insulated signaling events, in which only free GSK3 is subject to AKT-dependent inhibition, whereas Wnt activation exclusively inhibits Axin-bound GSK3 in a phosphorylation-independent manner (McNeill and Woodgett, 2010; Metcalfe and Brien, 2011). According to this model, AKT/GSK3 and Wnt/GSK3 should have no bearing on the respective other pathway.

However, several lines of evidence argue against complete pathway insulation. Firstly, and perhaps most controversially, several studies suggested that growth factors induce Wnt/β-catenin pathway activation directly via AKT-dependent GSK3 inhibition (Desbois-Mouthon et al., 2001; Fukumoto et al., 2001; Sharma et al., 2002). Conversely, it has been reported that Wnt ligands promote inhibitory GSK3
phosphorylation and thereby β-catenin signaling (Bikkavilli et al., 2008; Cook et al., 1996). However, a precise mechanism for these observations has not been elucidated. On the contrary, it is generally accepted that Axin-bound GSK3 is protected from kinase-dependent inhibition, and that cytosolic GSK3 does not contribute to β-catenin regulation (McNeill and Woodgett, 2010). These conflicting models may be reconciled by the observation that G protein activation, e.g. by colitis-associated prostaglandin E2 (PGE2), causes the dissociation of GSK3 from Axin, while at the same time activating GSK3 kinases (Castellone et al., 2005). Since Frizzled family Wnt receptors are G protein-associated as well, Wnt ligands may induce GSK3 phosphorylation indirectly, possibly via p38 MAPK (Bikkavilli et al., 2008). Thus, while Wnts and prostaglandins could trigger inhibitory GSK3 phosphorylation, this may be an epiphenomenon of G protein-dependent destruction complex disassembly, which is by itself sufficient to stabilize β-catenin.

This reciprocal regulation of GSK3 by Wnt and AKT is likely relevant for intestinal pathobiology. Miyoshi and colleagues observed that GSK3 inhibition via PGE2 and PKA promotes β-catenin nuclear translocation and thereby the differentiation of wound-associated intestinal epithelia, independent of Wnt signaling (Miyoshi et al., 2017). Moreover, Schmitt and colleagues recently reported that Stem Cell Factor (SCF), a cytokine increased in IBD, promotes the dedifferentiation of Paneth cells to drive epithelial restitution in the DSS colitis model (Schmitt et al., 2018). Paneth cell conversion in this study was associated with elevated AKT activity and inhibitory GSK3 phosphorylation, as well as increased β-catenin nuclear translocation. Based on these data, Schmitt et al. propose that SCF signaling facilitates the repopulation of the intestinal stem cell niche via AKT/GSK3-dependent β-catenin regulation. It should be noted, however, that these authors did not formally test the involvement of phospho-inhibited GSK3 in β-catenin stabilization.

In an additional mode of action, AKT bypasses GSK3 to activate β-catenin directly. AKT phosphorylates β-catenin at its C-terminus, thereby releasing it from cell junctions and promoting its nuclear translocation (Fang et al., 2007; He et al., 2007). In the gut, AKT-dependent β-catenin activation occurs in epithelial cells slightly above the crypt bottom, and initiates cell proliferation and possibly crypt budding (He et al., 2007; Nava et al., 2010). In this way, cytokine-mediated AKT activation may induce β-catenin signaling in epithelial reserve stem cells, which are thought to be excluded from Wnt stimulation (Richmond et al., 2018).

2.4. STAT signaling

Cytokine-induced STAT signaling is a central determinant of cell proliferation and survival during inflammation. Recent clinical trials have shown that inhibitors of Janus kinases (JAK), which act upstream of STAT transcription factors, are effective for the treatment of autoimmune disorders such as ulcerative colitis (Banerjee et al., 2017). Somewhat surprisingly then, despite the overlapping functions of Wnt and STAT signaling in the regulation of cell proliferation, there is limited evidence that these pathways intersect on the molecular level. On the contrary, a recent report by Oshima and colleagues suggests that a major STAT protein implicated in cellular homeostasis, STAT3, is dispensable for Wnt-dependent epithelial maintenance and tumorigenesis in the gut (Oshima et al., 2018). Nonetheless, there is some indication of interaction between Wnt and STAT signaling. It has been observed that WNT3A drives STAT3 gene transcription and induces STAT3 nuclear translocation (Fragoso et al., 2012; Hao et al., 2006), suggesting that cells with active Wnt signaling are sensitized to JAK/
STAT activation. Conversely, activation of STAT3 by inflammatory cytokines induces the Wnt inhibitor Dickkopf homolog (DKK) 1 (Li et al., 2011; Nava et al., 2010). DKK1 binds to and depletes the Wnt co-receptor Low-density lipoprotein receptor-related protein (LRP) 6, thereby reducing cell proliferation in intestinal epithelia.

