Review: Importance of the alternative NF-κB activation pathway in inflammation-associated gastrointestinal carcinogenesis.

J Yvette Merga[1,2], Adrian O'Hara[1,2], Michael D Burkitt[1], Carrie A Duckworth[1], Christopher S Probert[1], Barry J Campbell[1], D Mark Pritchard[1*].

[1] Gastroenterology Research Unit, Department of Molecular and Cellular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK.

[2] These authors contributed equally to this work

Keywords: NFκB, gastric cancer, inflammation, Helicobacter pylori, colitis

* Correspondence to:

D. Mark Pritchard

Department of Cellular and Molecular Physiology,

Institute of Translational Medicine,

University of Liverpool,

The Henry Wellcome Laboratory,

Nuffield Building,

Crown St.,

Liverpool.

L69 3GE

Tel: 0044 151 794 5772

e-mail: mark.pritchard@liverpool.ac.uk

Abstract

Chronic inflammation is a common factor in the development of many gastrointestinal malignancies. Examples include inflammatory bowel disease predisposing to colorectal cancer, Barrett's esophagus as a precursor of esophageal adenocarcinoma and *Helicobacter pylori*-induced gastric cancer.

The classical activation pathway of NF-κB signaling has been identified as regulating several sporadic and inflammation-associated gastrointestinal tract malignancies. Emerging evidence suggests that the alternative NF-κB signaling pathway also exerts a distinct influence on these processes. This review brings together current knowledge of the role of the alternative NF-κB signaling pathway in the gastrointestinal tract, with a particular emphasis on inflammation-associated cancer development.

Introduction

Members of the Nuclear Factor κ-light-chain-enhancer of activated B cells (NF-κB) family were initially described as transcription factors in B lymphocytes in 1986[1]. Since then, they have been shown to be widely expressed and are conserved across both vertebrates and invertebrates[2, 3].

The conventional model of NF-κB signaling proposes two main arms of the pathway. These share similar features, but are triggered independently and activate different target genes[4]. The classical (canonical) NF-κB activation pathway is triggered by Th1 cytokines and is typified by the action of RelA(p65)–NF-κB1(p50) heterodimers, whilst the alternative (non-canonical) activation pathway signals through the adaptor protein NF-κB-inducing kinase (NIK).

Activation of this mechanism leads to nuclear translocation of transcriptionally active v-rel avian reticuloendotheliosis viral oncogene homolog B (RelB)–NF-κB2(p52) heterodimers.

Signaling through either pathway can influence multiple different cellular functions and can exert effects that may appear contradictory. For example, both pro- and anti-apoptotic effects, as well as proliferation[5] and senescence[6] signals have been attributed to the classical activation pathway of NF-κB signaling. Because of the wide variation in outcomes following pathway activation, it is difficult to extrapolate the effects of NF-κB signaling from one context to another. Recent evidence has established that alternative activation pathway NF-κB signaling is important during the development of several gastrointestinal (GI) pathologies in mouse and man. This article seeks to review this evidence and to establish questions for future research.

Literature search strategy

A systematic search of the English language literature listed in the PubMed database up to 5th November 2015 was performed based on the following search terms:

(NF-κB2 OR NFκB2 OR NF-kappaB2 OR NFkappaB2 OR NF-kB2 OR NFkB2 OR p52 OR p100 OR "alternative NF-κB" OR "alternative NFκB" OR "alternative NF-kappaB" OR "alternative NFkappaB" OR "alternative NF-kB" OR "alternative NFkB" OR "non-canonical" OR relB OR NIK OR IKKa OR MAP3K14 OR CHUCK) AND (stomach OR gastric OR intestine OR intestinal OR gastrointestinal OR colon OR colonic OR colorectal OR colitis OR Crohn's OR bowel OR oesophagus OR oesophageal OR esophagus OR esophageal OR Barrett's).

This search generated 316 results, of which 115 articles were retained following review of titles and abstracts. The full texts of these articles were acquired and used as the basis of this article; retrieved article reference lists were searched manually for other relevant literature. The scope of the article has been limited to the importance of the alternative NF-κB signaling pathway in the gastrointestinal tract, particularly during the development of cancers and pre-malignant pathologies. Articles discussing the liver and pancreas only have not been included.

The alternative NF-κB activation pathway

The NF-κB/Rel family includes five members: RelA(p65), c-Rel, RelB, NF-κB1(p50/p105), and NF-κB2(p52/p100). Each possesses a structurally conserved 300 amino-acid sequence; the *REL* homology domain (RHD). In the unstimulated state, these proteins pool as homo- or heterodimers (of which there are at least 15 known combinations), predominantly within the cytoplasm[7]. Some of these dimers function exclusively in the classical NF-κB signaling pathway (figure 1a) and are beyond the scope of this article. Alternative NF-κB pathway activation leads to translocation of NF-κB2(p52)/RelB heterodimers into the nucleus, following which NF-κB2(p52) and RelB directly influence gene transcription.

