Immune System Efficiency in Cancer and the Microbiota Influence

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Abstract

The immune system plays a critical role in preventing cancer development and progression. However, the complex network of cells and soluble factor that form the tumor micro-environment (TME) can dictate the differentiation of tumor-infiltrating leukocytes and shift the antitumor immune response into promoting tumor growth. With the advent of cancer immunotherapy, there has been a reinvigorated interest in defining how the TME shapes the antitumor immune response. This interest brought to light the microbiome as a novel player in shaping cancer immunosurveillance. Indeed, accumulating evidence now suggests that the microbiome may confer susceptibility or resistance to certain cancers and may influence response to therapeutics, particularly immune checkpoint inhibitors. As we move forward into the age of precision medicine, it is vital that we define the factors that influence the interplay between the triad immune system-microbiota-cancer. This knowledge will contribute to improve the therapeutic response to current approaches and will unravel novel targets for immunotherapy.

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Introduction

The interplay between the immune system and cancer is unquestionable. Considered a key factor during tumorigenesis, the immune response against cancer has been extensively studied over the last decade. While it is now clear that the immune system plays crucial roles in controlling tumor growth, in some instances the activity of immune cells can also favor cancer progression [1–3]. Owing the improved understanding on the interaction between the immune system and cancer, in the last years we have witnessed a revolution in cancer treatment with the development of immunotherapies aimed at boosting or dampening key immune pathways to promote antitumor immune status [4–6]. This recent development in cancer treatment also brought to light key factors that modulate the immune response to cancer and therefore the efficacy of immunotherapies. One such factor is the microbiome, the complete collection of microorganisms (including bacteria, viruses, archaea, and fungi) and their genomes that colonize the different surfaces of the human body, such as the gut [7]. Indeed, recent exciting data demonstrated the critical role of the gut microbiota in modulating the antitumor immune response and the efficacy of the recently developed checkpoint blockade immunotherapies across different cancer types [8–11].

These data suggest that modulating the gut microbiota may represent a novel adjunct approach to current anti-cancer therapeutic modalities.

To understand how alterations in the composition and abundance of the microbiota, including of particular species that are commonly part of the human microbiota, influence cancer immune responses and the efficacy of immunotherapies requires a deep understanding of the key factors that govern the interaction between the triad immune system-microbiota-cancer. To help in this regard, here we discuss new advances in the understanding of the mechanisms underlying the interaction between the immune system and microbiota during carcinogenesis, as well as the factors whereby the microbiota impacts the immune response against cancer. While novel research is shedding light into the role of the virome and mycobiome in host immunity, the techniques currently in use to isolate and characterize bacterial communities of the microbiota are more developed and standardized. Therefore, in this review we will focus on the bacterial communities of the microbiota and discuss their impact in host immunity and cancer.

The Immune System and Cancer

A plethora of mechanisms control cellular growth and death. It is the failure in these processes, mostly caused by DNA mutations, that is the main cause for the development of aberrant cells that accumulate and form tumors [12, 13]. Despite these DNA mutations being a common feature of cellular growth and differentiation, they do not always cause cancer development. Indeed, the transformation of a normal cell into a malignant phenotype triggers intrinsic cell death pathways that limit the development of tumors [14–16]. Accordingly, one of the hallmarks of human cancers is their tendency to evade programmed cell death [13]. Additionally, neoplastic transformation can be associated with significant molecular alterations that trigger immune responses to rapidly eliminate malignant cells. Indeed, mounting evidence both from mice and from clinical epidemiology suggest that the immune system functions as a significant barrier to tumor formation and progression [17]. The hypothesis that the immune system is capable of recognizing and eliminating tumor cells was first postulated by Sir Frank Burnet and is currently known as cancer immunosurveillance [18, 19].

