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Pleiotropic functions of TNF-α in the regulation of the intestinal epithelial response to inflammation

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REVIEW

Abstract

An important function of intestinal epithelial cells (IECs) is to maintain the integrity of the mucosal barrier. Inflammation challenges the integrity of the mucosal barrier and the intestinal epithelium needs to adapt to a multitude of signals in order to perform the complex process of maintenance and restitution of its barrier function. Dysfunctions in epithelial barrier integrity and restoration contribute to the pathogenesis of inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis. Mucosal healing has developed to a significant treatment goal in IBD. In this review, we would like to highlight physiologic and pathologic adaptations of the intestinal epithelium to inflammation, exemplified by its responses to TNF-α. A large body of literature exists that highlights the diverse effects of this cytokine on IECs. TNF-α modulates intestinal mucus secretion and constitution. TNF-α stimulation modulates paracellular flow *via* tight junctional control. TNF-α induces intracellular signaling cascades that determine significant cell fate decisions such as survival, cell death or proliferation. TNF-α impacts epithelial wound healing in ErbB- and Wnt-dependent pathways while also importantly guiding immune cell attraction and function. We selected important studies from recent years with a focus on functional *in vivo* data providing crucial insights into the complex process of intestinal homeostasis.

Keywords: intestinal epithelium, mucosal immunology, tumor necrosis factor

Introduction

A central function of intestinal epithelial cells (IECs) is to maintain the integrity of the mucosal barrier separating the host from its environment. IECs not only constitute a multicellular wall but also are responsible for the integrity of multiple, biochemically distinct layers of the mucosal lining. They secrete glycoprotein-rich mucus into the intestinal lumen and provide a phospholipid membrane barrier, intercellular junctional complexes and the basolateral framework of the epithelium by producing central components of the basal membrane that contains collagen IV (1).

The integrity of the intestinal barrier is monitored not only by the epithelium itself but also by immune cell surveillance and stroma-producing mesenchymal cells and fibroblasts in the lamina propria. A constant intercellular cross-talk is necessary to maintain intestinal homeostasis. The epithelial layer is not static but is in fact a highly dynamic tissue with epithelial cell turnover, effectuated by cell shedding at the villus tip and constant proliferation of stem cells and transient amplifying cells along the crypt–villus axis. Homeostasis of the intestinal epithelium is strongly disturbed in the context of intestinal inflammation.

TNF-α is an important pro-inflammatory molecule with pleiotropic functions in intestinal inflammation. Monogenic diseases with an inflammatory bowel disease (IBD)-like phenotype associated with genes such as *NEMO* and *ADAM17* (2), as well as single nucleotide polymorphisms identified in genome-wide association studies indicating genes such as *RELA*, *NFKB1* and *TNFAIP3* as potential risk alleles (3), link alterations in TNF-α signaling to IBD. Antibodies targeting TNF-α have been the most successful introduction into the clinical treatment of IBD for many years, highlighting the importance and clinical relevance of TNF-α function in intestinal inflammation (4). In addition, mice over-expressing TNF-α as a result of a specific knock-in deletion of the *Tnf* 3′ untranslated region AU-rich elements ($Tnf^{\Delta ARE}$ mice) spontaneously develop a Crohn's-like IBD pathology, specifically in the terminal ileum providing strong *in vivo* evidence for a causative role of TNF-α in IBD pathogenesis (5).

TNF-α controls multiple cellular processes such as the production of inflammatory mediators, cell proliferation and survival and different modalities of cell death, which are

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intricately linked to the epithelial response to injury. A closer look at these diverse effects of TNF-α signaling in IECs exemplifies crucial necessities of barrier regulation in inflammation. The development of Villin-Cre and Villin-CreERT2 mice, which allow IEC-specific genetic alterations, has widely expanded the scientific tools in IEC biology (6). Cross-breeding with mouse strains expressing loxP sites flanking target genes allows a selective dissection of the TNF-α pathway in a cell type-specific manner.