In light of these findings, it is possible that activation of the STAT pathway serves as a negative regulator of Wnt-dependent cell proliferation. It is important to note, however, that STAT signaling is sufficient to promote epithelial regeneration during colitis (Kuhn et al., 2014; Pickert et al., 2009; Sugimoto et al., 2008). As we will discuss in the following chapter, inflammatory cytokines may thus support or replace Wnt signaling to drive epithelial homeostasis in IBD.

3. WNT pathway regulation during colitis

Given the prominent role of Wnt signaling in cell proliferation and tissue repair, one could reasonably expect to find robust, global changes in Wnt molecules during colitis, which drive mucosal regeneration. However, there is limited evidence to suggest that this is the case. You and colleagues observed altered expression of multiple Wnt ligands and receptors in UC biopsies compared to healthy controls (You et al., 2008), but the changes were independent of disease activity and Wnt target gene expression, and subsequent studies failed to replicate these earlier findings (Hughes et al., 2011). Nonetheless, Wnt/β-catenin pathway activation is a feature of chronic IBD and animal models thereof (Bradford et al., 2017; Serafini et al., 2014) (Fig. 2). These seemingly conflicting observations should not come as a surprise. Wnt ligand expression in the gut is highly compartmentalized (Gregorietti et al., 2005), and several recent studies have identified diverse pericryptal stromal cell populations that constitute an essential niche for intestinal epithelial stem cells. An in-depth review of non-epithelial Wnt sources in the gut and their role in tissue homeostasis can be found in this issue of Differentiation [Greicius and Vilushp, In press], as well as in our earlier contribution (Koch, 2017). Of relevance for IBD pathology, at least some of these cell populations dynamically adjust their abundance or ligand repertoire during colitis (Kinchen et al., 2018), and thus control adjoining stem cells without changing tissue-wide gene expression. However, recent developments in the field also suggest that inflammatory mediators take charge of epithelial maintenance and regeneration during colitis. Below, we will thus focus on the reciprocal regulation of Wnt and inflammatory signaling at the ligand level.

3.1. Regulation of intestinal epithelial stem cells by inflammatory cytokines

As discussed in the previous chapter, the Wnt/β-catenin pathway is highly interconnected with multiple other signaling pathways. Consistently, there is mounting evidence that immune cells govern epithelial stem cell dynamics via inflammatory cytokines, either directly or by regulating Wnt signaling (Table 1, and Fig. 3). The best-studied example for this are tumor necrosis factor (TNF-α) and interferon (IFN)-γ. TNF-α and IFN-γ are major pro-inflammatory cytokines that synergistically drive epithelial barrier dysfunction and apoptosis, particularly also during colitis (Nava et al., 2010). Individually, however, they exert discrete effects on epithelial stem cells in the gut. TNF-α activates β-catenin via AKT, and thus induces Wnt target gene expression in crypt base stem cells (Bradford et al., 2017). Accordingly, loss of TNF receptor in epithelial cells blunted Wnt signaling and wound healing in DSS-treated mice, indicating an integral role for TNF-α in mucosal regeneration. IFN-γ also triggers AKT-dependent β-catenin phosphorylation, and increases epithelial cell proliferation in the short term. In sharp contrast to TNF-α, however, IFN-γ induces the Wnt antagonist DKK1, presumably via STAT3, and thereby inhibits Wnt signaling in the gut (Li et al., 2011; Nava et al., 2010). Indeed, DKK1 levels are strongly increased in the intestinal mucosa during colitis, and depletion of DKK1 improves the recovery from DSS colitis (Frei et al., 2013; Koch et al., 2011; Nava et al., 2010). Interestingly, Richmond and colleagues recently observed that both TNF-α and IFN-γ activate mTERT-positive epithelial reserve stem cells during colitis, which reside outside of the Wnt signaling-active crypt niche (Richmond et al., 2018). This is likely mediated by STAT1, but not STAT3/5 signaling in these cells. At the same time, IFN-γ reduces the number of Lgr5-positive, active stem cells as well as Paneth cells (Raetz et al., 2013; Richmond et al., 2018), although the same has not been tested for TNF-α. Collectively, these studies suggest that TNF-α and IFN-γ have overlapping as well as contrasting effects on intestinal epithelia, which are facilitated by shared (AKT and STAT1) and divergent (NF-κB versus STAT3) signaling events.