The immune system comprises different lineages of cells, including macrophages, neutrophils, and lympho-

cytes, that act in a concerted manner to maintain homeostasis by protecting the host from external and, as postulated by Burnet, internal aggressions. Indeed, it has been now clearly demonstrated that the immune system detects and eliminates malignant cells including through antigen-specific responses initiated against antigens expressed by tumor cells [20–22]. Seminal studies from Kaplan and others show that the absence of one or several components of the immune system increased the incidence of cancer, both in mice and in humans [23–25]. One crucial observation that unraveled the relevance of the immune system in the fight against cancer was in mice lacking perforin, a component of cytolytic granules of cytotoxic T cells. In a set of different experimental approaches, perforin-dependent cytotoxicity was demonstrated to be a crucial mechanism of resistance to injected tumor cell lines but also to viral and chemical-induced carcinogenesis [26]. Also in mice, the blockade of interferon (IFN)- γ was associated with enhanced Meth-A tumor cell growth [27]. The critical role of the immune system in limiting carcinogenic events is also highlighted by several studies associating the presence of tumor-specific T cells in the blood, bone marrow, or tumors with good prognosis [28–34]. Despite this, preclinical studies have also shown that preconditioning of mice with IFN- γ resulted in increased B16 melanoma colonization of the lungs [35]. This enhanced colonization was attributed to the effects of IFN- γ in B16 cells, which caused a decreased sensitivity of these cells to the activity of natural killer (NK) cells [35]. In line with these data is the observation that in vitro IFN- γ induces the expression of markers that are usually associated with more aggressive phenotypes of melanoma cells [36]. When taken together, these studies suggest that the protective effect of the immune responses may be, at least in some cases, dependent upon the type of tumor [20]. Furthermore, the presence of different types of immune cells infiltrating the tumor has been associated with tumor progression [37–40]. To help untangling the complex interaction between the immune response with tumor cells, in this section we will discuss the involvement of the immune system in tumor control or progression (Fig. 1).

The Immune Response in Tumor Control

Cancers are heterogeneous masses of different types of cells, including tumor cells, immune cells, fibroblasts, and pericytes. This niche composed by cells and their by-products constitute the tumor microenvironment (TME). Soluble factors, such as cytokines, chemokines, and growth factors, mediate the intercellular communication between the different constituents of the TME [41].

Fig. 1. The antitumor and pro-tumor immune mechanisms generated toward cancer cells. IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; TGF, transforming growth factor; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells.

Tumor-infiltrating leukocytes are an important component of the TME. Among these leukocytes, tumor-associated macrophages (TAMs) are one of the most represented cells [42]. TAMs differentiate locally from recruited peripheral blood monocytes in response to cytokines and growth factors produced by stromal and tumor cells in the TME [42]. In responses to these factors, and as a result of their plasticity, macrophages can polarize toward an M1- or M2-like phenotype [43, 44]. Usually, M1-like macrophages are differentiated by pro-inflammatory mediators including lipopolysaccharides and IFN- γ , while M2-like macrophages are differentiated by the immuno-

modulatory cytokines interleukin (IL)-4, IL-10, and IL-13, and transforming growth factor (TGF)- β [44]. It is important to note that these 2 macrophage phenotypes are a simplistic view of the intricate mechanisms underlying macrophage differentiation [45]. Indeed, not only macrophage polarization is not definitive, as it can be altered in response to the microenvironment [46], but these macrophage phenotypes are not mutually exclusive and M1- and M2-like macrophages can coexist in the TME which is important to prevent dysregulated responses [45].

In the initial steps of the immune response, M1-like macrophages have been shown to play antitumor roles

through the production of pro-inflammatory cytokines such as IL-12, which promotes the differentiation of T-helper type 1 (Th1) cells [42, 47]. These cells produce IFN- γ and other pro-inflammatory cytokines and chemokines that enhance antigen presentation and promote the expression of inducible nitric oxide synthase, an enzyme actively associated with cytostatic and growth inhibitory effects [48]. On the other hand, M2-like macrophages are characterized by their pro-tumor role as discussed below [49].

In addition to macrophages, neutrophils are another important cellular component of the TME that has received increasing attention over the past years. In this regard, recent data show that neutrophils can also adopt 2 distinct phenotypes in response to the cytokines of the TME [50]. As discussed above for TAMs, neutrophils can also adopt antitumor (N1-like) or pro-tumor (N2-like) phenotypes [51]. N1-like neutrophils contribute to the antitumor immune response through the production of antimicrobial molecules with cytotoxicity against cancer cells [52–54], the inhibition of TGF- β [50], or by actively promoting the recruitment and stimulation of T cell proliferation [55] and IFN- γ secretion [56]. Indeed, recent studies using human samples of early-stage colorectal cancer (CRC) have associated the infiltration of neutrophils with good prognosis and increased overall survival [57, 58]. Furthermore, neutrophils have recently emerged as important targets of immunotherapy [59]. For example, a recent study from Zhang et al. [60] found that the ablation of TOLLIP, an innate immune-cell modulator, from neutrophils enhances the antitumor immune response in a mouse model of CRC induced by azoxymethane-dextran sulfate sodium salt. This study showed that TOLLIP-deficient mice exhibited a marked reduction in both microscopic and macroscopic polyps when compared with WT mice. This phenotype was associated with an increased expression of CD80 and a downregulation of immunosuppressive molecules by TOLLIP-deficient neutrophils, which enhanced the activation and survival of T cell [60]. While these data suggest a crucial role of neutrophils in the antitumor immune response, most studies investigating the role of these cells in cancer have relied on animal models or circulating human neutrophils. As such, only limited information is available on the roles of tumor-associated neutrophils (TANs) in cancer patients. Therefore, further studies are required to better define the mechanisms and pathways whereby neutrophils modulate tumor immunity.