TNF-α signaling pathways in IECs

IECs express both receptors that are known to transmit intracellular signaling of TNF-α: TNF-R1 (TNFRSF1A) and TNF-R2 (TNFRSF1B) (7). In intestinal inflammation, TNF-α is produced by invading immune cells and stromal cells and by the intestinal epithelium itself (8). In the following, a brief introductory description of TNF-α-induced intracellular signaling is provided.

Pro-TNF-α is formed as a transmembrane protein and signals in its membrane-bound form in a juxtacrine fashion. Cleavage by ADAM17 releases a 17 kDa ectopeptide, which assembles into a biologically active trimeric form (9). Many functions solely rely on the presence of TNF-R1, whereas the signaling pathways restricted to TNF-R2 remain less well studied. TNF-R1 signaling leads to a variety of intracellular events, finally activating two major transcription factors, NF-κB and c-Jun. Ligand-activated TNF-R1 is recognized by the adaptor protein TNF-R-associated death domain (TRADD), which recruits additional adaptor proteins such as receptor-interacting serine/threonine kinase 1 (RIPK1) and TNF-R-associated factor 2 (TRAF2) to form the membrane-bound 'complex I'. These proteins then recruit further key enzymes to TNF-R1.

TRAF2 provides the molecular link to p38 MAPK and ERK signaling, ultimately resulting in the activation of JNK and consecutive increases in the transcriptional activity of c-Jun. RIPK1 and TGF-β-induced kinase (TAK1) are essential for the activation of the transcription factor NF-κB. Inactive NF-κB is retained in the cytoplasm by members of the IκB protein family. Phosphorylation of IκB by the IκBα kinase (IKK) complex (comprising IKK-α, IKK-β and IKK-γ/NEMO) in response to TNF-α facilitates ubiquitination and leads to the degradation of IκB proteins, allowing nuclear translocation of NF-κB and consecutive regulation of canonical NF-κB target genes (10, 11). A20 (TNFAIP3) is an important regulator of ubiquitination (12, 13). TRADD furthermore interacts with the adaptor protein Fas-associated death domain (FADD) *via* death domains, leading to the recruitment of caspase-8 to the cytoplasmic 'complex II'. Caspase-8 plays a central role in the regulation of cell death pathways, such as apoptosis and necroptosis (see below) and is crucial for cell survival (14). Apart from the canonical NF-κB pathway described above, an alternative NF-κB pathway involving IKKα, NF-κB-inducing kinase (NIK) and RelB can be induced by other NF-κB-activating ligands than TNF-α (15). We will now provide a step-wise look at the intestinal barrier and its modulation by TNF-α starting at the first line of defense, the mucus layer.

TNF-α in the regulation of intestinal mucus

The mucosal surface of the gastrointestinal tract is covered by two stratified layers of mucus (Fig. 1). This mucus protects the intestinal epithelium from physical damage and limits the direct contact of IECs and the luminal flora. The structural integrity of the mucus layer is established by polymerizing the O-linked glycoproteins of the mucin family. A differentiated IEC type, the goblet cell, is the main source of mucin secretion. The outer layer of ~100 μm in mice is in direct contact with the luminal microflora. The inner, unstirred layer of ~50 μm is devoid of bacteria (16). The murine intestinal mucus consists mainly of the gel-forming mucin-2, encoded by the *Muc2* gene. Interestingly, both mucus layers are dominated by mucin-2. The inner layer is composed of a more densely packed mucin-2 assembly, which is proteolytically cleaved by multiple enzymes to form the outer layer. Intriguingly, by this model, biochemical alterations of the same structural protein lead to distinct layers with different functional properties.

The physiological importance of mucin-2 in barrier homeostasis has been demonstrated by the development of a spontaneous colitis in *Muc2*-deficient mice (17). Adding to its structural protection, the mucus is rich in anti-microbial peptides such as RegIII family members, lysozyme, cathelicidins and defensins (mouse cryptdins). Furthermore, secretory IgA is part of the mucus layer, providing an immunologic first line of defense.

