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Review

# The Fire Within: Microbes Inflame Tumors

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**The immune system and the microbiota mutually interact to maintain homeostasis in the intestine. However, components of the microbiota can alter this balance and promote chronic inflammation, promoting intestinal tumor development. We review recent advances in understanding the complex interactions between the microbiota and the innate and adaptive immune systems and discuss their potential to lead us in new directions for understanding cancer biology and treatment.**

## Introduction

As early as the 19th century, Robert Koch and Louis Pasteur had reported finding bacteria at tumor sites. In 1890, William Russell reported to the Pathological Society of London the discovery of a cancer parasite. However, during the first half of the 20th century, the theory that bacteria could cause cancer was considered heresy. Recently, a growing body of evidence has started to reveal that William Russell was right. Bacteria can drive tumor growth, and their interaction with the immune system can further fuel cancer progression. This is especially true at mucosal interfaces where bacteria are abundant and the immune system is highly reactive.

The number of bacteria in the human intestine is estimated to be around $1 \times 10^{14}$, outnumbering the eukaryotic cells by a factor of ten or more. Usually the microbiota does not elicit a proinflammatory immune response, as coevolution of the host mucosal immune system and commensal organisms developed multiple mechanisms for maintaining homeostasis. However, when these mechanisms are impaired and/or pathogenic bacteria are introduced into this tightly balanced ecosystem, the immune system responds to the microbiota and can trigger and/or sustain tumor growth in the intestine.

The reduced incidence of intestinal cancer in germ-free rodents that are genetically modified to promote tumor susceptibility, as compared to animals of the same genotype with normal microbiota, provides compelling evidence for microbiota's role in tumor growth (Reddy et al., 1975; Vannucci et al., 2008). The bacteria present in the intestine impact tumor development through multiple signaling pathways, some elicited by secretion bacterial-derived molecules. The mechanisms by which bacteria promote tumorigenesis can be direct or indirect (Table 1). As an example of a direct mechanism, deoxycholic acid (DCA), a gut bacterial metabolite, can directly cause DNA damage and, in turn, promote tumor development (Yoshimoto et al., 2013). In addition, *E. coli* NC101 can directly cause tumor growth in the intestine thanks to Colibactin, a peptide-polyketide hybrid genotoxin (Arthur et al., 2012). Finally, *Fusobacterium nucleatum* has recently been shown to directly promote intestinal tumorigenesis when its adhensin, FadA, binds to E-cadherin on epithelial cells and activates β-catenin signaling to promote epithelial cell proliferation (Rubinstein et al., 2013) (Kostic et al., 2013). Indirect mechanisms by which bacteria promote tumor growth involve the chronic activation of the immune system (Figure 1). In this Review, we will mainly focus on the indirect mechanisms by which the microbiota can initiate and/or sustain tumor development in the intestine through modulation of the innate and adaptive arms of the immune system.

## Innate Immune Receptors, Microbes, and Tumor Development

The innate immune system is a universal and evolutionarily conserved arm of the host immune defense system that enables rapid response to invading pathogens (Medzhitov, 2001). Signaling through pattern recognition receptors (PRRs), which include, among others, Nod-like receptors (NLRs) and Toll like receptors (TLRs), enables host immune sensing and reactivity toward diverse stimuli. Standing at the interface between microbiota and the immune system, the PPRs decode signals from the microbiota and help to shape the homeostatic host-microbiota interface. When the immune response does not properly control the microbiota—for example, due to a deficiency in these sensing platforms—alterations of commensal communities and emergence of normally suppressed bacteria can cause deleterious effects to the host. Throughout the text, we refer to this type of microflora as altered, or dysbiotic, microbiota. One of the main effects of dysbiosis is chronic unchecked activation of the immune system, thereby creating a proinflammatory milieu, which may favor the development and progression of neoplastic lesions in the intestine.

### *Nod-like Receptors*

The NLR family is a group of cytosolic receptor proteins that can be activated by a large variety of both endogenous and

**Table 1. Tumors' Associated Bacteria and Mechanism of Action**

| Bacteria | Mechanism | | Type of Tumor | Reference |
|---|---|---|---|---|
| | Direct | Indirect | | |
| *Clostridium Cluster* IX | Deoxycholic acid | | Liver cancer | Yoshimoto et al., 2013 |
| *pks*$^{+}$ *Escherichia coli* | PKS → Colibactin | | Colorectal cancer | Arthur et al., 2012 |
| *Dysbiosis/Prevotellaceae* | | Immune response<br>CCL5 | Colorectal cancer | Elinav et al., 2011;<br>Hu et al., 2013;<br>Sobhani et al., 2011 |
| Enterotoxigenic *Bacteroides fragillis* | | Immune response<br>Th17<br>Spermine oxidase | Colorectal cancer | Wu et al., 2009<br>Goodwin et al., 2011 |
| *Commensal bacteria* | | Immune response<br>IL-23 and IL-17 | Colorectal cancer | Grivennikov et al., 2012 |
| *Fusobacterium nucleatum* | Virulenc factor FadA | Immune response<br>NF-kB, IL-6, IL-8, IL-18 | Colorectal cancer | Rubinstein et al., 2013;<br>Kostic et al., 2013 |

exogenous triggers (Henao-Mejia et al., 2012). They represent the sensory component of a multiprotein complex called the inflammasome. Once formed, mature inflammasomes recruit and activate caspase enzymes that mediate the activation of the cytokines IL-1β and IL-18 (Henao-Mejia et al., 2012). Recent data show that control of the intestinal microbiota by the inflammasome is a central means of protecting gut homeostasis (Elinav et al., 2011). The inflammasome component NLRP6 was shown to have a protective role against the development of colitis-associated cancer via modulation of gut microbiota (Chen et al., 2011; Normand et al., 2011). Intense inflammatory responses and defective production of IL-18 were noted in the intestine in the absence of NLRP6 (Elinav et al., 2011) (Chen et al., 2011). Additionally, it was shown that NLRP6 defends the host against proliferation of certain bacteria (Anand et al., 2011; Anand and Kanneganti, 2013).