NF-κB mediated transcription is regulated by the inhibitors of NF-κB (IκB) (IκBα, IκBβ, IκBε, and Bcl-3) which are peptides that bind NF-κB protein dimers through residues containing 6 to 7 ankyrin repeats. Interaction of IκB proteins with NF-κB protein dimers prevents the nuclear translocation of the NF-κB protein dimers. NF-κB2 is synthesized as a 100kDa protein, p100, which has a RHD within its N-terminus and a seven ankyrin repeat domain at its C-terminus which confers intrinsic IκB activity (figure 1b) [8].

Alternative pathway NF-κB activation is characterized by processing of p100 into p52, both of which dimerize with RelB (Figure 1b). p100 is processed by at least two kinases, NIK and IκB kinase α (IKKα). These kinases can be activated by ligand interaction with several cell-surface receptors, including CD40 ligand binding to CD40, B-cell activating factor (BAFF) binding to its receptors, Lymphotoxin β interacting with LTβR, lipopolysaccharide (LPS) binding to Toll-like receptor 4 (TLR4) and Receptor activator of nuclear factor kappa-B ligand (RANKL) binding to RANK[9]. The pathway can also be activated by oncogenic viruses including Epstein-Barr Virus[10].

Upon ligand binding, tumour necrosis factor receptor-associated factors (TRAFs; such as TRAF2 and TRAF3) are recruited either directly or via other adaptor proteins to the intracellular domain of the activated receptor. Unlike TRAF2 and TRAF5 that also regulate classical NF-κB signaling, TRAF3 only regulates the alternative NF-κB signaling pathway and directly binds to NIK, regulating NIK turnover[11]. NIK is continually degraded by the proteasome as a result of polyubiquitination by the cellular inhibitors of apoptosis (c-IAP 1,2)/E2 ligase complex that is also recruited to TRAF3 (figure 1b). As a result of TRAF3 complex recruitment to the activated receptor, TRAF3 is targeted for degradation by the proteasome which allows NIK to accumulate in the cytosol (figure 1c) [12]. Cytosol accumulated NIK becomes phosphorylated at threonine 559 by an incompletely described mechanism that may involve both autophosphorylation[13], and phosphorylation via zinc-finger-protein-91[14]. Subsequently, Thr559-phospho-NIK phosphorylates p100 at serine (Ser) residues 866 and 870 acting as a docking molecule for recruitment of IKKα to p100. Recruitment of IKKα leads to phosphorylation of the N-terminus of NF-κB2(p100) at Ser 99, 108, 115 and 123 and at C-terminal Ser 872. Beta-transducin repeat-containing protein (beta-TrCP), a component of the

SCF ubiquitin ligase complex is then recruited to phosphorylated NF-κB2(p100) leading to polyubiquitination of NF-κB2(p100) at lysine (Lys) 855 which targets NF-κB2 for partial proteasomal degradation, leading to generation of NF-κB2(p52)[15].

Activation of the alternative NF-κB signaling pathway is generally slower (~6-8 hours) than the classical activation pathway, leading to a delayed but sustained response which integrates with the acute-phase (~1.5 hours) response driven by classical NF-κB pathway activation[9, 16]. Emerging evidence supports the concept that classical and alternative NF-κB activation pathways interact to induce coordinated and sustained immunological responses. Banoth and colleagues recently used systems level analysis to identify stimulus-specific crosstalk between the TLR4-activated canonical NF-κB activation pathway and LTβR-induced alternative NF-κB activation pathway signaling. They identified a positive-feedback loop that sustained the NF-κB response. Using mouse embryonic fibroblasts, they demonstrated that the LTβR signal prolonged the TLR4-induced RelA response, by targeting newly-synthesized p100 for processing to p52, allowing formation of p65(RelA)-p52(NF-κB2) dimers. This led to increased expression of pro-inflammatory cytokines and chemokines[17]. Understanding the integration of different mechanisms of NF-κB activation is a key challenge for this field.

Role of the alternative NF-κB activation pathway in gut development and repair

Whilst expression of the classical NF-κB activation pathway components RelA and c-Rel can be found in many tissues and cell types, in health the expression of alternative pathway NF-κB sub-units has more frequently been demonstrated in cells of the immune system. More recent data have however demonstrated constitutive expression of RelB within small intestinal Peyer's patches[18] and of RelB and NF-κB2 (p100) in murine gastric epithelium[19].

Animal models suggest that the alternative NF-κB activation pathway components NIK, NF-κB2 and RelB are required for the normal development of small intestinal Peyer's patches [20, 21]. Peyer's patches consist predominantly of immune cells which exist in close association with the specialized 'dome'-like epithelium described as follicle-associated epithelium (FAE). The presence of RelB-expressing cells also appears essential for the differentiation of enterocytes into microfold cells (M-cells) which represent approximately 5% of epithelial cells within FAE. Functionally, M-cells are specialized to perform luminal antigen sampling and immune priming[22]. M-cells have been implicated in the initiation of granulomatous lesions in Crohn's disease[23] and are thought to influence responses to gastrointestinal infections[24, 25], and colorectal carcinogenesis[26, 27]. Tahoun and colleagues found that stimulation of RelB-expressing FAE enterocytes with *Salmonella enterica* Typhimurium triggered autocrine activation by RANK ligand-RANK interaction induced the transcription factor Snail family zinc finger-2 (Slug) and resulted in transdifferentiation into M-cells[25]. The expression of glycoprotein-2 (GP2), a key receptor expressed on mature M-cells also appears to be dependent on the nuclear translocation of RelB[28].