NK cells are another innate cellular component of the TME [61]. This population has been shown to play criti-

cal roles in the elimination of abnormal cells [61, 62] both in mice [63, 64] and in humans [65–67]. The effector functions of NK cells to eliminate tumor cells relies in the expression of molecules that can act either directly on tumor cells, such as perforins [68–70] and granzymes [69, 70], or initiate the recruitment of other immune cells which contribute to tumor clearance [71–73].

In addition to the innate cellular components, lymphocytes are another important presence in the TME. The T cell populations that infiltrate the TME include CD4⁺ T cells, specifically the phenotypes Th1, Th17, and regulatory T cells (Tregs), and CD8⁺ cytotoxic T cells. The antitumor mechanism of action of CD8⁺ T cells is particularly important as it involves antigen-specific cytotoxicity, in addition to IFN- γ and tumor necrosis factor (TNF)- α secretion [74]. This mechanism is supported by CD4⁺ T cells, particularly Th1 cells through the production of IL-2, IFN- γ , and TNF- α [74]. In some circumstances, Th2 cells have also been also showed to prevent tumor progression [75] mainly through the recruitment of eosinophils. Indeed, the production of IL-18 by eosinophils has been shown to mediate the death of Colo-205 cells through the upregulation of adhesion molecules that facilitate the interaction between effector and target cells [76]. Additionally, eosinophils can also produce cytotoxins, namely, granzyme-A, that promote tumor elimination [77]. In addition to conventional T cells, NK T cells also participate in tumor clearance [78]. Their effector mechanism is dependent upon the secretion of granzymes and perforin that lyse tumor cells [79]. Additionally, NK-T cells also secrete IFN- γ , which promotes the activation of cells CD8⁺ T cells as well as the generation of M1-like macrophages [74]. The relevance of these populations in tumor control is highlighted by data showing that tumor-infiltrating lymphocytes are generally associated with good prognosis [74, 80].

Together, the above data show that protection conferred by the immune system is critical to prevent cancer development. Indeed, in recent years we have witnessed an increased development of cancer treatments that target different components of the immune system, including dendritic cell [81] and checkpoint blockade therapies [82–84]. However, the modulation that immune cells are subjected to in the TME can promote, rather than eliminate, cancer (Fig. 1).

The Immune Response in Tumor Progression

As discussed above, the immune system plays a crucial role in controlling tumor development and progression. However, tumors can develop even in the presence of a

functional immune system suggesting that tumor cells and the microenvironment that they create can modulate the antitumor function of the immune response. Indeed, the ability of tumor cells to avoid immune destruction has been now considered in the updated version of the “hallmarks of cancer” [13].

The induction of immune suppression mechanisms is a complex process that requires the involvement of tumor and immune cells that act synergistically to dampen anti-tumor immune response. As discussed in the previous section, the differentiation of TAMs in the TME can contribute for their pro-tumor characteristics [42, 85]. In this regard, several meta-analysis have clearly demonstrated that an increase in the number of TAMs is generally associated with poor prognosis [86, 87]. During cancer progression, several mechanisms have been identified to overcome antitumor response by TAMs including the secretion of prostaglandin E2 [88], the immunosuppressive cytokine IL-10 [89], the angiogenic factors such as IL-1 β , that through upregulation of HIF-1 α protein increases vascular endothelial growth factor (VEGF) secretion [90], VEGF [91], endothelin-2 [92], and epidermal growth factor family ligands [93]. Additionally, TAMs may also play a role in tumor aggressiveness by releasing matrix metalloproteases (namely, MMP-2 and MMP-9) that damage the extracellular matrix and the basement membrane, facilitating tumor invasion and metastases [93–95]. Using a mouse model of colon carcinoma, the expression of arginase and production of nitrogen monoxide by TAMs were also shown to contribute to tumor progression by inducing apoptosis of CD8⁺ cytotoxic T cells [96].