These data together suggest that NLRP6 plays an important role in regulating the gut microbiota communities by sensing and controlling potentially pathogenic species. In support of this notion, our group has shown that mice with perturbations in the NLRP6 inflammasome pathway develop an altered microbiota with overrepresentation of several bacterial taxa, including Prevotellaceae and TM7 (Elinav et al., 2011). This dysbiosis caused spontaneous intestinal inflammation and increased susceptibility to colitis (Elinav et al., 2011). Furthermore, the dysbiosis associated with NLRP6 inflammasome deficiency was able to promote colorectal carcinogenesis. Mechanistically, the dysbiosis promoted inflammation via the chemokine CCL5, which recruits a nonphysiological number of lymphocyte in the intestine. The resulting inflammatory state promotes epithelial cell proliferation through local activation of the IL-6 pathway (Elinav et al., 2011; Hu et al., 2013) (Figure 2). Interestingly, the dysbiotic microbiota is fully transferable to wild-type mice horizontally upon cohousing or vertically by cross-fostering. Whether the transmitted Prevotellaceae and TM7 have direct protumorigenic activity is not yet clear. Importantly, however, the dysbiotic microbiota in the NLRP6-deficient mice are competitively dominant over the endogenous microbiota and induce a stage of inflammation following prolonged cohabitation with wild-type mice (Elinav et al., 2011; Hu et al., 2013). These experiments demonstrate that enhanced susceptibility to colon cancer in this model is infectious and point to this dysbiotic microbiota as a driving force of cancer. In line with this, intestinal dysbiosis in mice deficient in Nod2, another NLR family member, was reported to promote transmissible inflammation-induced colorectal cancer (Couturier-Maillard et al., 2013). The molecular inputs by which the host modulates the microbiota and the cues by which representatives of the microbiota exert their effects on the host remain to be studied.

Interestingly, bacteria of the Prevotellaceae family are also found to be expanded in the fecal content of some patients with colorectal cancer as compared to control subjects (Sobhani et al., 2011), suggesting that the Prevotellaceae may contain certain pathogenic bacterial species that promote inflammation-associated intestinal carcinogenesis.

Other studies focused on different components of the inflammasome, such as Nlrp3, Capase-1, and IL-18, support the role of this complex in maintaining homeostasis in the intestine and in suppressing tumor development in an indirect manner (Hu et al., 2010, 2013; Allen et al., 2010; Henao-Mejia et al., 2012; Zaki et al., 2010). *Nlrp3*- and *Caspase-1*-deficient mice develop enhanced chronic colitis and colon rectal cancer in a mouse model of colitis-associated inflammation-induced colorectal cancer (Allen et al., 2010; Zaki et al., 2010). Likewise, *Il18*- and *Il18r*-deficient mice were shown to be hypersusceptible to DSS-induced colitis and colorectal tumorigenesis (Salcedo et al., 2010). Moreover, administration of exogenous IL-18 alleviated the severity of colitis and colitis-induced tumorigenesis in *Caspase1/11*- and *Nlrp3*-deficient mice (Zaki et al., 2010).

*Il1r*-deficient mice showed a similar tumor load in the AOM/DSS-induced colon rectal cancer model to that of WT mice. These differences in phenotypes between altered states of IL-1β or IL-18 signaling highlight the unique functions of these two cytokines during intestinal tumor development and progression. As such, preferential inflammasome activation of either IL-1β or IL-18, or their combined signaling, may lead to different downstream physiological and pathophysiological consequences. Differential activation of inflammasomes by microbiota or host-derived factors may contribute to or even drive net inflammasome-induced effector function. Thus, context-specific integrative inflammasome signaling in different cells and time points may induce differences in the inflammasome-activated

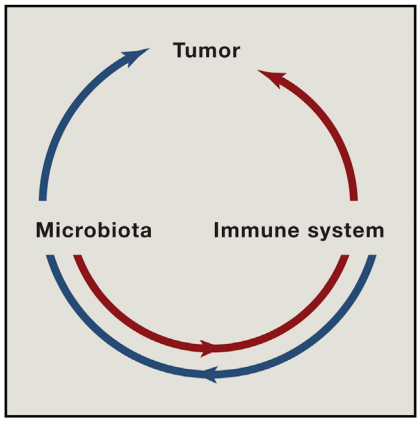


**Figure 1. The Immune System, Microbiota, and Tumors Are Tightly Connected**

The bacteria in the intestine can modulate tumor development through multiple different mechanisms that can be mainly divided into direct and indirect. The direct mechanisms (blue line) are those in which microbial products directly promote tumor growth. Of note, it has been shown that some deficiencies in the immune system can allow the growth of certain procarcinogenic bacteria. The indirect mechanisms (red line) are those in which the bacteria per se are not able to promote tumor initiation and growth unless they interact with the immune system, which ultimately promotes cancer. Finally, it is also possible that deficiencies in specific mechanisms of the immune response allow the expansion of certain bacteria, which, in turn, activate a protumorigenic immune response (blue + red line).

cytokine profile, resulting in differential downstream phenotypes. These cell- and tissue-specific interplays between different inflammasomes at different stages of tumor formation and growth will be exciting to characterize.

