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Permissive/Protective Interplay of Microbiota with T Cell Adaptive Immune Response in Colon Cancer

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Abstract

Colon microbiota, as a complex and diverse population, has been shown to be either pro- or anti-tumorigenic, depending on its content. The composition of microbiota critically determines the differentiation, activation, and expansion of T cells by which pro- or anti-tumorigenic effects of microbes are frequently reported to be mediated. In this review study, we specified an imbalance in microbiota and T cells in particular regulatory T cells and Th17 cells in colon cancer. We also aimed to discuss evidence, suggesting the contribution of microbiota to carcinogenesis or anti-carcinogenesis through influencing T cells.

Keywords: Colon Cancer, Microbiota, T Cells, Tregs, Th17

1. Introduction

The immune system can be divided into innate and adaptive immunity (1, 2). Innate immunity refers to defense mechanisms, acting non-specifically after the initial appearance of an antigen in the body. These mechanisms consist of physical barriers (e.g., skin and mucosal surfaces), chemicals, and innate immune system cells, including neutrophils, eosinophils, basophils, mast cells, macrophages, dendritic cells, and natural killer cells (1, 2).

Adaptive immunity consists of two arms, i.e., humoral immunity comprised of B cells producing antibodies and T cell-mediated immunity. T cells are divided into two broad subsets: cytotoxic CD8+ T lymphocytes and CD4+ T helper (Th) cells with two main Th1 and Th2 cell subgroups (1, 2). Th1 cells produce interleukin (IL)-2, gamma-interferon (IFNγ), and tumor necrosis factor-alpha (TNF-α). They also express signal transducer and activator of transcription 4 (Stat4), Stat1, and T-box transcription factor.

CD4+ Th1 cells are part of a type-1 immune response and are involved in priming and expanding cytotoxic CD8+ T cells. A Th1 immune response is mainly related to immunity against intracellular microbes and cancer. On the other hand, CD4+ T cells, differentiating into Th2 cells, express GATA3 and Stat6 and produce more IL-4, IL-5, and IL-13. In addition, CD4+ Th2 cells are part of a type-2 immune response, i.e., a type of immunity which is mainly involved in the removal of helminthes and extracellular parasites and facilitates B-cell antibody secretion (3-5).

Recent studies have progressively revealed the role of recently recognized CD4+ subsets, i.e., Th17 cells and T regulatory cells (Tregs), in immunity, particularly at mucosal surfaces where large and diverse numbers of microbes (also known as microbiomes) reside (5, 6). Th17 cells, as an inflammatory subset of CD4+ cells, are the main source of IL-17A, IL-17F, and IL-22 (7). These cells are most frequently found in the gastrointestinal tract, especially in the intestinal lamina propria. Tregs play a critical role in the maintenance of immunological self-tolerance and immune homeostasis at sites of inflammation, especially mucosal surfaces (8, 9). TGF-β and IL-2 are two crucial cytokines, which are involved in the differentiation of naïve T cells from Treg cells. Tregs express fork-head lineage-specific transcription factor (FoxP3) proteins and are able to secrete immunomodulatory cytokine, IL-10 (10). In addition to an imbalance in Th1/Th2 cells, a dysregulated immune response, related to Th17 and FoxP3+ Tregs, has been linked to certain types of cancer, particularly colon cancer (3-5).

Colon carcinoma is among the leading causes of cancer-related death throughout the world, with a rising incidence rate in recent years (11). Colon is the site where the greatest density and number of microbes can be found. Colon cancer may be one of the major types of cancer regarding the interplay between the immune system and microbes, as it develops in the presence of gut flora, as well as myeloid and lymphoid cells, which induce and produce pro-inflammatory cytokines (12).

It is well documented that microbes play a pivotal role in both cancer formation/progression and/or prevention/regression, depending on the host model, microenvironment, and infectious elements (12-14). As well rec-

ognized in previous reviews, contribution of microbiota to cancer could be mediated through a variety of mechanisms, such as chronic inflammation, innate immunity (e.g., macrophages and dendritic cells), and DNA damage (6, 15). One of these mechanisms, which has been less reviewed, is the interplay between T-cell adaptive immune responses and microbiota. In this review study, we specified T cells and their effector subsets, as well as microbiota in the colon. We also aimed to discuss evidence on how microbiota contributes to carcinogenesis or anti-carcinogenesis through influencing T cells.