Similar to these cytokines, IL-6 and IL-22 also exert subtly different functions on intestinal epithelial stem cells (Jeffery et al., 2017; Lindemans et al., 2015). However, in contrast to TNF-α and IFN-γ, this is not achieved by downstream signal separation, but rather by differential receptor presentation on epithelial cells. Jeffery et al. reported that the IL-6 receptor is exclusively expressed in small intestinal Paneth cells, but not their directly adjacent stem cells (Jeffery et al., 2017). Consistently, these authors observed that IL-6 activated STAT3 only in Paneth cells, which caused increased proliferation of Lgr5-positive stem cells presumably via induction of Wnt ligands in Paneth cells. On the other hand, Lindemans and colleagues observed IL-22 receptor expression specifically in Lgr5-positive cells, but not Paneth cells (Lindemans et al., 2015). Here, IL-22 also activated STAT3 and increased stem cell proliferation, but this did not result from Wnt ligand induction or activation of Wnt-dependent gene transcription. Although the mechanisms by which STAT signaling controls epithelial cells are not fully understood, these authors noted that STAT3 was required to maintain a stem cell gene expression signature in Lgr5-positive cells. Paneth cells are only found in small intestinal crypts, and consistently,

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Acts on</th>
<th>Effect on Wnt signaling</th>
<th>Effect on stem cells</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>NF-κB, MAPK, JAK/STAT</td>
<td>Activates β-catenin via AKT</td>
<td>Activates reserve stem cells via STAT; induces stem cell genes</td>
<td>(Bradford et al., 2017; Nava et al., 2010; Richmond et al., 2018)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>JAK/STAT, AKT</td>
<td>Activates β-catenin via AKT; Inhibits Wnt/β-catenin signaling via DKK1</td>
<td>Reduces active stem cell numbers; activates reserve stem cells via STAT</td>
<td>(Nava et al., 2010; Richmond et al., 2018)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>NF-κB, MAPK</td>
<td>Activates Wnt/β-catenin signaling via NF-κB</td>
<td>Increases proliferation via STAT3 in Paneth cells</td>
<td>(Kaler et al., 2009)</td>
</tr>
<tr>
<td>IL-4</td>
<td>NF-κB, MAPK, JAK/STAT, AKT, MAPK</td>
<td>Activates Wnt/β-catenin signaling via WISP1</td>
<td>Increases proliferation via ERK</td>
<td>(Quiros et al., 2017)</td>
</tr>
<tr>
<td>IL-6</td>
<td>JAK/STAT</td>
<td>Activates Wnt/β-catenin signaling via WISP1</td>
<td>Increases proliferation via STAT3</td>
<td>(Lindemans et al., 2015)</td>
</tr>
<tr>
<td>IL-17</td>
<td>NF-κB, MAPK</td>
<td>Activates Wnt/β-catenin signaling via WISP1</td>
<td>Increases proliferation via STAT3</td>
<td>(Mahapatro et al., 2016)</td>
</tr>
</tbody>
</table>
IL-22 but not IL-6 is required for epithelial regeneration following DSS colitis (Pickert et al., 2009). Thus, epithelial injury responses in different parts of the intestine may be fine-tuned using the same signaling modules, simply through divergent cytokine receptor expression.

Furthermore, the prototypical anti-inflammatory cytokine IL-10 promotes mucosal wound healing at least in part by activating Wnt signaling in epithelial cells. Quiros and colleagues reported that IL-10 drives WNT1-inducible signaling protein 1 (WISP-1) expression during colitis (Quiros et al., 2017). These authors observed that WISP-1 activates Wnt signaling and c-Myc expression in the mucosa, which was associated with increased epithelial cell proliferation and wound closure. Interestingly, WISP-1 may require IL-10-dependent STAT activation, although the precise mechanism remains to be determined.