Whilst there is substantial evidence that RelB is involved in the development of Peyer's patches and M-cells within FAE, the mechanisms involved remain unclear. Wang and colleagues demonstrated that Peyer's patch development was dependent on stimulation of TNFR and LTβR[29], however the precise ligands for these receptors and the downstream effects of signaling via this pathway during the development of Peyer's patches, have not yet been defined.

There is also evidence that alternative pathway NF-κB signaling may influence gastric epithelial homeostasis. Mice lacking the COOH-terminal ankyrin repeat domain of p100 synthesize a truncated form of NF-κB2, which lacks the IκB activity of p100 but is able to dimerize with other NF-κB protein family members and can translocate to the nucleus to influence transcription. This models unimpeded alternative pathway NF-κB activation and leads to persistent translocation of NF-κB2-containing dimers to the nucleus. These animals exhibit massive gastric antral hyperplasia, have increased lymphocyte proliferation and die during early postnatal life due to complications arising from gastric outlet obstruction[30].

Alternative pathway NF-κB signaling in cancers of the gastrointestinal tract

Inflammatory mediators influence many cellular functions that may promote development of a malignant phenotype[31]. Since NF-κB signaling regulates innate immune responses, influencing the expression of a set of target genes in epithelial and immune compartments including cytokines (e.g. TNF, interleukin (IL)-6 and IL-1β)[6, 32, 33], their receptors (TNF superfamily receptors) and other inflammatory mediators, including cell adhesion molecules such as vascular cell adhesion protein 1 (VCAM-1)[34, 35] as well as genes involved in regulating the cell cycle such as cyclins and cyclin-dependent kinases[36-38] it is unsurprising that these processes also influence tumorigenesis.

The majority of studies investigating NF-κB and cancers of the gastrointestinal tract have focused upon the classical activation pathway family members NF-κB1, RelA (p65) and c-Rel. However there is emerging evidence that alternative NF-κB activation pathway family members also play important roles during carcinogenesis throughout the GI tract.

Esophageal cancer

Two histological subtypes of esophageal cancer exist, both of which develop on a background of chronic inflammation. Esophageal squamous carcinoma is associated with inflammation induced by extrinsic stimuli including cigarette smoking and alcohol consumption. Esophageal adenocarcinoma is also associated with these extrinsic risk factors, but is also associated with chronic gastroesophageal reflux, and occurs on a background of columnar metaplasia (Barrett's esophagus). The epidemiology and pathophysiology of these conditions are distinct, with the incidence of both conditions increasing in western populations.

In normal squamous esophageal tissue, the expression of NF-κB proteins is relatively low. However tissue samples from patients with esophageal squamous cell carcinoma showed increased expression of NF-κB1(p50/105), NF-κB2(p52/100) and RelA up to 18-fold[39]. In addition the anti-tumor activity of the hypo-methylating chemotherapeutic drug decitabine has been associated with increased expression of NF-κB2[40].

There are few data addressing the role of alternative pathway NF-κB signaling in gastro-esophageal reflux disease, Barrett's esophagus or esophageal adenocarcinoma in humans, however a recent study examined the role of reflux and smoking on the expression of alternative pathway NF-κB signaling components in the esophageal epithelium of mice. Using immunohistochemical techniques, it was demonstrated that the abundance of NIK was increased in the presence of either cigarette smoke alone or reflux alone[41]. One of the challenges of investigating the function of NF-κB pathways is the need to differentiate the abundance of proteins that signal within the pathway and their functional effects. These data provide inconclusive evidence that alternative pathway NF-κB signaling is involved in esophageal adenocarcinoma development, but support the need for further investigation of alternative pathway NF-κB signaling in this context.

Gastric Cancer

The most important etiological factor during gastric carcinogenesis is infection with *Helicobacter pylori*[42-46]. *H. pylori* infection results in stereotypical pre-malignant gastric pathology progressing from atrophic gastritis through metaplasia into dysplasia and cancer. In humans this process develops over several decades; mouse infection with the closely related bacterium *Helicobacter felis* recapitulates this sequence of pathology over an accelerated timescale. We recently showed that, despite heavy colonization with *H. felis*, *Nfkb2*$^{-/-}$ mice developed minimal inflammatory cell infiltration, demonstrated a blunted cytokine response and did not develop the pre-malignant lesions associated with chronic *H. felis* infection[19] (Figure 2a-d).

Other data have demonstrated *in-vitro* relevance for this pathway in response to *H. pylori* in both gastric epithelial and immune cell culture systems[19, 45, 46]. Hirata and colleagues showed that IKKα accumulates in AGS cells following *H. pylori* infection, but this did not lead to NF-κB2(p100) processing to NF-κB2(p52). The consequences of this are not clear, however it has been postulated that accumulation of cytosolic NF-κB2(p100) may inhibit nuclear NF-κB2(p52) interaction with chromatin, leading to an altered transcriptional fingerprint[47].

Separately, *H. pylori* activation of NF-κB was shown to occur via a pathway requiring IKKα, IKKβ, and NIK in MKN45 and KATO III human gastric cancer cell lines. In this context, activation of NIK also required signaling via the adapter proteins TRAF2 and TRAF6[48]. Further evidence for the involvement of NIK was provided by Neumann and colleagues [41] who demonstrated that activation of NF-κB by *H. pylori* required the formation of a complex between p21-activated kinase 1 (PAK1) and NIK[49].