2. T Cells and Microbiota in the Colon

Body surfaces, e.g., intestinal and respiratory mucosa and skin, are colonized by a vast number of microorganisms (either helpful or pathogenic), which represent the so-called microbiota. Five dominant species of intestinal microbiota in normal adults are *Firmicutes, Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (6, 15, 16). However, intestinal microbiota can be influenced by a variety of factors, such as diet (17, 18), acute or chronic inflammation, and antibiotics (19-21).

Approximately 70% of the human microbiota is composed of bacteria which cannot be cultivated by current microbiological methods, and only genomic next-generation sequencing analysis has been successfully employed in their characterization (17, 22). According to the literature, microbiota has a substantial influence on the host's immune responses and differentiation of various types of T cells (23).

It has been previously shown that the number of T cells remarkably decreases in germ-free mice (mice without intestinal microbiota), suggesting that T cells accumulate following recruitment by bacterial materials (23-25). The specificity of these cells is not known at present, although it seems that many of them are probably exclusive to commensal microbiota and the products to prevent damage caused by pathogens or toxins (22).

Based on recent studies, the content of microbiota substantially shapes the balance of Treg/Th17 cells (26). A notable microorganism in human colonic microbiota is *Bacteroides fragilis*, which has been linked to the development of FoxP3+ Tregs and suppression of inflammation in the gut. According to the literature, monocolonization of germ-free animals with this bacterium significantly enhances the number and suppressive activity of Tregs, thus inducing the production of IL-10 while decreasing Th17 responses (27). Further analysis indicated that polysaccharide A (PSA), an immunomodulatory molecule of *Bacteroides fragilis*, is the component responsible for the augmentation of Foxp3+ Tregs and production of IL-10. Interestingly, researchers showed that PSA is not only able to prevent, but also treat experimental colitis in animals (27, 28).

There are some pathogenic bacteria which favor the induction of Th17 cells rather than Tregs. For instance, Th17 cells become abundant in the colon of mice upon colonization with some specific bacteria or their products, especially segmented filamentous bacterium (SFB), which is a component of *Clostridia*-related species, and flagellin-positive bacteria. The difference in IL-17 and IL-22 production has been only observed in Th17 cells, not IL-17 or IL-22, which produce innate immune cells; this suggests the specific effect of SFB on the differentiation and/or recruitment of Th17 cells (29-31).

It has been also shown that Th17 response is important for protection against mucosal pathogens, such as *Klebsiella pneumonia* (32), *Salmonella typhimurium* (33), and *Citrobacter rodentium* (29). Mice deficient in Th17 cells show serious pathology during infection with these pathogens and demonstrate increased translocation of bacteria into lymph nodes (29).

3. Microbiota in Favor of Tumorigenesis

There are not many well-documented reports to confirm the association between a single microbe and cancer. Nevertheless, some studies have revealed the relationship between gastric cancer and *Helicobacter pylori* (34), cervical cancer and human papillomavirus (35), bladder cancer and *Schistosoma haematobium* (36), bile duct cancer and *Clonorchis sinensis* and *Opisthorchis viverrini* (37), and finally liver cancer and hepatitis B and C viruses (38, 39).

There is a general consensus regarding the contribution of multiple members of colonic bacterial/microbial community to the induction of tumor formation and progression in colon cancer (40-42). In general, contribution of microbiota to tumorigenesis has been demonstrated in a number of colon cancer animal models in a germ-free environment. Also, its influence on treatment with broad-spectrum antibiotics for the elimination of all microorganisms in the gut has been revealed.

Vannucci et al. investigated tumor development and growth in a rat model of colorectal cancer in a germ-free environment and compared the results with a similar rat model under regular conditions and normal colonic microbiota. As the results indicated, cancer developed in 50% of germ-free mice, while its incidence was approximately 80% in mice kept under regular conditions (43).

The higher risk of cancer in germ-free colon cancer models compared to their normal microbiota counterparts has been illustrated in several studies in both genetically modified and chemically induced colorectal can-

cers (44, 45). Depletion of all gut microorganisms by broad-spectrum antibiotics in animal models predisposed to colon cancer is another example of experiments, analyzing the association between whole microbiota and colon cancer. In general, antibiotic treatment of mice significantly decreases tumor formation and progression (46, 47).