TANs may also adopt an immunosuppressive phenotype in the context of tumors, including by producing chemokines that promote the recruitment of Tregs [50, 97]. N2-like neutrophils also produce cathepsin G and arginase [51], further promoting immunosuppressive status. As described above, most studies on the role of neutrophils in cancer have relied on mouse models. As such, it is still not clear if the N1-/N2-like profile described in mouse models can be directly applied to humans. However, some of the genes reported to be associated with the phenotype and function of neutrophils in mice have also been reported in humans. For example, the chemokines CCL2 and CCL17 that were originally found as part of the N2-like neutrophils signature in mice were also associated with increased tumor progression in humans [98, 99]. Accordingly, patients with low CCL2 or CCL17 TAN counts had substantially better outcomes than those with higher numbers of these cells [97]. Furthermore, patients diagnosed in advance stages of cancer

have been reported to present neutrophilia, which was associated with poor prognosis [100, 101]. Additionally, TANs produce several molecules that promote invasion and metastasis including MMPs and VEGF [102, 103]. Accordingly, it has been shown that neutrophils promoted lung metastases by breast cancer cells [104] and their depletion in murine models of melanoma and fibrosarcoma reverts tumor growth, angiogenesis, and metastasis [104, 105]. More studies are required to determine the impact of neutrophils, and neutrophil polarization, particularly in human cancers.

In addition to TAMs and TANs, myeloid-derived suppressor cells (MDSCs) are another population associated with the suppression of antitumor immune response [106]. The suppressive capacity of these cells appears to be enhanced as they infiltrate tumors, pointing to the crucial role of the TME in modulating their function [107]. MDSCs are characterized by their ability to suppress the immune response through different mechanisms, including the inhibition of T cell functionality associated with the expression and activity of arginase-1 [108, 109], promoting the differentiation of Tregs [110], and the differentiation of pro-tumor TAMs [111, 112]. Recent work in humans also showed that MDSCs can promote angiogenesis via MMP-9 secretion [113].

In the context of the adaptive immune response, Tregs have been identified as central suppressors of antitumor immune responses [114, 115]. The immunosuppressive function of Tregs is mediated through the secretion of IL-10 and TGF- β , which hamper the activity of effector T cells and their ability to eliminate tumor cells. Accordingly, increased accumulation of Tregs in the TME associates with poor prognosis, in multiple types of cancer [80, 116, 117]. In addition to Tregs, Th2 response has also been demonstrated to favor tumor progression through different mechanisms, including the production of IL-10 [118–120] that inhibits antitumor immune responses as well as the production of arginase-1 by M2-like macrophages [121], differentiated in a context of a Th-2 response, that inhibits cytotoxic T cell activity.

While the data described above show that there are multiple immune mechanisms associated with the inhibition of antitumor immune responses, it is important to note that this inhibition is not only accomplished through suppressive mechanisms. Indeed, an exaggerated immune response can promote dysregulated inflammation which favors cancer development. It was Virchow that suggested the first association between inflammation and cancer, over 150 years ago [122]. After this initial observation, several pieces of evidence confirmed the associa-

Fig. 2. The antitumor and pro-tumor impact of the microbiota in cancer.

tion between prolonged inflammation and increased risk of cancer [123, 124]. As the inflammatory process promotes the further recruitment of inflammatory immune cells to damaged sites, the constant production of angiogenic factors, pro-inflammatory cytokines, and reactive oxygen species creates the conditions to drive normal cells into tumorigenic [125, 126]. As discussed later in this review, infection by pathogens instigates inflammation that promotes the development of cancer. Indeed, infections have been estimated to be the cause of approximately 20% of all cancers [127, 128].

The Microbiota in Cancer

After birth, microorganisms colonize all the surfaces of the human body exposed to the external environment. These communities of microorganisms (microbiota) and their collective genomes (microbiome) have long been recognized to play crucial roles in the digestion of nutri-

ents, the production of vitamins, and other essential molecules [129]. However, more recent data show that the microbiota plays a critical role in the development and function of the immune system [130–132].