Taken together, these studies support the pivotal role of the inflammasome in regulating gut homeostasis and tumor development. However, it still remains to be proven that an altered microbiota is responsible for the phenotype observed in these knock out mouse models.

### *Toll-like Receptors*

TLRs recognize a variety of conserved pathogen-associated molecular patterns (PAMPs) and endogenous stress signals (Medzhitov, 2001; Palm and Medzhitov, 2009). Recognition of the various microbial PAMPs by their cognate TLRs can activate downstream signaling pathways, such as NF-κB, which leads to the production of proinflammatory cytokines and chemokines and enhances tumor cell proliferation and survival (Rakoff-Nahoum and Medzhitov, 2009). Supporting this notion, administration of TLR agonists such as lipopolysaccaride (LPS) increases inflammation in the tumor microenvironment (Rakoff-Nahoum and Medzhitov, 2009). Accordingly, deficiency in the TLR-signaling adaptor molecule MyD88 and in one of the components of the TLR family, TLR4, was found to be protective in different mouse models of colon cancer (Rakoff-Nahoum and Medzhitov, 2009). These results provided the first clue that the TLR-mediated innate immune sensing of the microbiota in the intestine is important in the regulation of tumorigenesis (Rakoff-Nahoum and Medzhitov, 2009). Furthermore, genetic deficiency of TLR causes alteration of the microbiota composition (Jin and Flavell, 2013) and thereby might promote the outgrowth of certain bacteria species with pathogenic potential. Alteration of gut microbiota may promote inflammation through enhanced PAMP presentation to intestinal immune cells. For example, mice lacking TLR2 or TLR5 have been shown to develop an altered gut microbiota, which can cause spontaneous intestinal inflammation and increased LPS in circulation. This has been associated with the development of metabolic syndrome featuring insulin resistance and obesity (Hu et al., 2013; Jiang et al., 2013; Jin and Flavell, 2013; Wu et al., 2013). Given the close association between inflammation and colorectal cancer, it is conceivable that the colitogenic flora in these *TLR2*/*TLR5*-deficient mice may also enhance intestinal tumorigenesis. Finally, increased penetration of commensal flora into the tumor as a result of diminished intestinal integrity in a mouse model of colorectal tumorigenesis has also been shown to sustain tumor growth through the activation of TLRs (Grivennikov et al., 2012). In particular, bacterial components such as flagellin bind to TLR5 and promote the release of the proinflammatory cytokine IL-23 (Kinnebrew et al., 2012), which is highly expressed in both mouse and human intestinal tumors (Grivennikov et al., 2012). When IL-23 is constitutively released at a high level, it promotes a state of chronic inflammation, which can abet the growth of the tumors (Grivennikov et al., 2012; Figure 3).

## Microbially Influenced Adaptive Immune Response Affecting Tumor Development

The adaptive arm of the host immune system is comprised of cells that respond selectively and specifically to infectious agents that the innate cells have collected and presented to them. Some of these responses can be protumorigenic. For example, upon contact with specific bacteria, $CD4^+$ T cells can produce protumorigenic cytokines. The following paragraphs describe how certain members of the microbiota alter the adaptive immune response and, in turn, promote tumor growth. Finally, we describe two examples of how the adaptive immune system can keep its protumorigenic features in check.

### *Th17 Cells and Protumorigenic Cytokines*

$CD4^+$ T cells that express RORγt and, consequently, secrete large amounts of IL-17 are called Th17 cells. The presence of Th17 cells in the intestine is essential to control microbial invasion, but specific compensatory mechanisms are required to control Th17 cells. If those mechanisms fail, Th17 cells become pathogenic and can induce chronic inflammation and autoimmune disease. A growing body of evidence suggests that Th17 cells and their cytokines also have a strong protumorigenic potential in the intestine. Considering the capacity of certain components of the microflora to modulate Th17 cell biology (see below), we propose that this cell type is one of the main instruments by which the microbiota can indirectly promote tumor growth.

IL-17A expression has been observed in several tumors and appears to have pro- and antitumorigenic activities depending on the type of tumor (Grivennikov et al., 2012; Muranski et al., 2008). In the intestine, several lines of evidence support the concept that IL-17A favors the development of colorectal cancer. Mice that are genetically predisposed to develop tumors in the intestine ($APC^{min/+}$) show a drastic impairment in intestinal tumorigenesis when crossed with IL-17A-deficient mice (Chae et al., 2010). Additionally, it has been shown that $APC^{min/+}$

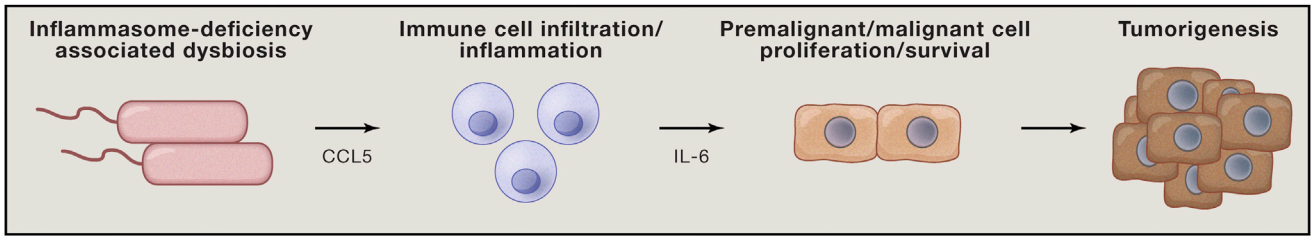


**Figure 2. Dysbiosis Associated with Inflammasome Deficiency Can Drive Inflammation-Induced Colorectal Cancer**
The altered elements in the microbiota induce local colonic inflammation through epithelial reprogramming and induction of CCL5 transcription. This, in turn, results in local induction of IL-6 secretion and resultant proliferative signaling on intestinal epithelial cells, culminating in tumor formation.

mice that cannot respond to IL-17 develop fewer tumors in the colon (Grivennikov et al., 2012).