In addition to the evidence that *H. pylori* can activate NF-κB via the alternative activation pathway in epithelial tissue, *in-vitro* processing of NF-κB2(p100) to NF-κB2(p52) has also been demonstrated in B lymphocytes in response to *H. pylori* infection[50]. This may have implications for the development of gastric MALT lymphomas.

Further evidence that classical and alternative pathway NF-κB activation is relevant to *Helicobacter* induced pathology comes from the evidence that clarithromycin, one of the antibiotics used most frequently to eradicate *H. pylori*, modulates *H. pylori*-induced activation of NF-κB via both activation pathways in gastric cancer cells[51].

Colorectal cancer

Sporadic colorectal cancers most commonly develop from adenomatous polyps[52]. These lesions develop as a consequence of the acquisition of stereotypical mutations over time. They occur in an otherwise non-inflamed colonic mucosa, but an inflammatory response to adenoma development is frequently identified. Despite this, few data regarding the importance of the alternative activation pathway of NF-κB signaling during sporadic colorectal carcinogenesis exist.

Amongst the best characterized pathways involved in colorectal carcinogenesis is the WNT signaling pathway. Activation of this pathway leads to stabilization of β-catenin, which is observed in many colonic adenomas. There is increasing evidence for crosstalk between the NF-κB and WNT signaling pathways, with evidence that activation of NF-κB pathways may accelerate the loss of APC during colorectal adenoma development[53]. However there is little direct evidence to suggest that alternative pathway NF-κB signaling is involved.

There is circumstantial evidence that alternative pathway NF-κB signaling may exert an effect on sporadic colonic carcinogenesis. MicroRNA miR-518a-3p was observed to be down-regulated in colon cancers in proportion to their size and TNM stage. This microRNA targets NIK for degradation, therefore its down-regulation leads to increased NIK abundance and subsequent activation of NIK dependent NF-κB pathways[54].

In contrast to sporadic colon cancer, more has been published regarding the impact of alternative pathway NF-κB signaling during the development of colitis-associated colorectal cancer. Individuals with chronic inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, have an increased risk of colorectal cancer[55]. In these patients, colorectal tumors develop on the background of an inflamed colonic mucosa, and rather than developing as discrete polyps, usually arise within fields of flat dysplastic mucosa[52].

The most established murine model of colitis-associated cancer uses a single dose of the DNA-damaging agent azoxymethane (AOM) followed by several doses of dextran sulfate sodium (DSS) to induce chronic colitis (AOM/DSS)[56]. We have reported that *Nfkb2*$^{-/-}$ mice are resistant to AOM/DSS-induced colitis-associated adenoma development[57]. Transgenic mice developed fewer dysplastic colonic lesions, were less susceptible to DSS-induced colitis (Figure 2i-p), and had a more robust apoptotic response following DNA damage compared to wild-type controls.

In contrast to *Nfkb2*$^{-/-}$ mice, *Nlrp12*$^{-/-}$ mice exhibited increased susceptibility to AOM/DSS induced colitis-associated colorectal cancer; polyps isolated from these mice had increased activation of the alternative NF-κB activation pathway. *Nlrp12*$^{-/-}$ primary dendritic cells showed elevated NIK expression, p100 processing to p52 and reduced abundance of TRAF3.

This provides evidence for NLRP12 acting as a checkpoint on signaling via the alternative NF-κB activation pathway during inflammation and tumorigenesis[58].

Alternative pathway NF-κB signaling in conditions of the GI tract that predispose to cancer

Gastrointestinal tract infections

Chronic bacterial and helminth infections are established risk factors for the development of malignancy. The best-characterized organism is *H. pylori*, which plays a major role in gastric carcinogenesis as discussed above. Several lines of evidence have identified that alternative pathway NF-κB signaling is important in the host response to gastrointestinal pathogens.

Infection with the foodborne pathogen *Salmonella* Typhimurium has been shown to promote the differentiation of M-cells in the small intestine through induction of RANK, and its ligand RANKL, leading to increased differentiation of M-cells and increased uptake of the pathogen. In addition to the evidence described above that *S*. Typhimurium may induce M-cell transdifferentiation via an alternative activation NF-κB pathway dependent mechanism Tahoun and colleagues[25] have also demonstrated that translocation of *S*. Typhimurium was significantly reduced in the presence of the NF-κB inhibitor SN50, suggesting that NF-κB signaling plays a role in enhancing *S*. Typhimurium translocation across the epithelium. Whilst this organism is not directly associated with gastrointestinal carcinogenesis, these mechanisms offer a paradigm by which other carcinogenic organisms may influence epithelial events via NF-κB signaling.

Banoth and colleagues demonstrated that C57BL/6 mice infected with *Citrobacter rodentium* developed colitis with epithelial accumulation of p100. *Nfkb2*$^{-/-}$ mice infected with *C.*

rodentium were more susceptible to developing colitis than wild-type mice. Using reciprocal bone marrow transfer experiments, *Nfkb2*$^{-/-}$ mice given wild-type mouse bone marrow lost weight and had 100% mortality before day 10, whereas wild-type mice given either wild-type or *Nfkb2*$^{-/-}$ bone marrow did not lose weight or show increased mortality. These findings indicate that NF-κB2 expression within the non-myeloid compartment is involved in limiting the severity of *C. rodentium* associated colitis[17].