TNF-α influences the constitution of the mucus layer in multiple ways. On the one hand, exogenous TNF-α administration, at a dose sufficient to cause massive IEC death, leads to a TNF-R1-dependent loss of mucin-producing goblet cells, while mRNA production of *Muc2* is increased in a TNF-R2-dependent pathway (18). This study nicely introduces dose-dependent and receptor-dependent mechanisms to understand apparently contradicting effects of TNF-α (18). In isolated HT29 IECs, *Muc2* mRNA is up-regulated in response to TNF-α in a MAPK-dependent pathway (19). In another study, TNF-α stimulation induced a goblet-cell-like phenotype in a human adenocarcinoma cell line (20), pointing at a role of TNF-α in the differentiation toward the secretory lineage. Adding a level of complexity, sulfation of mucins was altered in response to TNF-α (21).

TNF-α not only modifies mucin expression, secretion and composition but also regulates other components of the intestinal mucus. TNF-α has been shown to support the expression of the polymeric immunoglobulin receptor (pIgR), which is necessary for the transcytosis of secretory IgA into the mucus (22). For further reading on IgA, we recommend the article by Suzuki and Nakajima in this issue of *International Immunology*.

In conclusion, TNF-α modification of intestinal mucus is a complex process, in which TNF-α induces protective and destructive pathways dependent on dosage and secondary signals. The mucus barrier function strongly relies on its high viscosity, which depends on its glycoprotein and water content. The transepithelial paracellular flow of water is achieved and regulated by intercellular contacts. Next, we will focus on the impact of TNF-α in this process.

TNF-α in tight-junction modulation

Intercellular contacts, mainly tight junctions, establish a sealed epithelial barrier function, limiting paracellular leakage

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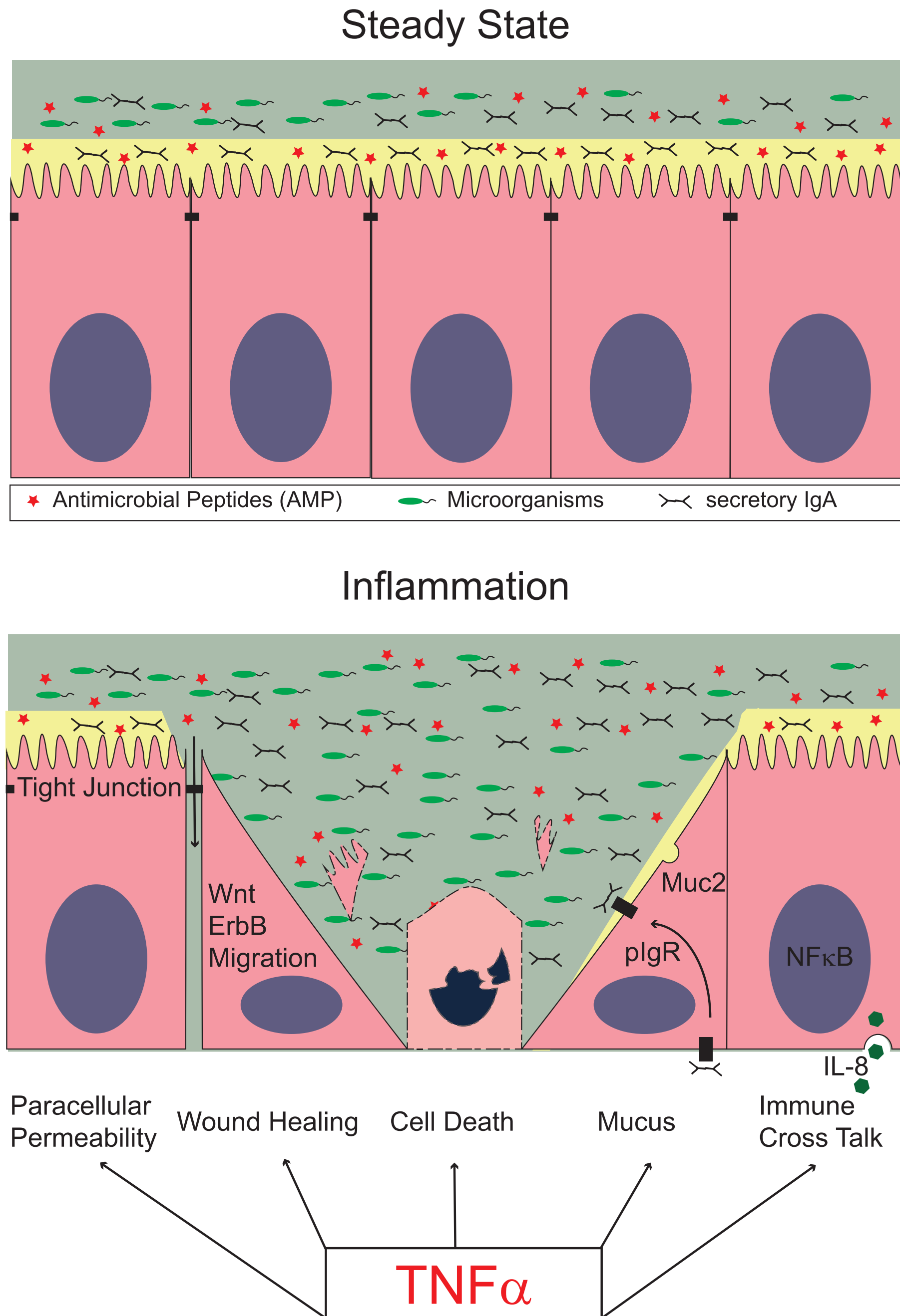