Th17 cells produce other cytokines besides IL-17, such as IL-22. IL-22 is a cytokine of the IL-10 family, which has been linked to intestinal tumorigenesis in murine studies (Kirchberger et al., 2013; Huber et al., 2012b). In addition, studies have linked IL-22 to human colon cancer (Jiang et al., 2013) and, in particular, a chemo-resistant state of colorectal cancer (Wu et al., 2013).

Myeloid cells produce the cytokine IL-23 in response to molecules from the microbiota, such as flagellin (Kinnebrew et al., 2012). This, in turn, promotes the expression of the cytokines discussed above—IL-17A and IL-22—by Th17 cells (Huber et al., 2012a). IL-23 is highly expressed in human colon cancer samples compared to adjacent normal tissue (Grivennikov et al., 2012; Langowski et al., 2006). It should be noted also that innate lymphoid cells 3 (ILC3) are an important innate source of IL-17 and IL-22 and a target of IL-23 (Figure 4) (Walker et al., 2013). Accordingly, it has been recently shown that ILC3 can sustain colon cancer through the production of IL-22 (Kirchberger et al., 2013).

Importantly, germ-free mice lack Th17 cells, suggesting that endogenous microbiota are critical for the induction of this type of cell. It has been shown that commensal bacteria can produce and release adenosine 5′-triphospahate (ATP), which by stimulating lamina propria dendritic cells (DC) to produce IL-6, TGF-β, and IL-23, induces Th17 cell development (Atarashi et al., 2008). Strikingly, the colonization of germ-free mice with a specific member of commensal microbiota, the segmented filamentous bacteria (SFB), promotes the induction of Th17 cells (Ivanov and Honda, 2012). The presence of SFB in the small intestine promotes the induction/expansion of Th17 cells via their stimulation of production of serum amyloid A, which, in turn, promotes the differentiation of Th17 cells (Ivanov et al., 2009). However, whether SFB can promote tumor growth through Th17 cells remains to be evaluated.

More direct evidence for a role of bacterially stimulated tumor growth via Th17 cells comes from studies of enterotoxigenic *Bacteroides fragilis*. This bacterium secretes *B. fragilis* toxin (BFT) and causes human inflammatory diarrhea. Mice that are predisposed to develop tumors in the intestine, when colonized with this specific subgroup of *B. fragilis*, have a remarkable Th17 cell tumor infiltration that results in an increased tumor growth compared to noncolonized mice (Wu et al., 2009).

Overall these data support the role of Th17 cells and their cytokines, IL-17 and IL-22, in intestinal carcinogenesis. If Th17 cells are overstimulated by a specific component of the microbiota, such as SFB and flagellin-positive bacteria, they can promote a state of chronic inflammation mediated by the release of IL-17 and IL-22, which likely favors the development of intestinal tumor growth (Figure 4).

An opposite and surprising microbiota-mediated effect was recently noted with respect to the effect of the Th17 response on a variety of solid and hematopoietic tumors. Interestingly, this Th17 response was causally linked to microbial regulation of chemotherapeutic treatment. Treatment with the alkylating agent cyclophosphamide induced dysbiosis and enhanced intestinal penetration of Gram-positive commensal bacteria that resulted in a potent tumor suppressive Th17 response (Viaud et al., 2013). Likewise, a microbiota-mediated antitumorigenic effect mediated through regulation of treatment was recently noted in mice administered with an innate immune therapy consisting of CpG oligodeoxyribonucleotides and anti-IL-10 receptor antibodies, affecting tumor infiltrating myeloid cells (Iida et al., 2013). In antibiotic-treated or germ-free mice, myeloid cell response to this combined immunotherapy was impaired, leading to enhanced tumor growth and establishing the microbiota as an important factor determining chemotherapeutic responsiveness.

Tumor-suppressive Th17 cell subsets can therefore mediate opposing chemotherapy-induced effects through alteration of the gut microbiota, resulting in a net antitumorigenic response. It is plausible that integration of such multiple signals through their cumulative effects on specific commensal microbial populations and the various immune arms will result in context-specific effects on tumor growth, establishing the microbiota as a critical hub integrating host and environmental signals participating in tumor formation or suppression.

### *Anti-Inflammatory Control Mechanisms*

Th17 cells and their cytokine production can be kept in check by regulatory T cells (Tregs) that produce the anti-inflammatory cytokine IL-10 and dendritic cells that secrete IL-22-binding protein (IL-22BP). Thus, although some bacteria are able to induce Th17 cells, we favor the idea that others could actually promote control of Th17 cells and thereby limit carcinogenesis. For example, *Lactobacillus*, *Bifidobacteria*, and *Clostridium genera* induce a specific subset of regulatory T cells in the intestine called Foxp3$^+$ Treg cells (Honda and Littman, 2012). These Foxp3$^+$ Treg cells, through the secretion of IL-10, can control