The development of new methodologies for nucleic acid sequencing unraveled the diversity and function of the microbiota, particularly bacterial communities [133]. These methodologies showed that the composition of the gut microbiota stabilizes after 3 years of age and remains constant during adolescence and adulthood [134]. Hosting >1,000 different bacterial species, the gastrointestinal tract harbors the most complex and well-studied microbiota of the human body [134]. The upper part of the gastrointestinal track, including stomach, duodenum, and jejunum, is enriched in aerobic gram-positive bacteria from the *Lactobacillus* and *Enterococcus* genera. In the ileum, the concentration of bacteria increases, with the representation of coliforms. The distal region of gastrointestinal tract (cecum and colon) harbors the most diversity with species of bacte-

ria belonging to the genera *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* [134].

Although the microbiota composition remains relatively stable, changes in lifestyle, such as diet [135, 136], or consumption of antibiotics [137, 138] can lead to microbiota imbalances, a condition known as dysbiosis [139]. With the recent body of literature pointing toward the critical role of the microbiota as determinant of health or pathologic conditions, including cancer [140], in the following sections we will discuss the interaction between gut microbiota and cancer development or control.

The Microbiota in Tumor Control

The microbiota plays crucial roles in controlling tumor development and progression through the production of metabolites that fuel the immune responses or promote the development of antitumor environments (Fig. 2). Specifically, fiber-rich foods have been suggested to have health benefits through the maintenance of a healthy microbiota capable of production of short-chain fatty acids (SCFA) [141–143]. Accordingly, recent data show that butyrate and propionate, 2 SCFA produced by different microbiota species, display antitumor capacity against CRC [144] or hepatic cancer [145]. The protective capacities of these metabolites have been shown to be mediated directly in tumor cells or indirectly by modulating the immune response and the TME. Specifically in tumor cells, several in vitro studies show that these metabolites inhibit the activity of histone deacetylases, which are critical to control of gene transcription [146–148]. In line with these data, Zagato et al. [149] elegantly showed that the endogenous murine microbiota member *Faecalibaculum rodentium* and its human homolog, *Holdemanella bififormis*, were involved in CRC prevention. This study showed that the production of SCFA, specifically butyrate, was inhibiting calcineurin-mediated NFATc3 transcription factor activation through its inhibitory effect at the histone deacetylase level [149]. These data corroborated the results from Peuker et al. [150] that identified the contribution of calcineurin activity to tumor growth and proliferation. In what regard the indirect effects of SCFA, a recent study showed that butyrate plays a key role in regulating colonic inflammation and therefore CRC development [151]. In this study, the protective role of butyrate was mediated by promoted anti-inflammatory properties in colonic macrophages and dendritic cells which promoted the differentiation of Treg cells and IL-10-producing T cells [151]. Accordingly, mice deficient in the receptor for butyrate in the colon (GPR109A encoded by *Niacr1*) were more susceptible to development

of colon cancer. Importantly, Niacin, a pharmacological Gpr109a agonist, suppressed colitis and colon cancer [151].

In addition to SCFA, microbiota also produce other molecules with antitumor potential. For example, the production of pyrodoxine, from the vitamin B group, by a wide group of bacteria stimulates the antitumor immune response [152, 153]. Additionally, Konishi et al. [154] showed that the production of ferricrheme by *Lactobacillus casei* induces the apoptosis of colon-derived tumor cells through the activation of the JNK-mediated apoptosis. Lactic acid-producing bacteria have also been shown to play key roles in controlling tumor cells growth by stimulating antitumor immune responses [155–157]. Specifically, the supplementation of *L. casei* in a chemical-induced model of intestine injury was associated with increased production of lactate which decreased the production of myeloperoxidases and TNF- α [158]. These data were corroborated by another study wherein *L. casei* supplementation improved chronic inflammatory bowel disease by downregulating the production of pro-inflammatory cytokines such as IL-6 and IFN- γ by intestinal lamina propria mononuclear cells [159].

Another important mechanism whereby microbiota promotes regulation of inflammation, and therefore tumor control, is through the production of metabolites that are agonists of the aryl hydrocarbon receptor (AhR). This receptor is expressed at barrier sites, such as gastrointestinal tract, acting as a sensor of environmental chemicals, including dietary compounds. Recent data show that mice treated with AhR antagonists develop severe symptoms upon induction of colitis and that patients with ulcerative colitis had reduced expression of AhR [160]. Alexeev et al. [161] showed that the levels of indole-3-propionic acid, a metabolite of the microbial tryptophan catabolism recently identified as an AhR agonist, were reduced in both ulcerative colitis patients and mice. Importantly, the supplementation of mice with indole-3-propionic acid enhanced the production of IL-10 and inhibited the production of pro-inflammatory cytokines [161].