Fig. 1. The intestinal barrier in health and disease. Upper panel: The intestinal barrier in the steady state is maintained by the intestinal mucus and a single-cell lining of intestinal epithelial cells (shown in pink) sealed together by apical junctional complexes including tight-junctions. Intestinal mucus comprises two distinct layers, one of which (shown in yellow) is devoid of bacteria and contains bioactive molecules such as IgA and anti-microbial peptides. Lower panel: The intestinal barrier is breached in the context of inflammation. Cellular mechanisms targeted by TNF-α are depicted including alteration of paracellular permeability and TNF-α-induced cell death. TNF-α contributes to the restitution and regeneration of the epithelial lining by supporting epithelial cell migration and proliferation and to the reconstitution of the intestinal mucus by increasing expressions of pIgR and Muc2. Immune cell attraction is actively controlled by cytokine and chemokine cross-talk.

and supporting epithelial cell polarity. Tight junctions consist of multiprotein complexes, in membrane areas of specific lipid composition, including occludin and claudin family members and junction adhesion molecules (JAMs) such as JAM-A. Claudins are transmembrane proteins. The extracellular domains of claudins of two adjacent cells interact in order to achieve a close intercellular link. Tight junctions are connected to the intracellular actin cytoskeleton by plaque proteins such as ZO-1/TJP1 (zona occludens-1/tight-junction protein-1) (23). Paracellular permeability is regulated by the contraction of a perijunctional actomyosin ring. The phosphorylation of the regulatory light chain of myosin II by myosin light chain kinase (MLCK) induces ring contraction and increases paracellular permeability.

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Many studies have implicated tight-junction regulation in TNF-α-induced epithelial barrier loss. TNF-α stimulation of epithelial monolayers leads to a loss of transepithelial impedance, illustrating an altered barrier function. Interestingly, this is achieved in part by activation of MLCK by TNF-α by at least two different mechanisms: TNF-α increases both MLCK expression and its enzymatic function. The intracellular pathway involved in MLCK up-regulation likely involves NF-κB and AP-1 transcription factors (24). In a relevant recent study, TNF-R2 has been shown to mediate MLCK up-regulation and colitis development upon transfer of normal $CD4^{+}CD45RB^{hi}$ cells to *Tnfr2*$^{-/-}$*Rag1*$^{-/-}$ recipient mice (25).