There are studies on colon cancer linking a single bacterium, e.g. *Fusobacterium nucleatum*, to colon cancer (48, 49). Mima et al. measured the amount of this bacterium in colorectal carcinoma tissues via quantitative polymerase chain reaction assay. They found that *Fusobacterium nucleatum* was positive in 13% of tumor tissues and 3.4% of adjacent non-tumor tissues (49). Other investigations have attempted to find a relationship between a group of bacteria and colon cancer rather than microbiota as a whole or single bacterium. Sears and Pardoll first proposed the "alpha-bug hypothesis", which integrates the single microbe and microbiome community views of microbial carcinogenesis (40). This hypothesis suggests that certain microbiota members, such as enterotoxigenic *Bacteroides fragilis*, *Streptococcus gallolyticus* (also known as *Streptococcus bovis*), superoxide-producing *Enterococcus faecalis*, and *Escherichia coli*, can be pro-tumorigenic (40).

There are plenty of mechanisms which link microbiota to tumorigenesis, including DNA damage and genome instability, alterations of mucosal permeability (facilitating the translocation of bacteria or their toxins, e.g., lipopolysaccharides), replacement of anti-cancer colonic bacteria with pathogenic ones, and deviation of the immune response (both innate and adaptive immunity) to a pro-tumorigenic one (6, 40, 47-49).

3.1. Microbiota in Favor of Tumorigenesis Through T Cells

An aberrant immune response against intestinal flora antigens is thought to be associated with several pathological conditions of the colon, ranging from inflammatory bowel diseases, such as Crohn's disease, to cancer (23, 50, 51). Emerging evidence suggests that in the majority of colon cancer cases, the cause of aberrant immune response is intestinal flora or an antigen rather than a primary defect in the immune system (12, 50).

An effective anti-tumor immune response is generated by both CD4+ and CD8+ T cells and their interactions; also, CD4+ Th1 cells induce tumor-specific CD8+ T cells (52-54). As the study of human colon cancer samples has confirmed, Th1 adaptive immunity and presence of tumor-specific cytotoxic CD8+ cells are associated with better clinical outcomes and less tumor recurrence (55, 56). On the other hand, experimental animal models have presented firm evidence indicating the influence of microbiota on tumor-specific CD8+ T cell responses (46).

Additionally, Bhattacharya et al. investigated the mentioned association in a mouse model of colon cancer with deficiency in colonic all-trans-retinoic acid. The authors showed that all-trans-retinoic acid supplementation could reduce the tumor burden in these mice. According to the findings, the advantage of all-trans-retinoic acid treatment was mediated by cytotoxic CD8+ T cells, which were activated due to major histocompatibility complex class-I upregulation (CD8 ligands) by all-trans-retinoic acid on tumor cells (46).

Consistent with the abovementioned findings, increased colonic expression of all-trans-retinoic-acid-catabolizing enzyme, CYP26A1, was found to be correlated with the reduced frequency of tumoral cytotoxic CD8+ T cells and poor disease prognosis. Researchers finally illustrated that pretreatment with broad-spectrum antibiotics, which deplete the whole microbiota, completely prevents all-trans-retinoic acids in this mouse model. Interestingly, human colon cancer specimens have also shown deficiency in all-trans-retinoic acid, similar to the animal model (46). Nevertheless, further investigation is required to determine if this deficiency in human colon cancer is related to microbiome and whether it can be reversed by all-trans-retinoic acid supplementation towards a better immune response and prognosis.

The classical Th1/Th2 immune response pattern has been challenged by the identification of Tregs and Th17 cells, giving way to a new era presenting the additional involvement of CD4+ T cells in anti-tumor immunity (57, 58). According to the literature, in most types of cancer, Treg cells increase in the peripheral blood, accumulate in lymph nodes and tumor tissues, and take part in immunosuppression and inhibition of efficient anti-tumor immunity (59-61). However, regarding colon cancer, more data is available supporting an opposite hypothesis which considers a protective role for Tregs. In fact, it has been suggested that Tregs prevent cancer by inhibiting bacteria-driven inflammation (62, 63).

Enrichment of Th17 cells in the peripheral blood and tumor samples from colon cancer patients has been confirmed in more recent studies (7). In other types of cancer, association of Th17 cells with tumor immunity remains controversial, as both pro-tumor and anti-tumor effects have been reported (59, 60, 64, 65). The stronger contribution of increased Th17 cells and decreased Tregs to colon cancer compared to other cancer types again confirms the inflammatory cause of colon cancer.