While the above data show that microbiota metabolites can regulate inflammation, the expression of AhR can be an indicator of poor prognosis in certain types of cancer [162–165]. Accordingly, Shimba et al. [163] reported that A549 tumor cells expressing high levels of AhR had accelerated cell growth due to shortening late M to S phases of cell cycle. Additionally, Yin et al. [164] described shortened cell cycles and increased proliferation of gastric cancer cell lines due to the activity of the AhR.

The activity of AhR also blocked the efficiency of tyrosine kinase inhibitors on lung adenocarcinoma, thus favoring tumor growth [166]. Together, these data show that while the activity of the AhR may be beneficial in regulating inflammatory contexts it can also enhance tumor cell growth and resistance to therapy.

The Microbiota in Tumor Progression

As discussed above, uncontrolled chronic inflammatory contexts are characterized by increased production of pro-inflammatory cytokines and other molecules that promote tumor development and progression (Fig. 2). The microbiota play a key role in these contexts as the outgrowth of certain commensal bacteria has been associated with chronic inflammation and cancer development [167–169]. Indeed, infectious agents are currently estimated to be responsible for ~20% of human cancers [127, 128]. While a large percentage of these occurrences are due to viral infections, such as the human papillomavirus (HPV) that causes cervical cancer, the contribution of bacteria is usually relegated to a second plane [170, 171]. However, in recent years an increasing number of studies have identified an important role of bacterial infections in carcinogenesis [167, 172]. As an example, bacterial vaginosis has been associated with the prevalence of HPV infection [173–175]. Although the mechanisms underlying this association are not fully understood, it is likely that the disruption of the vaginal milieu by bacterial vaginosis prompts a decrease in protective species of the genus *Lactobacillus* and an increase in both strict and facultative anaerobic bacteria, namely, *Gardnerella vaginalis* [173–175]. The presence of these bacteria in the vaginal mucosa is likely to promote the activation of downstream inflammatory pathways that influences mucosal susceptibility for HPV [176–179].

One of the first associations between cancer and bacteria was reported in the 1980s [180]. These initial studies identified *Helicobacter pylori* as a causative agent of infectious gastritis, a chronic inflammation of the stomach [180]. This chronic inflammatory state promotes a niche that prompts tumor development. Accordingly, this infectious agent is now classified by the World Health Organization as a carcinogen class I [181]. Disseminated through the planet, it has been estimated that >50% of world population harbors these bacteria [182]. Owing to its ability to neutralize gastric acidity, through the expression of urease, *H. pylori* is able to survive and proliferate in the stomach and infect the gastric mucosa [183]. The expression of particular cytotoxins, mostly VacA and CagA, promotes the production of the 2 classical pro-in-

flammatory cytokines IL-8 and IL-6 by gastric epithelial cells [184–187] and activates the NF- κ B pathway [188, 189], thus enhancing and perpetuating the inflammatory response.

Another common member of the human microbiota that has been shown to enhance tumorigenesis through the production of an enterotoxin is *Bacteroides fragilis* [190, 191]. Haghi et al. [192] reported a higher prevalence of *B. fragilis* in CRC patients when compared to control group. Interestingly, the authors also showed an increased expression of the *B. fragilis* enterotoxin gene in advanced stages of the disease [192]. In line with these data, Purcell et al. [193] associated the presence of enterotoxigenic *B. fragilis* to the abundance of early stage carcinogenic lesions in colorectal tissue. Further, *B. fragilis* enterotoxin has been shown to induce persistent colitis in mice promoting chronic inflammatory status and cancer development [194]. This inflammation is mediated by increased IL-8 production and b-catenin signaling [195, 196]. Recently, *B. fragilis* toxin was also shown to modulate the Wnt pathway and to promote cell proliferation [197].