Alternative pathway NF-κB signaling is also implicated in the orchestration of immune responses to gastrointestinal helminth infestation. C57BL/6 mice generate a marked Th2 immune response following colonization with the whipworm *Trichuris muris.* This induces rapid expulsion of the helminth and clearance of infestation within 35 days of colonization. In contrast, *Nfkb2*$^{-/-}$ mice develop a chronic, persistent infestation with an impaired Th2 cytokine response[59]. More recently, an immunosuppressed man with histoplasmosis and extra-intestinal *Hymenolepis nana* infection was found to possess abnormal proliferative cells which displayed malignant characteristics. Upon investigation, the cells were found to be consistent with tapeworm stem cells; this was the first report of parasite-derived cancer cells in a human, further demonstrating the link between infection and cancer, and highlighting its relevance when considering GI exposure to helminths[60].

Inflammatory bowel disease

Chronic idiopathic inflammatory bowel diseases that affect the colon increase an individual's risk of developing colorectal cancer in proportion to the duration of disease and the extent of inflammation[61-64], and evidence suggests that treatments that decrease an individual's inflammatory burden, including azathioprine[65] and 5-amino salicylic acid preparations [66] may decrease the risk of developing colitis-associated colon cancer.

The alternative NF-κB activation pathway has not to date been targeted specifically for the development of novel therapeutics for IBD. This is likely due to the fact that most studies in IBD have found activation of the classical activation pathway, but not the alternative pathway[67-69]. For example, Ardite and colleagues used electromobility shift assays to detect NF-κB components in colonic biopsy samples from patients with active IBD who had been treated with steroids. The classical NF-κB activation pathway subunit p50 was found in inflamed tissue from IBD patients but p65, p52, c-Rel and RelB were not detected[67].

In contrast to the findings in humans, mouse models of colitis induced by carrageenan and DSS have demonstrated that classical and alternative NF-κB signaling pathways are activated[70-73] during colonic inflammation. One of the earliest events in IBD is thought to be impairment of enteric mucosal barrier function. We have modeled this by administration of LPS from *Escherichia coli* to C57BL/6 mice and mice lacking NF-κB2. We demonstrated that *Nfkb2*$^{-/-}$ mice were more resistant to LPS-induced small intestinal epithelial cell shedding compared to wild-type mice[71] (Figure 2e-h). Separately and as described above, we demonstrated that the severity of DSS-induced acute colitis was reduced in *Nfkb2*$^{-/-}$ mice relative to wild-type mice[57](figure 2i-p).

Our studies, and those of Banoth suggest that there may be a function for these pathways in the regulation of enteral inflammation, suggesting that targeting alternative pathway NF-κB signaling in IBD may be a fruitful avenue for further research.

One element of IBD therapy in which tangential evidence for alternative pathway NF-κB signaling has been accrued is the role of functional foods. This is of particular interest in the context of Crohn's disease, where dietary modification with liquid feeding has been shown to offer therapeutic benefits, particularly in pediatric patients.

A study examining the role of parenteral nutrition in IBD demonstrated that a lack of enteral stimulation in mice led to globally reduced expression of NF-κB proteins in Peyer's patches[74]. Given the data that implicate RelB in Peyer's patch development, and the role that Peyer's patches play in immune priming these findings suggest that diet induced NF-κB signaling may influence GI immune tolerance.

In contrast the use of dietary flaxseed to attempt to ameliorate experimental colitis was found to exacerbate DSS-induced colitis in mice, which was associated with an increase in expression of RelB in colonic tissue[72]. Together these studies highlight the functional effect that foods may have on NF-κB alternative pathway signaling in the gastrointestinal tract.

Celiac Disease

Gluten sensitive enteropathy, or celiac disease, confers an increased risk of small bowel adenocarcinoma and small bowel lymphoma, however due to the rarity of these cases it is difficult to accurately quantify the degree of increased risk[75]. Recent data have identified that increased expression of NFKB2 in patients with celiac disease, irrespective of their adherence to a gluten free diet[76]. Currently it is not clear whether this association has any impact on the prevalence or biology of small bowel malignancies.

Discussion

Several recent studies have highlighted the importance of alternative pathway NF-κB signaling in addition to the more widely studied classical NF-κB signaling pathway during the development and progression of both inflammation-associated cancers of the GI tract and sporadic cancers which have an inflammatory element. Some elements of NF-κB signaling pathways and their implications for disease have been well characterized, but investigation in other areas is lacking. In particular, gaps exist in our understanding of these pathways in the formation of esophageal cancers and the role, if any, played by the gastrointestinal microbiome in regulating them.

Most of the studies reported to date have relied on relatively imprecise tools to characterize the function of NF-κB signaling pathways, for instance the observation that NF-κB activation events may be localized to specific anatomical structures within in the GI tract (e.g. Peyer's patches) suggests that previous studies which have assessed NF-κB activation events in whole tissue preparations should be interpreted with caution. Many investigations have also relied on either transgenic mice in which the stoichiometry of different pathway members is fundamentally altered, or have used semi-quantitative analyses of protein abundance to try to explain the complexities of these pathways.