A different mechanism of TNF-α tight-junction permeability regulation has been described. Thereby occludin is endocytosed from the tight junction in response to TNF-α, allowing a size-selective paracellular pore of up to 62 Å radius (26). Paracellular transport through regulated tight junctions is therefore size-selective and allows the passive gradient-directed flow of solutes and water. Larger particles or microorganisms may not pass this way. Occludin deficient mice do not show overt signs of a disturbed epithelial barrier (27). Results from claudin-deficient mice have also pointed to non-tight junctional structural regulatory functions of these proteins (28, 29). In addition, TNF-α affects the distribution of JAM-A in the tight junctions (30) and *Jama*$^{-/-}$ mice show disturbed epithelial barrier function and are highly susceptible to dextran sulfate sodium (DSS)-induced colitis (31, 32). Therefore, the mechanistic implications of TNF-α-induced alterations in epithelial tight junctions and paracellular permeability in IBD pathogenesis require further investigation.

The intercellular tight-junction barrier is additionally stressed in the context of epithelial cell shedding. Shedding occurs in homeostatic tissue at the villus tip, whereas epithelial shedding is markedly increased in the context of inflammatory stimuli. In homeostatic epithelial cell shedding, barrier integrity is maintained by a rapid redistribution of tight-junctional proteins to the basolateral side of the single shed cell and a zipper-like replacement by the neighboring cells. TNF-α induces a different form of epithelial cell shedding accompanied by epithelial cell death pathway activation, in which multiple adjacent epithelial cells lose tissue contact. This inflammatory shedding leaves microerosions that cannot be adequately substituted by tight junctional re-arrangement. These events have been demonstrated by elegant *in vivo* imaging studies in mice and humans (33, 34). These microerosions or epithelial gaps might play an important pathophysiological role as a crucial step in barrier dysfunction. Moreover, gaps identified by confocal laser endomicroscopy in IBD patients are useful in predicting the occurrence of the next relapse.

For therapeutic interventions, it is crucial to understand how TNF-α influences epithelial cell death on a molecular level. In the following section, we will give an overview of molecular aspects of TNF-α-induced cell death pathways.

TNF-α and IEC death

For many years, epithelial cell death has been known to be a consequence of TNF-α-mediated stimulation. It has been debated whether cell death occurs as a consequence of shedding or whether dying cells are shed (35). In recent years, there has been a renewed interest in the mechanisms of TNF-α-induced cell death pathways. This interest has been heralded by the demonstration of caspase-independent cell death pathways leading to a regulated form of necrosis called necroptosis (36). To date, the physiological importance of the necroptosis pathway remains incompletely understood.

It has been known for many years that TNF-α induces extrinsic caspase-dependent apoptosis. A widely accepted model states that the pro-survival complex I bound to the membrane is converted to a cytosolic complex II in response to endocytotic TNF-R1 internalization. A homodimer of caspase-8 is released from complex II, then cleaves and activates caspase-3, thereby activating the canonical extrinsic apoptosis pathway with characteristic morphology. Pharmacological, viral or genetic inhibition of caspase-8 still results in cell death, yet with a necrotic phenotype (necroptosis). This caspase-independent cell death pathway is constitutively inhibited by the proteolytic activity of caspase-8 (37). Necroptosis as of now cannot be positively identified. So far, it is defined as a necrotic cell death in the absence of caspase cleavage in response to external triggers. A central molecule in necroptosis is receptor-interacting serine/threonine kinase 3 (RIPK3). When caspase-8 activity is inhibited, deubiquitylated RIPK1 interacts with RIPK3, leading to phosphorylation of mixed lineage kinase domain-like protein (MLKL). It has been suggested that these molecules form a complex called the necroptosome inducing downstream pathways possibly involving PGAM5 and DRP1 (38, 39). It has been demonstrated that cFLIP isoforms guide the crucial cell fate decision in complex II: cFLIP competes with procaspase-8 and thus regulates the proteolytic activity of caspase-8. Experimental evidence suggests that the complex between cFLIP-L and procaspase-8 only partially inhibits caspase-8 activity and favors cell survival under certain conditions, whereas the heterodimer of cFLIP-S and procaspase-8 blocks the proteolytical activity of caspase-8 completely and thereby potentially facilitates necroptosis (40). However, this model has not yet been verified *in vivo*. Further functional studies will clarify the role of cFLIP isoforms in immune homeostasis of the gut.