While the above examples demonstrate direct cytokine effects on intestinal epithelial cells, there is also evidence for paracrine activation of epithelial Wnt signaling by inflammatory mediators. Cosín-Roger et al. showed that IL-4 induces the expression of various Wnt ligands, including the major stromal ligand WNT2B, by polarizing mucosal macrophages towards a pro-resolving M2a phenotype (Cosín-Roger et al., 2013, 2016). Polarized macrophages activated Wnt signaling in intestinal epithelial cell lines, and M2a macrophages were also observed adjacent to regenerating epithelia in IBD patient samples. Wnt induction was mediated by STAT6 in these studies, suggesting that STAT signaling may be a key determinant of epithelial homeostasis in the gut, through integration of converging signaling pathways in a cell type-dependent manner. Considering the recent discovery of distinct pericryptal mesenchymal cells that produce Wnt agonists in the gut, it appears worthwhile to investigate if these cells are controlled by inflammatory cytokines or STAT signaling as well.

### 3.2. Control of inflammatory cytokine production by Wnt ligands

On the other hand, a considerable body of studies has shown that inflammatory cytokines and chemokines are themselves regulated by Wnt ligands (Table 2); however, the underlying mechanisms are generally less clear. The strongest evidence outlines a central role of WNT5A in the regulation of cytokines in various cell types, including epithelia, endothelia, and immune cells. Interestingly, WNT5A is a non-canonical Wnt ligand, i.e. it does not signal through β-catenin. Instead, non-canonical Wnts activate alternative downstream pathways, including planar cell polarity (PCP) and calcium signaling (Niehrs, 2012). In the context of intestinal pathobiology, WNT5A is one the few Wnt ligands whose expression is strongly increased in chronic colitis (Fig. 2B, C). WNT5A has been shown to promote crypt budding in wound-associated epithelia, presumably by synergizing with the transforming growth factor (TGF)-β pathway (Miyoshi et al., 2012). Moreover, WNT5A is required for the Th1 differentiation of CD4 T cells, most likely via PCP signaling (Sato et al., 2015).

In addition to these functions, numerous studies have shown induction of major inflammatory cytokines by WNT5A, partly in co-operation with pattern recognition receptor signaling, e.g. via lipopolysaccharide/toll-like receptor (TLR) 4/NF-κB. For example, WNT5A induces IL-1 and IL-6 in endothelial cells, lung epithelial cells, and macrophages, possibly via Wnt/calcium signaling (Jang et al., 2017; Kim et al., 2010; Pereira et al., 2008). At the same time, WNT5A may suppress IL-6, and induce IL-10 and TGF-β in dendritic cells (Oderup et al., 2013), suggesting that the same Wnt ligand can exert pro- as well as anti-inflammatory functions depending on the cell type. Curiously, another classical non-canonical Wnt ligand, WNT11, suppresses rather than induces IL-1β and IL-6 in macrophages exposed to bacterial antigens (Morishita et al., 2016). Morishita et al. observed that WNT11 reduces NF-κB activity in a mouse macrophage cell line, which suggests that different non-canonical Wnt ligands may have opposing effects on the NF-κB pathway.

Given the high degree of integration between the Wnt/β-catenin and inflammatory signaling pathways, it seems feasible that canonical Wnt ligands contribute to cytokine regulation as well. Indeed, Gatica-Andrades et al. observed that inhibition of β-catenin/TCF interaction, and to a lesser extent Wnt secretion, reduced the induction of several major inflammatory cytokines in LPS-challenged mice (Gatica-Andrades et al., 2017). However, few studies have shown a direct link between Wnt signaling upstream of β-catenin and the expression of...
Table 2
Regulation of inflammatory mediators by Wnt ligands in different cell types.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Regulated cytokines and chemokines</th>
<th>Cell type</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNT1</td>
<td>Induces IL-8</td>
<td>Endothelial cells</td>
<td>(Longo et al., 2002; Masckauchan et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Induces IL-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNT2</td>
<td>Represses bacteria-induced IL-8</td>
<td>Pre-adipocytes</td>
<td></td>
</tr>
<tr>
<td>WNT3A</td>
<td>Induces TGF-β</td>
<td>Intestinal epithelia</td>
<td>(Liu et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Induces IL-1β, IL-6</td>
<td>Dendritic cells</td>
<td>(Amüller et al., 2013; Oderup et al., 2013)</td>
</tr>
<tr>
<td>WNT4</td>
<td>Induces IL-6, IL-8, CCL2, CCL5</td>
<td>Lung epithelia</td>
<td>(Heijink et al., 2013)</td>
</tr>
<tr>
<td>WNT5A</td>
<td>Synergizes with LPS in IL-1β, IL-6 induction</td>
<td>Macrophages</td>
<td>(Jang et al., 2017; Kim et al., 2010; Oderup et al., 2013; Pereira et al., 2008; Sato et al., 2015; Sato and Tamura, 2016)</td>
</tr>
<tr>
<td></td>
<td>Induces IL-6, IL-8</td>
<td>Stromal cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synergizes with LPS in IL-12, IL-23 induction; suppresses LPS-induced IL-6 expression; induces IL-10, TGF-β</td>
<td>Dendritic cells</td>
<td></td>
</tr>
<tr>
<td>WNT5B</td>
<td>Induces IL-6, IL-8</td>
<td>Fibroblasts</td>
<td>(van Dijk et al., 2016)</td>
</tr>
<tr>
<td>WNT7A</td>
<td>Synergizes with LPS in IL-6 induction</td>
<td>Macrophages</td>
<td></td>
</tr>
<tr>
<td>WNT11</td>
<td>Represses bacteria-induced IL-8</td>
<td>Macrophages</td>
<td></td>
</tr>
</tbody>
</table>