Banoth and colleagues have demonstrated a different approach to research in this field by employing systems medicine techniques to characterize interactions between multiple components of this complex transcriptional regulating machinery. In the future, increasingly complex mathematical modeling will be required to better understand how inflammation is regulated by NF-κB signaling in real-time, and to apply these models to complex systems, including the pathogenesis of cancer. To achieve this, close collaboration between researchers with diverse backgrounds including clinician scientists, molecular biologists and systems biologists will be necessary.

Acknowledgments

This work was supported by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement No 305564 (SysmedIBD). AO'H was funded by a North West Cancer Research grant. CAD was funded by The Physiological Society and the Fiona Elizabeth Agnew Trust. MDB was supported by a BSG/CORE development award.

References

1. Sen, R. and D. Baltimore, *Multiple nuclear factors interact with the immunoglobulin enhancer sequences.* Cell, 1986. **46**(5): p. 705-716.
2. Antonova, Y., et al., *The role of NF-kappaB factor REL2 in the Aedes aegypti immune response.* Insect Biochem.Mol.Biol., 2009. **39**(4): p. 303-314.
3. Ghosh, S., M.J. May, and E.B. Kopp, *NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses.* Annu.Rev.Immunol., 1998. **16**: p. 225-260.
4. Vallabhapurapu, S. and M. Karin, *Regulation and function of NF-kappaB transcription factors in the immune system.* Annu Rev Immunol, 2009. **27**: p. 693-733.
5. Campbell, K.J. and N.D. Perkins, *Regulation of NF-kappaB function.* Biochem Soc Symp, 2006(73): p. 165-80.
6. Shakhov, A.N., et al., *Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages.* J Exp Med, 1990. **171**(1): p. 35-47.
7. Silverman, N. and T. Maniatis, *NF-kappaB signaling pathways in mammalian and insect innate immunity.* Genes & development, 2001. **15**(18): p. 2321-42.

8. Nishikori, M., *Classical and Alternative NF-. KAPPA. B Activation Pathways and Their Roles in Lymphoid Malignancies.* Journal of Clinical and Experimental Hematopathology, 2005. **45**(1): p. 15-24.
9. Dejardin, E., *The alternative NF-kappaB pathway from biochemistry to biology: pitfalls and promises for future drug development.* Biochem Pharmacol, 2006. **72**(9): p. 1161-79.
10. Luftig, M., et al., *Epstein-Barr virus latent infection membrane protein 1 TRAF-binding site induces NIK/IKK alpha-dependent noncanonical NF-kappaB activation.* Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(1): p. 141-6.
11. Hacker, H., P.H. Tseng, and M. Karin, *Expanding TRAF function: TRAF3 as a tri-faced immune regulator.* Nature reviews. Immunology, 2011. **11**(7): p. 457-68.
12. Vallabhapurapu, S., et al., *Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling.* Nat Immunol, 2008. **9**(12): p. 1364-70.
13. Sun, S.C., *Controlling the fate of NIK: a central stage in noncanonical NF-kappaB signaling.* Sci Signal, 2010. **3**(123): p. pe18.
14. Jin, H.R., X. Jin, and J.J. Lee, *Zinc-finger protein 91 plays a key role in LIGHT-induced activation of non-canonical NF-kappaB pathway.* Biochemical and biophysical research communications, 2010. **400**(4): p. 581-6.
15. Xiao, G., A. Fong, and S.C. Sun, *Induction of p100 processing by NF-kappaB-inducing kinase involves docking IkappaB kinase alpha (IKKalpha) to p100 and IKKalpha-mediated phosphorylation.* J Biol Chem, 2004. **279**(29): p. 30099-105.

16. Saccani, S., S. Pantano, and G. Natoli, *Modulation of NF-kappaB activity by exchange of dimers.* Molecular cell, 2003. **11**(6): p. 1563-74.

17. Banoth, B., et al., *Stimulus-selective crosstalk via the NF-kappaB signaling system reinforces innate immune response to alleviate gut infection.* Elife, 2015. **4**.

18. Li, Q. and I.M. Verma, *NF-kappaB regulation in the immune system.* Nature reviews. Immunology, 2002. **2**(10): p. 725-34.

19. Burkitt, M.D., et al., *Signaling mediated by the NF-kappaB sub-units NF-kappaB1, NF-kappaB2 and c-Rel differentially regulate Helicobacter felis-induced gastric carcinogenesis in C57BL/6 mice.* Oncogene, 2013. **32**(50): p. 5563-73.

20. Yilmaz, Z.B., et al., *RelB is required for Peyer's patch development: differential regulation of p52-RelB by lymphotoxin and TNF.* EMBO J, 2003. **22**(1): p. 121-30.

21. Fagarasan, S., et al., *Alymphoplasia (aly)-type nuclear factor kappaB-inducing kinase (NIK) causes defects in secondary lymphoid tissue chemokine receptor signaling and homing of peritoneal cells to the gut-associated lymphatic tissue system.* The Journal of experimental medicine, 2000. **191**(9): p. 1477-86.