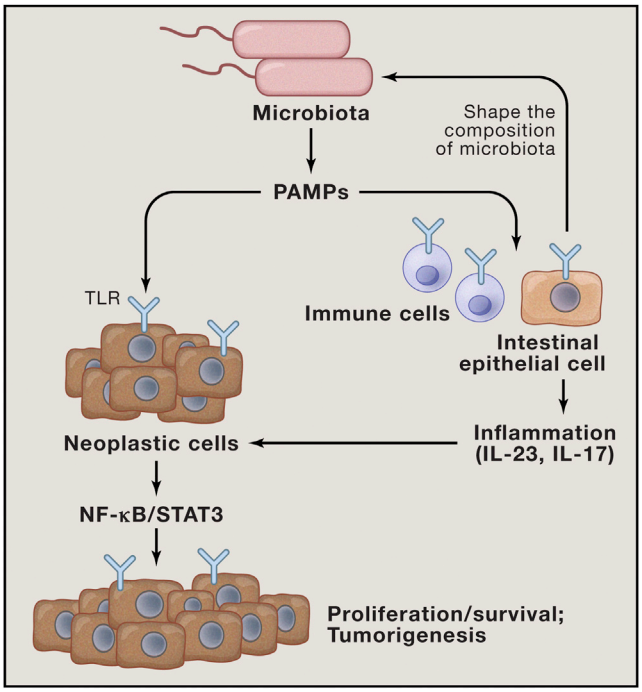


**Figure 3. Microbes, TLRs, and Tumorigenesis**
Microbial PAMPs activate TLR signaling in a variety of cell types, leading to cytokine production and NF-κB-mediated inflammation, which can fuel tumor growth. These events can also alter the gut microbiota such that it can feed back to TLR signaling augmenting inflammation.

the production of IL-17A and the proliferation of Th17 cells (Figure 4) (Huber et al., 2011). We hypothesize that these interactions can have antitumorigenic activity in the intestine. In support of this notion, it is known that Foxp3$^+$ Treg cells can actively block intestinal tumor growth through the production of IL-10 (Erdman et al., 2003; Erdman et al., 2005). Whether this is due to the suppression of Th17 cell protumorigenic activity remains to be determined.

*Bacteroides fragilis* releases polysaccharide A (PSA) and blocks intestinal inflammation. IL-10 and type 1 regulatory T (Tr1) cells, a distinct subset of regulatory T cells characterized by the secretion of high concentration of IL-10 and surface expression of CD49b and LAG-3, are required for this beneficial effect (Mazmanian et al., 2008). Conversely, IL-10-deficient mice develop chronic inflammation that is exacerbated when the mice are colonized by *Helicobacter hepaticus*. *H. hepaticus* not only promotes inflammation in the colon, but is also associated with a high incidence of tumor development in these mice (Fox et al., 2011). However the hypothesis that Tr1 cells and their secreted IL-10 can have antitumorigenic capacity via suppressing Th17 cells remains to be tested. Of note, *Bifidobacterium longum*, one of the commensal bacteria in mice, induces moderate levels of Tr1 cells, and feeding mice with *Bifidobacterium breve*, which is normally not present in the intestine of mice, strongly promotes the accumulation of Tr1 cells in the colon (Jeon et al., 2012). Interestingly, cancer patients have a significantly reduced population of *Bifidobacterium longum* compared to patients with inflammatory bowel disease (Gueimonde et al., 2007).

Recently, a selected mixture of Clostridia strains was identified from the human microbiota that is able to attenuate disease in preclinical models of colitis through the induction of IL-10$^+$ regulatory T cells (Atarashi et al., 2013). These data may reveal a potential antitumorigenic role of a certain component of the microbiota and suggest that therapeutic colonization with specific strains of human-associated bacteria may have the potential to reduce tumorigenesis, but definitive preclinical and clinical data are still missing.

DC present in the intestine continuously release a soluble IL-22 receptor, IL-22-binding protein (IL-22BP, IL-22Ra2). IL-22BP binds to IL-22 and limits its bioavailability by prohibiting the binding of IL-22 to the membrane-bound IL-22 receptor (IL-22R1). Mice that are deficient for IL-22BP show increased IL-22-mediated tumorigenesis in colitis-associated and spontaneous colon cancer models. The integrity of the intestinal epithelial cell barrier is required for high IL-22BP expression in the intestine (Huber et al., 2012b). Conversely, tissue damage and bacteria infiltration lead to downregulation of IL-22BP. This mechanism is controlled by inflammasomes (NRLP3/6) that promote the release of IL-18, which, in turn, downregulates IL-22BP expression by DC (Huber et al., 2012b). On the other hand, bacterial components can also stimulate DC to produce IL-23 (Kinnebrew et al., 2012), which induces IL-22 production by ILC3 (Huber et al., 2012a; Kirchberger et al., 2013). IL-22 then favors wound healing and limits bacteria-driven inflammation (Hanash et al., 2012; Sonnenberg et al., 2011). Of note, IL-22BP is re-expressed during the wound recovery phase, thereby dampening the IL-22-induced regenerative program, which, if uncontrolled, can promote tumor formation (Huber et al., 2012b) (Figure 4).

## Alteration of the Human Intestinal Microbiota as a Therapeutic Approach?

It is largely accepted that patients with chronic inflammation in the intestine have a higher propensity to develop intestinal tumors. The data reviewed here suggest that chronic inflammation in the intestine could be, at times, the result of a pathogenic interaction of the microbiota with multiple components of the immune system. We also interpret the data reviewed in the previous section to indicate that the microbiota can be leveraged to limit inflammation and thereby mitigate cancer risk. Thus, the possibility of retraining tumor progression by controlling intestinal microbiota in patients seems logical to envision. Below, we highlight evidence that the human microbiota can be therapeutically manipulated to control intestinal inflammation. However, an effect on tumor development has not been established.