TNF-α-induced cell death pathways have been studied in the intestinal epithelium in elegant functional studies *in vivo*. Piguet *et al*. (41) were the first to show that acute exogenous TNF-α administration at a lethal dose leads to extensive IEC death and we have shown that this occurs specifically *via* intestinal epithelial TNF-R1 (8). The crucial pro-survival function of TNF-α-induced NF-κB signaling in IECs has been highlighted because IEC-specific inhibition of NF-κB through conditional ablation of *Nemo*, or of both *Ikk1* and *Ikk2* subunits essential for NF-κB activation, spontaneously caused severe chronic intestinal inflammation in mice. NF-κB deficiency led to apoptosis of colonic epithelial cells and translocation of bacteria into the mucosa (42). Enterocyte-specific deletion of *Tak1*, which also inhibits epithelial NF-κB activation, results in colonocyte cell death and spontaneous colitis as in *Nemo*$^{\Delta IEC}$ and *Ikk1*/*Ikk2*$^{\Delta IEC}$ mice (43).

Deficiency in *Fadd* or *Casp8* in IECs might at the time have been expected to prevent caspase-8-mediated epithelial apoptosis. Yet, *Fadd*$^{\Delta IEC}$ colonocytes die in a microbiota-TLR- and TNF-α-dependent fashion, resulting in chronic intestinal inflammation. Further analysis revealed that *Fadd*$^{\Delta IEC}$-induced epithelial cell death was dependent on RIPK3 and showed

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morphologic features of necrosis (44). Therefore, although classic extrinsic apoptosis and caspase activation is blocked, FADD deficiency in IECs still allows epithelial cell death *via* necroptosis in a RIPK3-dependent way. In *Casp8*$^{\Delta IEC}$ mice, canonical extrinsic apoptosis is disturbed as well. *Casp8*$^{\Delta IEC}$ mice are viable and do not suffer from spontaneous colitis, yet display a high susceptibility to TNF-α-induced cell death and colitis models. In these mice, TNF-α induces a RIPK1/RIPK3-dependent cellular necrosis pathway that can be pharmacologically blocked by necrostatin.

Casp8$^{\Delta IEC}$ mice furthermore develop a spontaneous chronic ileitis accompanied by specific RIPK1/RIPK3-mediated necroptosis in Paneth cells of the small-intestinal crypt bottom. Interestingly, this finding might show a differential susceptibility of different IEC types to TNF-α-induced cell death pathways (14). The importance of cFLIP in intestinal homeostasis has been demonstrated by two groups (45, 46). *cFlip*$^{\Delta IEC}$ mice are not viable. A tamoxifen-inducible deletion of *cFlip* in IECs (*cFlip*$^{i\Delta IEC}$) results in extensive cell death among enterocytes following induced deletion. This cell death was accompanied with a marked activation of caspase-8 and caspase-3 activity. Death of *cFlip*-depleted IECs was regulated extrinsically and required the presence of death receptor ligands but was independent of RIPK3 (45, 46).