Also associated with increased CRC incidence is *Fusobacterium nucleatum* [198, 199]. The pathogenesis of these bacteria has been associated with the expression of FadA and Fap2 virulence factors [200, 201]. These virulence factors induce the expression of oncogenes and promote the growth of cancer cells [200]. A recent study identified a tumor-based immune evasion mechanism that is dependent on the interaction of Fap2 protein with the inhibitory receptor T-cell immunoglobulin and ITIM domain, expressed by all human NK cells and some T cell populations, inhibiting their function [202]. Additionally, the expression of pro-inflammatory cytokines, such as IL-8, TNF, and IL-6, associated with high levels of *F. nucleatum* may also contribute to establishing a microenvironment amenable for cancer progression [198], including the recruitment of myeloid-derived suppressor cells, and promoting the differentiation of M2-like macrophages and tumor-associated neutrophils which sustain the immunosuppressive environment [203, 204].

The Immune System as a Target: Immunotherapy

The diversity of cells that are part of the tumor as well as the heterogeneity of the mutations that result in malignant cell differentiation results in altered molecules and pathways that are prime targets for therapeutic approach-

es. The combination of different approaches is also used to further enhance the potential of cancer therapy [205, 206]. As discussed above, the immune system plays crucial roles in multiple carcinogenic scenarios. As such, cancer therapies that aim to boost the antitumor immune response, known as immunotherapies, have brought a new wave of enthusiasm in the fight against cancer [207, 208]. Immunotherapies aim to boost the function of specific subsets of cells, which is usually accomplished through reeducation, as it is the case for dendritic cell therapies, or modulation of the immune response, as it is the case of blockade antibodies or cytokines which target critical immune pathways [4].

The first immunotherapy approved was based on the administration of high doses of IL-2 to treat metastatic renal cell carcinoma and metastatic melanoma [209]. The potential of this therapy was associated with ability of IL-2 in prompting the activation and differentiation of naïve T cells into effector and memory T cells and by stimulation of activation-induced cell death pathways [210, 211]. In addition to IL-2, IFN- α has been also approved for adjuvant treatment of completely resected high-risk melanoma patients and several refractory malignancies [212, 213]. Despite the success of these therapies to activate the immune system of cancer patients, cytokine therapy has been difficult to implement in clinical settings. Indeed, while multiple cytokines are still under evaluation in preclinical setting and clinical trials (including granulocyte-macrophage colony-stimulating factor, IFN- γ , IL-7, IL-12, and IL-21), cytokines in monotherapy have not fulfilled the promise of efficacy seen in preclinical experiments. This is likely associated with their dose-limiting toxicities and pleiotropic functions. However, as our knowledge in the role of specific cytokines increases and novel methodologies for the administration and engineering of these molecules are developed, we will certainly see a rise in the use of cytokines as cancer therapies [214].

One central mechanism whereby the TME contributes to the suppression of antitumor immunity is through the induction of immune checkpoint inhibitors that act as “brakes” of the acquired immune response. While immune checkpoint inhibitors play key roles in preventing autoimmunity and tissue damage during an immune response, their dysregulation during tumorigenesis can hamper the activation of immune cells and prevent their antitumor activity. It was in this context that the recent development and clinical use of monoclonal antibodies that block the function of the immune checkpoint inhibitors cytotoxic T-lymphocyte-associated protein 4

(CTLA-4), program-death 1 (PD-1), and programmed death-ligand 1 (PD-L1) revolutionized cancer therapy [215]. Both CTLA-4 and PD-1 are negative regulators of T-cell immune function [216, 217]. CTLA-4 is a CD28 homolog expressed by T cells that binds to B7 molecules in antigen presenting cells. Contrary to the stimulatory signal provided by the interaction CD28-B7, binding of CTLA-4 to B7 does not produce a stimulatory signal. As affinity of CTLA-4 for B7 is much higher than CD28, the CTLA-4-B7 binding prevent the costimulatory signal normally provided by CD28, thus preventing T cell activation and effector differentiation [216, 217]. Similar to CTLA-4 signaling, the binding of PD-1 with its ligands PD-L1 or PD-L2 also has an inhibitory effect on T cells, specifically in T cell proliferation and cytokine production [218, 219]. However, unlike CTLA-4 ligands expressed only by antigen-presenting cells, PD-1 ligands are more widely expressed. This pattern of expression enables PD-1 functions to impair T cell activity during the effector phase of the response in peripheral tissues, specifically tumors [216, 217].