22. Mabbott, N.A., et al., *Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium.* Mucosal Immunol, 2013. **6**(4): p. 666-77.

23. Barnich, N. and A. Darfeuille-Michaud, *Adherent-invasive Escherichia coli and Crohn's disease.* Curr Opin Gastroenterol, 2007. **23**(1): p. 16-20.

24. Etienne-Mesmin, L., et al., *Interactions with M cells and macrophages as key steps in the pathogenesis of enterohemorrhagic Escherichia coli infections.* PloS one, 2011. **6**(8): p. e23594.

25. Tahoun, A., et al., *Salmonella transforms follicle-associated epithelial cells into M cells to promote intestinal invasion.* Cell host & microbe, 2012. **12**(5): p. 645-56.

26. Allen-Vercoe, E. and C. Jobin, *Fusobacterium and Enterobacteriaceae: Important players for CRC?* Immunol Lett, 2014.

27. Martin, H.M., et al., *Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer.* Gastroenterology, 2004. **127**(1): p. 80-93.

28. Kimura, S., et al., *Visualization of the entire differentiation process of murine M cells: suppression of their maturation in cecal patches.* Mucosal Immunol, 2015. **8**(3): p. 650-60.

29. Wang, J., et al., *TNFR and LTbetaR agonists induce follicle-associated epithelium and M cell specific genes in rat and human intestinal epithelial cells.* Cytokine, 2009. **47**(1): p. 69-76.

30. Ishikawa, H., et al., *Gastric hyperplasia and increased proliferative responses of lymphocytes in mice lacking the COOH-terminal ankyrin domain of NF-kappaB2.* J Exp Med, 1997. **186**(7): p. 999-1014.

31. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.

32. Hiscott, J., et al., *Characterization of a functional NF-kappa B site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop.* Mol Cell Biol, 1993. **13**(10): p. 6231-40.

33. Libermann, T.A. and D. Baltimore, *Activation of interleukin-6 gene expression through the NF-kappa B transcription factor.* Mol Cell Biol, 1990. **10**(5): p. 2327-34.

34. Iademarco, M.F., et al., *Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1).* J Biol Chem, 1992. **267**(23): p. 16323-9.

35. Santee, S.M. and L.B. Owen-Schaub, *Human tumor necrosis factor receptor p75/80 (CD120b) gene structure and promoter characterization.* J Biol Chem, 1996. **271**(35): p. 21151-9.

36. Hinata, K., et al., *Divergent gene regulation and growth effects by NF-kappa B in epithelial and mesenchymal cells of human skin.* Oncogene, 2003. **22**(13): p. 1955-64.

37. Iwanaga, R., et al., *Activation of the cyclin D2 and cdk6 genes through NF-kappaB is critical for cell-cycle progression induced by HTLV-I Tax.* Oncogene, 2008. **27**(42): p. 5635-42.

38. Lee, H.H., et al., *NF-kappaB-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes.* Proc Natl Acad Sci U S A, 1999. **96**(16): p. 9136-41.

39. Kang, M.R., et al., *NF-kappaB signalling proteins p50/p105, p52/p100, RelA, and IKKepsilon are over-expressed in oesophageal squamous cell carcinomas.* Pathology, 2009. **41**(7): p. 622-5.

40. Liu, W.H., et al., *Low-dose decitabine induces MAGE-A expression and inhibits invasion via suppression of NF-kappaB2 and MMP2 in Eca109 cells.* Biomed Pharmacother, 2014. **68**(6): p. 745-50.

41. Aiyer, H.S., et al., *Molecular changes in the esophageal epithelium after a subchronic exposure to cigarette smoke in the presence of bile-acid reflux.* Inhal Toxicol, 2011. **23**(5): p. 304-11.

42. *NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease.* JAMA, 1994. **272**(1): p. 65-9.

43. Forman, D., et al., *Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation.* BMJ, 1991. **302**(6788): p. 1302-5.

44. Graham, D.Y., et al., *Effect of treatment of Helicobacter pylori infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study.* Ann Intern Med, 1992. **116**(9): p. 705-8.

45. Nomura, A., et al., *Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii.* N Engl J Med, 1991. **325**(16): p. 1132-6.

46. Parsonnet, J., et al., *Helicobacter pylori infection and the risk of gastric carcinoma.* N Engl J Med, 1991. **325**(16): p. 1127-31.

47. Hirata, Y., et al., *Helicobacter pylori induces IkappaB kinase alpha nuclear translocation and chemokine production in gastric epithelial cells.* Infection and immunity, 2006. **74**(3): p. 1452-61.

48. Maeda, S., et al., *H. pylori activates NF-kappaB through a signaling pathway involving IkappaB kinases, NF-kappaB-inducing kinase, TRAF2, and TRAF6 in gastric cancer cells.* Gastroenterology, 2000. **119**(1): p. 97-108.

49. Neumann, M., et al., *The PAK1 autoregulatory domain is required for interaction with NIK in Helicobacter pylori-induced NF-kappaB activation.* Biol Chem, 2006. **387**(1): p. 79-86.

50. Ohmae, T., et al., *Helicobacter pylori activates NF-kappaB via the alternative pathway in B lymphocytes.* J Immunol, 2005. **175**(11): p. 7162-9.