Strategies to manipulate the microbiota therapeutically include oral administration of certain bacteria strains ("probiotics"), dietary interventions to alter microbiota composition ("prebiotics"), and fecal transplantation. Examples of such interventions include the administration of *Escherichia coli* Nissle 1917, a bacterium that was isolated from the stool of a World War I soldier who, in contrast to his comrades, did not suffer from diarrhea. This bacterium was suggested to be an efficient therapy in patients suffering from ulcerative colitis (Kruis et al., 2004). Dietary interventions have been shown to be efficient in reducing the inflammatory response in the *IL-10*-deficient mice, which spontaneously develops colitis (Devkota et al.,

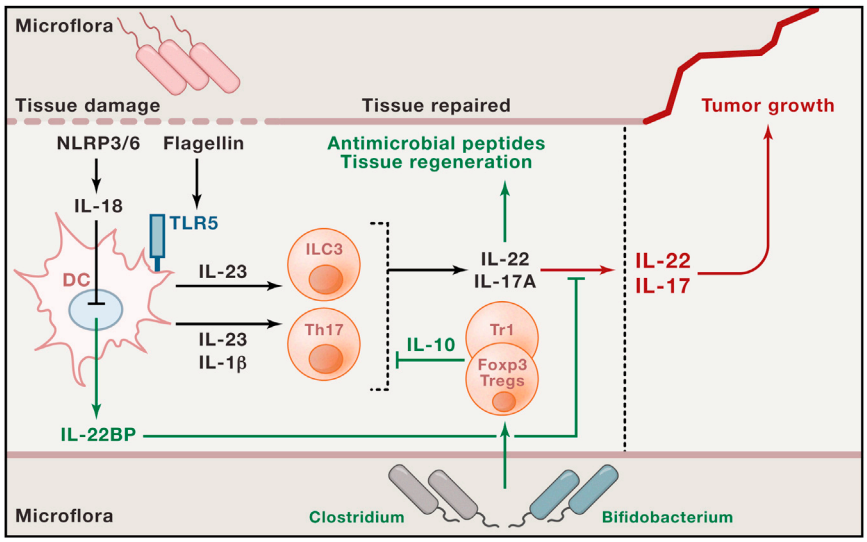


**Figure 4. Interplay between Microbes, Proinflammatory, and Anti-Inflammatory Immune Cells and Cytokines that May Influence Tumor Growth**

Upon tissue damage and bacterial invasion, NLRP3/NLRP6 activate IL-18, which downregulates IL-22BP production by intestinal DC. Moreover, when DC sense bacterial components via TLR5, they produce IL-23. This cytokine acts on ILCs and, together with IL1β on Th17 cells, promotes the production of IL-17A and IL-22. Adequate levels of these two cytokine promote antimicrobial peptide secretion and tissue regeneration; their levels are kept in check by IL-22BP and IL-10 from regulatory T cells, which, in turn, may be induced by specific commensal bacteria. If IL-17A and IL-22 are not controlled, they can promote tumorigenesis.

2012). Finally, fecal transplantation showed efficacy in treating patients suffering from chronic *Clostridium difficile* infection (van Nood et al., 2013). Similar approaches are currently being tested in patients with inflammatory bowel disease. However, the results of the first small studies are conflicting: whereas fecal transplantation was shown to be efficient in children and young adults with ulcerative colitis (Kunde et al., 2013), another study did not show efficacy in patients with chronic active ulcerative colitis (Kump et al., 2013). Larger studies will be essential to clarify this point.

## Concluding Remarks and Future Perspectives

The immune system is able to trigger and sustain tumor cell growth. The microbiota shapes the immune system and thereby can indirectly modulate the development of tumors through the immune system. We believe that developing methods to selectively manipulate components of the microbiota and ultimately target tumor initiation and progression represents a complex yet exciting challenge in the field. Although the intestinal microbiota is mainly localized to the gut and was originally suggested to display regulatory functions within the intestinal mucosa, recent evidence indicates that functional microbiota alterations may also have systemic effects impacting other parts of the body (Henao-Mejia et al., 2012). As such, we hypothesize that the microbiota and its interactions with the host could also be important in tumorigenesis in other organs. This approach requires first the identification of the relevant bacteria in humans, their altered interactions with the human immune system, and their ultimate effects on tumor initiation and progression. Second, we need to identify methods to selectively manipulate the microbiota. Such modalities could represent novel therapeutic interventions to be coupled with conventional treatments for the therapy of tumors and other multifactorial human diseases.

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## REFERENCES

Allen, I.C., TeKippe, E.M., Woodford, R.M., Uronis, J.M., Holl, E.K., Rogers, A.B., Herfarth, H.H., Jobin, C., and Ting, J.P. (2010). The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J. Exp. Med. *207*, 1045–1056.

Anand, P.K., and Kanneganti, T.D. (2013). NLRP6 in infection and inflammation. Microbes Infect. *15*, 661–668.

Anand, V.S., Kavitha, C., and Subbarao, C.V. (2011). Effect of Cavity Design on the Strength of Direct Posterior Composite Restorations: An Empirical and FEM Analysis. Int. J. Dent. *2011*, 214751.

Arthur, J.C., Perez-Chanona, E., Mühlbauer, M., Tomkovich, S., Uronis, J.M., Fan, T.J., Campbell, B.J., Abujamel, T., Dogan, B., Rogers, A.B., et al. (2012). Intestinal inflammation targets cancer-inducing activity of the microbiota. Science *338*, 120–123.

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K., and Takeda, K. (2008). ATP drives lamina propria T(H)17 cell differentiation. Nature *455*, 808–812.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature *500*, 232–236.

Chae, W.J., Gibson, T.F., Zelterman, D., Hao, L., Henegariu, O., and Bothwell, A.L. (2010). Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. Proc. Natl. Acad. Sci. USA *107*, 5540–5544.

Chen, G.Y., Liu, M., Wang, F., Bertin, J., and Núñez, G. (2011). A functional role for Nlrp6 in intestinal inflammation and tumorigenesis. J. Immunol. *186*, 7187–7194.