Certain microbes could induce Th17 response and suppress Treg cells; through this mechanism, these microbes might be pro-tumorigenic in colon cancer. Stimulation of tumor growth by bacteria is clearly linked to Th17 cells, based on the study of enterotoxigenic *Bacteroides fragilis*, a

causative microbe for human inflammatory diarrhea; this pathogenic bacterium secretes *B. fragilis* toxin. Also, animal models have linked colonization with enterotoxigenic *B. fragilis* to colon cancer, but not non-toxigenic *B. fragilis* (66). *B. fragilis* stimulates the production and activation of STAT3, characterized by a selective Th17 response; in fact, blockage of IL-17 prevents colonic hyperplasia and tumor formation in these mice (66).

IL-17A signaling can be tumorigenic through several pathways (7, 67, 68). IL-17A induces mitogen-activated protein kinase (MAPK) and NFκB signaling, the key pathways related to the production of pro-inflammatory cytokines. In the IL-17A-deficient colon cancer mouse model, lower levels of IL-6, STAT3, IFNγ, and TNF-α were expressed and smaller tumors were produced, compared to wild-type mice. Moreover, IL-17A-deficient mice showed decreased numbers of β-catenin positive cells within the intestinal crypts, in addition to reduced key cell cycle regulators, e.g., cyclin D1, indicating a role for IL-17A in tumor progression, as well as tumor formation (67).

Th17 cells produce other cytokines, such as IL-22, which has been associated with carcinogenesis through the stimulation of STAT3 activation and MAPK family members (69, 70). Similar to IL-17A, Jiang et al. also showed that high levels of IL-22 promote the growth of human colon cell lines, transplanted into immunodeficient mice; they found that this effect was mediated by STAT3 activation and cyclin D1 (71).

4. Microbiota Contribution in Favor of Tumor Protection

Contribution of microbiota as a health-promoting factor is best exemplified by the administration of probiotics, a group of live microorganisms which are shown to be beneficial for the host (72). Disorders ranging from inflammatory diseases to several types of cancer, including colon cancer, are believed to benefit from probiotics (72, 73). In fact, a combination of microorganisms seems to act much more efficient than a single microorganism. Strains of lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium* species, are commonly added as probiotics to dairy products such as yogurt (72).

Another example of microbiota contribution to protection against colorectal cancer is the positive effect of short-chain fatty acid butyrate (produced by non-absorbed carbohydrates through colonic microbiota) on the prevention and inhibition of colorectal cancer (74). Short-chain fatty acids (SCFAs), predominantly acetate, propionate, and butyrate, are the main metabolites produced during the catabolism of carbohydrates (75). Multiple mechanisms have been suggested, relating microbiota to colon cancer protection. These mechanisms include alteration of microbiota content, induction of epithelial cell apoptosis, reduced bacterial translocation, improved epithelial defense barrier, modulation of mucosal inflammation, oxidative status, and shift of T cells towards an anti-tumor phenotype (72, 74, 76).

4.1. Microbiota Contribution in Favor of Tumor Protection Through T Cells

B. fragilis is among intestinal resident microbiotas, inducing Treg differentiation and immune homeostasis (26). In this regard, Dwivedi et al. reviewed mechanisms through which probiotics induce Treg cells: i) inhibition of dendritic cell (DC) maturation and activation of tolerogenic DCs; ii) probiotic-induced activation of FoxP3, TGF-β, cytotoxic T lymphocyte antigen-4, and IL-10, as Treg-associated molecules; and iii) stimulation of toll-like receptors (TLRs), expressed on gut lymphoid and epithelial cells by probiotic ligands which are involved in Treg induction (77).

A recent study also reported Treg polarization from Th0 by heat-killed probiotic *Lactobacillus casei Lbs2* (78). In addition, SCFAs, PSA production by specific probiotic species, and microbial metabolites (generated through food digestion) have been introduced to be involved in Treg induction by probiotics (77). *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacteria infantum* administration in a rat model of colon cancer was also reported to exert anti-cancer effects through TLR2 signaling (14). Therefore, Treg activation by probiotics has been delineated as one of the triggered mechanisms to suppress abnormal inflammation and subsequent complications, including colorectal cancer (77, 78).

5. Conclusion

In conclusion, microbiota is believed to have the potential to provide a pro- or anti-tumor microenvironment. These vast colonies of microorganisms act through several mechanisms to exert such properties; their effects on T cells seem a major factor in this respect. Administration of probiotics aimed at shifting the immune system response towards an anti-tumor phenotype is a practical example, suggesting the importance of information about microbiota.

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