Inflammatory mediators. Amüller and colleagues reported that WNT3A induces IL-1β and IL-6 in lung epithelial cells, similar to WNT5A (Amüller et al., 2013). Although β-catenin target genes were induced concomitantly, consistent with Wnt pathway activation, it is unclear whether cytokine expression in this setting requires TCF-dependent transcription. WNT3A also induces TGF-β, but not IL-10, in dendritic cells (Oderup et al., 2013), which further suggests that canonical and non-canonical Wnt signaling have similar but non-identical functions in cytokine regulation. Finally, multiple Wnt ligands control IL-8 levels in different cell types, including intestinal epithelial cells (Liu et al., 2012, 2011; Masckauchan et al., 2005; Pereira et al., 2008). IL-8 is a potent chemoattractant whose expression is strongly increased during intestinal infection and inflammation. Interestingly, Liu et al. observed that Salmonella infection of intestinal epithelia up-regulated WNT2 and WNT11 expression, and both ligands reduced bacteria-induced IL-8 levels and epithelial injury (Liu et al., 2012, 2011). Thus, as-yet unknown Wnt signaling events may contribute to host defense, by regulating inflammatory pathways in response to epithelial injury. It should be noted, however, that few of the above studies have provided a compelling explanation for how Wnt ligands achieve immune pathway control, although the available data suggest that non-canonical signaling may play an unexpectedly large role.

4. Conclusions

The Wnt/β-catenin signaling pathway is a highly conserved organizer of tissue development and regeneration. Research particularly in recent years has revealed that Wnt signaling is intimately linked with numerous inflammatory pathways on virtually every level. It thus seems increasingly clear that the Wnt pathway moonlights as a regulator of inflammation. However, since few investigators have explored Wnt signaling specifically in this context, major questions remain to be resolved. For example, if and how Wnt/β-catenin signaling actually contributes to wound healing during colitis has yet to be formally established. In fact, recent observations suggest that Wnt ligands may act in quite unexpected ways in the early stages of crypt regeneration. Both in the case of DSS-induced epithelial injury and parasite infection, wound-associated crypts transiently adopt a “fetal-like” state, in which cellular homeostasis is independent of β-catenin/TCF signaling (Nusse et al., 2018; Yui et al., 2018). However, cell conversion apparently requires combinatorial signaling via integrins and Wnt ligands to activate YAP/TAZ-dependent gene transcription. Interestingly, IFN-γ triggers epithelial reprogramming as well (Nusse et al., 2018), which outlines yet another converging mechanism by which Wnts and cytokines drive mucosal regeneration.

To complicate matters further, using a multi-omics approach, Lyons and colleagues observed surprisingly little concordance between gene expression, protein levels, and protein phosphorylation in a mouse colitis model, as well as between expression data from mouse and human colitis (Lyons et al., 2018). Thus, it will require considerable future efforts to elucidate the exceedingly complex signaling networks that shape inflammatory bowel diseases and related disorders. However, given how little we currently understand about signal integration and its impact on human health, addressing this challenge will likely result in major breakthroughs in preclinical and translational research.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.diff.2019.01.002.

References

L. Moparthi, S. Koch