Couturier-Maillard, A., Secher, T., Rehman, A., Normand, S., De Arcangelis, A., Haesler, R., Huot, L., Grandjean, T., Bressenot, A., Delanoye-Crespin, A., et al. (2013). NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. J. Clin. Invest. *123*, 700–711.

Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B., and Chang, E.B. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in $Il10^{-/-}$ mice. Nature *487*, 104–108.

Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., Peaper, D.R., Bertin, J., Eisenbarth, S.C., Gordon, J.I., and Flavell, R.A. (2011). NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell *145*, 745–757.

Erdman, S.E., Rao, V.P., Poutahidis, T., Ihrig, M.M., Ge, Z., Feng, Y., Tomczak, M., Rogers, A.B., Horwitz, B.H., and Fox, J.G. (2003). CD4(+)CD25(+) regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. Cancer Res. *63*, 6042–6050.

Erdman, S.E., Sohn, J.J., Rao, V.P., Nambiar, P.R., Ge, Z., Fox, J.G., and Schauer, D.B. (2005). CD4+CD25+ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice. Cancer Res. *65*, 3998–4004.

Fox, J.G., Ge, Z., Whary, M.T., Erdman, S.E., and Horwitz, B.H. (2011). Helicobacter hepaticus infection in mice: models for understanding lower bowel inflammation and cancer. Mucosal Immunol. *4*, 22–30.

Goodwin, A.C., Destefano Shields, C.E., Wu, S., Huso, D.L., Wu, X., Murray-Stewart, T.R., Hacker-Prietz, A., Rabizadeh, S., Woster, P.M., Sears, C.L., and Casero, R.A., Jr. (2011). Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc. Natl. Acad. Sci. USA *108*, 15354–15359.

Grivennikov, S.I., Wang, K., Mucida, D., Stewart, C.A., Schnabl, B., Jauch, D., Taniguchi, K., Yu, G.Y., Osterreicher, C.H., Hung, K.E., et al. (2012). Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature *491*, 254–258.

Gueimonde, M., Ouwehand, A., Huhtinen, H., Salminen, E., and Salminen, S. (2007). Qualitative and quantitative analyses of the bifidobacterial microbiota in the colonic mucosa of patients with colorectal cancer, diverticulitis and inflammatory bowel disease. World J. Gastroenterol. *13*, 3985–3989.

Hanash, A.M., Dudakov, J.A., Hua, G., O'Connor, M.H., Young, L.F., Singer, N.V., West, M.L., Jenq, R.R., Holland, A.M., Kappel, L.W., et al. (2012). Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity *37*, 339–350.

Henao-Mejia, J., Elinav, E., Strowig, T., and Flavell, R.A. (2012). Inflammasomes: far beyond inflammation. Nat. Immunol. *13*, 321–324.

Honda, K., and Littman, D.R. (2012). The microbiome in infectious disease and inflammation. Annu. Rev. Immunol. *30*, 759–795.

Hu, B., Elinav, E., Huber, S., Booth, C.J., Strowig, T., Jin, C., Eisenbarth, S.C., and Flavell, R.A. (2010). Inflammation-induced tumorigenesis in the colon is regulated by caspase-1 and NLRC4. Proc. Natl. Acad. Sci. USA *107*, 21635–21640.

Hu, B., Elinav, E., Huber, S., Strowig, T., Hao, L., Hafemann, A., Jin, C., Wunderlich, C., Wunderlich, T., Eisenbarth, S.C., and Flavell, R.A. (2013). Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. Proc. Natl. Acad. Sci. USA *110*, 9862–9867.

Huber, S., Gagliani, N., Esplugues, E., O'Connor, W., Jr., Huber, F.J., Chaudhry, A., Kamanaka, M., Kobayashi, Y., Booth, C.J., Rudensky, A.Y., et al. (2011). Th17 cells express interleukin-10 receptor and are controlled by $Foxp3^{-}$ and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. Immunity *34*, 554–565.

Huber, S., Gagliani, N., and Flavell, R.A. (2012a). Life, death, and miracles: Th17 cells in the intestine. Eur. J. Immunol. *42*, 2238–2245.

Huber, S., Gagliani, N., Zenewicz, L.A., Huber, F.J., Bosurgi, L., Hu, B., Hedl, M., Zhang, W., O'Connor, W., Jr., Murphy, A.J., et al. (2012b). IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature *491*, 259–263.

Iida, N., Dzutsev, A., Stewart, C.A., Smith, L., Bouladoux, N., Weingarten, R.A., Molina, D.A., Salcedo, R., Back, T., Cramer, S., et al. (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science *342*, 967–970.

Ivanov, I.I., and Honda, K. (2012). Intestinal commensal microbes as immune modulators. Cell Host Microbe *12*, 496–508.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Jeon, S.G., Kayama, H., Ueda, Y., Takahashi, T., Asahara, T., Tsuji, H., Tsuji, N.M., Kiyono, H., Ma, J.S., Kusu, T., et al. (2012). Probiotic Bifidobacterium breve induces IL-10-producing Tr1 cells in the colon. PLoS Pathog. *8*, e1002714.

Jiang, R., Wang, H., Deng, L., Hou, J., Shi, R., Yao, M., Gao, Y., Yao, A., Wang, X., Yu, L., and Sun, B. (2013). IL-22 is related to development of human colon cancer by activation of STAT3. BMC Cancer *13*, 59.

Jin, C., and Flavell, R.A. (2013). Innate sensors of pathogen and stress: linking inflammation to obesity. J. Allergy Clin. Immunol. *132*, 287–294.