A20 (TNFAIP3) is a ubiquitin-editing molecule of high importance in NF-κB signaling in response to TNF-α. It has been identified as a disease susceptibility gene for IBD. A20 can lead to the deubiquitination of RIPK1 at K63 and the ubiquitination at K48, leading to a degradation of RIPK1. A20-deficient mice and enterocyte-specific A20$^{\Delta IEC}$ mice suffer from an increased enterocyte cell death in response to TNF-α (13), pointing at an important protective role of this molecule in intestinal homeostasis. The exact molecular events of enterocyte cell death in A20$^{\Delta IEC}$ mice await further characterization.

In conclusion, multiple cell death modalities occur in IECs in response to inflammatory triggers. The executing pathways are currently being delineated more precisely in order to better understand epithelial damage in the context of inflammation.

TNF-α and intestinal epithelial wound healing

Epithelial injury and TNF-α-induced cell death results in epithelial erosions that have to be repaired as quickly as possible in order to limit influx of the luminal content into mucosal tissue. This complex process requires multiple cellular processes such as migration, reorganization of the cytoskeleton, transient dedifferentiation and proliferation. Re-establishment of epithelial integrity has been classified into two different steps: restitution and regeneration. Restitution involves cellular spreading and migration along the basement membrane and dedifferentiation and reorganization of the epithelial cytoskeleton, whereas regeneration includes cellular redifferentiation and proliferation. These steps take place in parallel and involve intertwined molecular mechanisms (47). Epithelial wound healing is an especially vulnerable situation, in which cellular proliferation is supported and crucial checkpoints of intestinal epithelial cell cycle control are altered, providing an increased susceptibility to de-regulated cellular proliferation as seen in inflammation-induced carcinogenesis (48).

Prominent players in the epithelial wound healing response are members of the epidermal growth factor (EGF) family and lipid mediators such as COX-2-derived prostaglandin E2. IL-22 has also been shown to regulate intestinal homeostasis in a STAT3-dependent manner (49). TNF-α promotes epithelial migration at low doses in a TNF-R2-dependent pathway involving focal adhesion kinase (FAK) (50). Mizoguchi *et al.* (7) demonstrated, *in vivo*, that TNF-R2 signaling in IECs promotes cell survival and proliferation in the setting of chronic intestinal inflammation. IEC proliferation is strongly guided by the Wnt pathway. Wnts ligate LRP6 and/or Frizzled (Fz) on target cells such as transient amplifying cells along the crypt–villus axis and block degradation of β-catenin, which then accumulates in the nucleus in a complex with the transcription factor Tcf (T-cell factor) and regulates gene expression. Dickkopf proteins, such as Dkk-1, inhibit Wnt-induced β-catenin accumulation (51). Importantly, Dkk-4 has been linked to chemoresistance in colorectal carcinoma (52).

TNF-α significantly modulates the intestinal wound healing response. TNF-α can support wound healing and it protects from intestinal epithelial apoptosis by transactivation of the ErbB family of EGF receptors (53). The interaction between TNF-α and ErbB pathways is executed on multiple levels: transactivation is supported by TNF-α-induced ADAM17, which liberates ErbB ligands to induce signaling (53). Furthermore, ErbB signaling is activated intracellularly by TNF-α-induced receptor phosphorylation. Additionally, TNF-α induces ErbB receptor expression as exemplified by EGF receptor, ErbB2 and ErbB4. ErbB signaling then activates the PI3K/Akt pathway, induces COX-2 and protects from IEC apoptosis (54, 55). Inflammatory cytokines such as IFN-γ and TNF-α have also been shown to impact Wnt signaling synergistically. Thereby, while initially activating β-catenin, IFN-γ finally reduces epithelial proliferation by inducing Dkk-1 and thereby inhibiting Wnt signaling (56, 57). Given the data from Ebert *et al.* (52), it may be suggested that inflammation also importantly influences response to tumor therapy.