51. Peng, Y.C., et al., *Clarithromycin modulates Helicobacter pylori-induced activation of nuclear factor-kappaB through classical and alternative pathways in gastric epithelial cells.* Clin Exp Med, 2014. **14**(1): p. 53-9.

52. Arvelo, F., F. Sojo, and C. Cotte, *Biology of colorectal cancer.* Ecancermedicalscience, 2015. **9**: p. 520.

53. Shaked, H., et al., *Chronic epithelial NF-kappaB activation accelerates APC loss and intestinal tumor initiation through iNOS up-regulation.* Proceedings of the National Academy of Sciences of the United States of America, 2012. **109**(35): p. 14007-12.

54. Qu, L.L., et al., *Downregulation of miR-518a-3p activates the NIK-dependent NF-kappaB pathway in colorectal cancer.* Int J Mol Med, 2015. **35**(5): p. 1266-72.

55. Triantafillidis, J.K., G. Nasioulas, and P.A. Kosmidis, *Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies.* Anticancer research, 2009. **29**(7): p. 2727-37.

56. Cooper, H.S., et al., *Clinicopathologic study of dextran sulfate sodium experimental murine colitis.* Laboratory investigation; a journal of technical methods and pathology, 1993. **69**(2): p. 238-49.

57. Burkitt, M.D., et al., *NF-kappaB1, NF-kappaB2 and c-Rel differentially regulate susceptibility to colitis-associated adenoma development in C57BL/6 mice.* J Pathol, 2015.

58. Allen, I.C., et al., *NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-kappaB signaling.* Immunity, 2012. **36**(5): p. 742-54.

59. Artis, D., et al., *Differential requirement for NF-kappa B family members in control of helminth infection and intestinal inflammation.* J Immunol, 2002. **169**(8): p. 4481-7.

60. Muehlenbachs, A., et al., *Malignant Transformation of Hymenolepis nana in a Human Host.* New England Journal of Medicine, 2015. **373**(19): p. 1845-1852.

61. Eaden, J.A., K.R. Abrams, and J.F. Mayberry, *The risk of colorectal cancer in ulcerative colitis: a meta-analysis.* Gut, 2001. **48**(4): p. 526-35.
62. Gillen, C.D., et al., *Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis.* Gut, 1994. **35**(11): p. 1590-2.
63. Rutter, M.D., et al., *Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk.* Gut, 2004. **53**(12): p. 1813-6.
64. Rutter, M., et al., *Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis.* Gastroenterology, 2004. **126**(2): p. 451-9.
65. Gong, J., et al., *Use of Thiopurines and Risk of Colorectal Neoplasia in Patients with Inflammatory Bowel Diseases: A Meta-Analysis.* PloS one, 2013. **8**(11): p. e81487.
66. van Staa, T.P., et al., *5-Aminosalicylate use and colorectal cancer risk in inflammatory bowel disease: a large epidemiological study.* Gut, 2005. **54**(11): p. 1573-8.
67. Ardite, E., et al., *Effects of steroid treatment on activation of nuclear factor kappaB in patients with inflammatory bowel disease.* British journal of pharmacology, 1998. **124**(3): p. 431-3.
68. Schmid, R.M., G. Adler, and S. Liptay, *Activation of NFkappaB in inflammatory bowel disease.* Gut, 1998. **43**(4): p. 587-8.
69. Schreiber, S., S. Nikolaus, and J. Hampe, *Activation of nuclear factor kappa B inflammatory bowel disease.* Gut, 1998. **42**(4): p. 477-84.
70. Bhattacharyya, S., P.K. Dudeja, and J.K. Tobacman, *Carrageenan-induced NFkappaB activation depends on distinct pathways mediated by reactive oxygen species and Hsp27 or by Bcl10.* Biochim Biophys Acta, 2008. **1780**(7-8): p. 973-82.

71. Williams, J.M., et al., *A mouse model of pathological small intestinal epithelial cell apoptosis and shedding induced by systemic administration of lipopolysaccharide.* Dis Model Mech, 2013. **6**(6): p. 1388-99.

72. Zarepoor, L., et al., *Dietary flaxseed intake exacerbates acute colonic mucosal injury and inflammation induced by dextran sodium sulfate.* Am J Physiol Gastrointest Liver Physiol, 2014. **306**(12): p. G1042-55.

73. Bhattacharyya, S., et al., *Specific effects of BCL10 Serine mutations on phosphorylations in canonical and noncanonical pathways of NF-kappaB activation following carrageenan.* Am J Physiol Gastrointest Liver Physiol, 2011. **301**(3): p. G475-86.

74. Lan, J., et al., *Parenteral nutrition impairs lymphotoxin beta receptor signaling via NF-kappaB.* Ann Surg, 2011. **253**(5): p. 996-1003.

75. Catassi, C., I. Bearzi, and G.K. Holmes, *Association of celiac disease and intestinal lymphomas and other cancers.* Gastroenterology, 2005. **128**(4 Suppl 1): p. S79-86.

76. Fernandez-Jimenez, N., et al., *Coregulation and modulation of NFkappaB-related genes in celiac disease: uncovered aspects of gut mucosal inflammation.* Hum Mol Genet, 2014. **23**(5): p. 1298-310.