Kinnebrew, M.A., Buffie, C.G., Diehl, G.E., Zenewicz, L.A., Leiner, I., Hohl, T.M., Flavell, R.A., Littman, D.R., and Pamer, E.G. (2012). Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. Immunity *36*, 276–287.

Kirchberger, S., Royston, D.J., Boulard, O., Thornton, E., Franchini, F., Szabady, R.L., Harrison, O., and Powrie, F. (2013). Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. J. Exp. Med. *210*, 917–931.

Kostic, A.D., Chun, E., Robertson, L., Glickman, J.N., Gallini, C.A., Michaud, M., Clancy, T.E., Chung, D.C., Lochhead, P., Hold, G.L., et al. (2013). Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe *14*, 207–215.

Kruis, W., Fric, P., Pokrotnieks, J., Lukás, M., Fixa, B., Kascák, M., Kamm, M.A., Weismueller, J., Beglinger, C., Stolte, M., et al. (2004). Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut *53*, 1617–1623.

Kump, P.K., Gröchenig, H.P., Lackner, S., Trajanoski, S., Reicht, G., Hoffmann, K.M., Deutschmann, A., Wenzl, H.H., Petritsch, W., Krejs, G.J., et al. (2013). Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. Inflamm. Bowel Dis. *19*, 2155–2165.

Kunde, S., Pham, A., Bonczyk, S., Crumb, T., Duba, M., Conrad, H., Jr., Cloney, D., and Kugathasan, S. (2013). Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. J. Pediatr. Gastroenterol. Nutr. *56*, 597–601.

Langowski, J.L., Zhang, X., Wu, L., Mattson, J.D., Chen, T., Smith, K., Basham, B., McClanahan, T., Kastelein, R.A., and Oft, M. (2006). IL-23 promotes tumour incidence and growth. Nature *442*, 461–465.

Mazmanian, S.K., Round, J.L., and Kasper, D.L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. Nature *453*, 620–625.

Medzhitov, R. (2001). Toll-like receptors and innate immunity. Nat. Rev. Immunol. *1*, 135–145.

Muranski, P., Boni, A., Antony, P.A., Cassard, L., Irvine, K.R., Kaiser, A., Paulos, C.M., Palmer, D.C., Touloukian, C.E., Ptak, K., et al. (2008). Tumor-specific Th17-polarized cells eradicate large established melanoma. Blood *112*, 362–373.

Normand, S., Delanoye-Crespin, A., Bressenot, A., Huot, L., Grandjean, T., Peyrin-Biroulet, L., Lemoine, Y., Hot, D., and Chamaillard, M. (2011). Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. Proc. Natl. Acad. Sci. USA *108*, 9601–9606.

Palm, N.W., and Medzhitov, R. (2009). Pattern recognition receptors and control of adaptive immunity. Immunol. Rev. *227*, 221–233.

Rakoff-Nahoum, S., and Medzhitov, R. (2009). Toll-like receptors and cancer. Nat. Rev. Cancer *9*, 57–63.

Reddy, B.S., Narisawa, T., Maronpot, R., Weisburger, J.H., and Wynder, E.L. (1975). Animal models for the study of dietary factors and cancer of the large bowel. Cancer Res. *35*, 3421–3426.

Rubinstein, M.R., Wang, X., Liu, W., Hao, Y., Cai, G., and Han, Y.W. (2013). Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe *14*, 195–206.

Salcedo, R., Worschech, A., Cardone, M., Jones, Y., Gyulai, Z., Dai, R.M., Wang, E., Ma, W., Haines, D., O'hUigin, C., et al. (2010). MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J. Exp. Med. *207*, 1625–1636.

Sobhani, I., Tap, J., Roudot-Thoraval, F., Roperch, J.P., Letulle, S., Langella, P., Corthier, G., Tran Van Nhieu, J., and Furet, J.P. (2011). Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS ONE *6*, e16393.

Sonnenberg, G.F., Fouser, L.A., and Artis, D. (2011). Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat. Immunol. *12*, 383–390.

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., Visser, C.E., Kuijper, E.J., Bartelsman, J.F., Tijssen, J.G., et al. (2013). Duodenal infusion of donor feces for recurrent Clostridium difficile. N. Engl. J. Med. *368*, 407–415.

Vannucci, L., Stepankova, R., Kozakova, H., Fiserova, A., Rossmann, P., and Tlaskalova-Hogenova, H. (2008). Colorectal carcinogenesis in germ-free and conventionally reared rats: different intestinal environments affect the systemic immunity. Int. J. Oncol. *32*, 609–617.

Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D., Enot, D.P., Pfirschke, C., Engblom, C., Pittet, M.J., et al. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science *342*, 971–976.

Walker, J.A., Barlow, J.L., and McKenzie, A.N. (2013). Innate lymphoid cells—how did we miss them? Nat. Rev. Immunol. *13*, 75–87.

Wu, S., Rhee, K.J., Albesiano, E., Rabizadeh, S., Wu, X., Yen, H.R., Huso, D.L., Brancati, F.L., Wick, E., McAllister, F., et al. (2009). A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat. Med. *15*, 1016–1022.

Wu, T., Cui, L., Liang, Z., Liu, C., Liu, Y., and Li, J. (2013). Elevated serum IL-22 levels correlate with chemoresistant condition of colorectal cancer. Clin. Immunol. *147*, 38–39.

Yoshimoto, S., Loo, T.M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., Iwakura, Y., Oshima, K., Morita, H., Hattori, M., et al. (2013). Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature *499*, 97–101.

Zaki, M.H., Boyd, K.L., Vogel, P., Kastan, M.B., Lamkanfi, M., and Kanneganti, T.D. (2010). The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity *32*, 379–391.