Guanylate binding protein-1 (GBP-1) is also induced by both inflammatory cytokines and inhibits Wnt-driven epithelial proliferation (58). Surprisingly, it has been proposed that NF-κB signaling may promote intestinal epithelial dedifferentiation in the context of intestinal tumorigenesis (59). It may be hypothesized that there is a physiologic role of this effect in the context of epithelial wound healing.

Intestinal epithelial TNF-α and NF-κB guiding the intestinal immune response

Apart from apical mucus and cell-intrinsic regulation, TNF-α signaling importantly mediates local tissue inflammation by interacting with immune cell populations. TNF-α regulates cytokine and chemokine (e.g. CXCL-1, CXCL-5, and IL-8) production and thereby differentially modulates chemoattraction and immune effector mechanisms (60). Mice with an IEC-specific deletion of IKK-β show reduced levels of thymic stromal lymphopoietin (TSLP) and increased levels of TNF-α, IL-12/23p40, IFN-γ and IL-17 in the intestine and fail to mount a protective T_h2-dependent immunity (61). Guma *et al.* (62) developed a model of constitutive activation of IKK2 and thereby NF-κB in the intestinal epithelium. In this model, an increased amount

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of inflammatory cells infiltrate the mucosa, yet tissue damage is largely absent. It is noteworthy that constitutive activation of IKK2 leads to a high susceptibility to endotoxin challenge, in the case of additional activation of the p38 MAPK pathway.

Although the focus of this review is restricted to the intestinal epithelial response to TNF-α, a sidenote is necessary to highlight the fact that TNF-α in non-epithelial cells is also of crucial importance in intestinal inflammation. Pioneering work by Kontoyiannis *et al.* (5) established that defective TNF-α mRNA silencing due to the absence of genomic AU-rich elements (ΔARE) induces spontaneous IBD in mice.

It was shown that specific expression of TNF-R1 in IECs alone is not sufficient to induce chronic intestinal inflammation by TNF-α (8). Furthermore, selective functional TNF-R1 signaling in mesenchymal cells is sufficient to induce intestinal inflammation in $Tnf^{\Delta ARE}$ mice (63), whereas IEC-specific TNF-α overexpression leads to an early activation of subepithelial intestinal myofibroblasts and is sufficient to drive full IBD pathogenesis in this model (8). Therefore, an indirect effect of TNF-α in epithelial homeostasis mediated by mesenchymal cells is alternatively proposed to underlie TNF-α-driven IBD pathogenesis.

Conclusions and outlook

In this review, we tried to give a concise overview on the multiple and diverse effects of TNF-α on the intestinal epithelium in the context of inflammation and mucosal injury (Fig. 1). TNF-α modulates intestinal mucus secretion and constitution. TNF-α stimulation modulates solute inflow and outflow *via* tight-junctional control. TNF-α induces intracellular signaling cascades that determine crucial cell fate decisions such as survival, cell death or proliferation. TNF-α impacts epithelial wound healing in ErbB- and Wnt-dependent pathways. Finally, TNF-α regulates interactions between IECs and immune cells. Indirect effects of TNF-α on IECs mediated by TNF-α signaling in intestinal myofibroblasts also have been demonstrated in TNF-α-driven Crohn's-like IBD in the mouse (8, 63).

The mechanisms described highlight the fact that TNF-α and downstream NF-κB signaling may have fundamentally different outcomes in regard to functionality. These effects differ widely and are most often apparently contradicting. Yet, many of these differences may be explained by dose dependency, time point dependency and in a context-dependent way, taking interacting signaling pathways into account (50, 62). Future systems-biology-based approaches may improve our understanding of TNF-α signaling in IECs (64). Furthermore, the increased use of genetic tools to modify signaling pathways in a cell-specific manner will allow reassessment of many *in vitro* findings for their *in vivo* relevance in the context of the tissue microenvironment. Genetic analysis might also provide a more detailed look into specific effects on different epithelial cell populations and their respective functional consequences. This way, important regulatory axes may be identified and controversies arising from different model systems may be put aside.

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