Pioneering studies conducted by James Allison provided evidence that the inhibition of CTLA-4 through the use of monoclonal antibodies was associated with tumor regression in mice [220]. Subsequent studies also highlighted the antitumor potential of CTLA-4 blockade in adjunct therapies, with vaccines or chemotherapy [221, 222]. In clinical settings, ipilimumab, a commercialized form of CTLA-4 monoclonal antibody, has been proven efficient in the treatment of metastatic melanoma [223]. Additionally, a longitudinal follow-up study of 177 patients indicated a curative regression of metastatic melanoma in a small percent of patients [224]. The potential of anti-CTLA-4 in cancer treatment led to the approval of ipilimumab treatment for metastatic melanoma. In addition to the overall increase in the survival of patients treated with ipilimumab, other studies demonstrated a synergistic effect of ipilimumab and gp100 peptide vaccine, which is indicated for melanoma treatment [223]. However, these treatments were also associated with adverse effects. Therefore, future studies and clinical trials will be crucial to assess the relevance of CTLA-4 blockade in cancer treatment [223, 225].

In addition to CTLA-4, PD-1 and its ligand PD-L1 raised interest as immunotherapeutic targets. During tumor progression, cancer cells can increase their expression of PD-L1 that upon binding to PD-1 expressed by T cells inhibits their response [226]. Indeed, this pathway has been shown to play a key role in the escape of tumors from immunosurveillance as T cells express high levels of

PD-1 upon tumor infiltration [227]. Accordingly, PD-L1 expression has been reported in several types of cancer including bladder, lung, colon, and melanoma. Importantly, the administration of anti-PD-1/PD-L1 monoclonal antibodies has been successful in controlling tumor progression and it has been approved for the treatment of several types of cancer [228–232]. Moreover, it has been reported that PD-1 blockade in melanoma [233] and lung cancer [234] is more efficient in cases of highly mutated cells. It has been hypothesized that this enhanced efficiency is associated with the increased expression of neoantigens by tumor cells, which in combination with anti-PD-1 further enhance the response of T cells [234]. The efficiency of these therapies has led to the combination of CTLA-4 and PD1/PD-L1. While their combination showed increased efficiency, it also resulted in worse adverse effects [235–239].

In the last decade, several other immune checkpoint receptors including TIM-3 and LAG-3 have been in the frontline to be validated as therapeutic targets, showing promising preclinical and clinical results [240–243]. Nonetheless, not all the individuals respond to immunotherapy nor they are free from relapsing, which opens the door for the constant development of new strategies to cancer treatment. While heterogeneity of tumors and preexisting tumor immune response can dictate the success of immunotherapies, the impact of commensal microbiota has been under scrutiny in the last years.

Microbiota Modulation of Cancer Therapy

The impact of the microbiota in the tumor immune response is undeniable. Although its importance has recently been reinvigorated, older studies showed the therapeutic potential of controlled doses of bacteria, or bacterial products, to potentiate the immune response and achieve antitumor immune status [244–246]. More recently, the microbiota has been shown to play a critical role in the efficacy of immunotherapy, with 2 recent preclinical studies reporting that alterations in microbiota composition dictated the efficiency of CTLA-4 and PD-1 blockade [8, 247]. Vétizou et al. [8] reported that anti-CTLA-4 therapy did not inhibit tumor growth in germ-free or antibiotic-treated mice, pointing to a key role of the microbiota in the efficacy of anti-CTLA4. Further experiments showed that *Bacteroidales* and *Burkholderiales* were crucial for the antitumor activity of anti-CTLA-4 therapy [8]. Indeed, oral transplantation of *B. fragilis* in combination with *B. thetaiotaomicron* or *B. cepacia* restored the efficiency of anti-CTLA-4 as did the fecal transplants from human samples with

elevated levels of *Bacteroides* species [8]. Mechanistically, the microbiota promoted the generation of antitumor antigen-specific Th1 responses which controlled tumor growth both in mice and patients, likely by producing cross-reactive antigens and enhancing antitumor T cell responses [8].

The impact of microbiota is not specific to anti-CTLA4 therapy. Indeed, Sivan et al. [247] reported discrepancies in tumor growth and anti-PD-1 therapy when comparing genetically similar mice harboring distinct microbiota. Interestingly, fecal transplants from responsive to unresponsive mice restored the efficacy of PD-1 blockade, showing that microbiota composition plays a critical role in the anti-PD-1 therapy. Importantly, the microbiota of mice prone to respond to anti-PD-1 treatment was enriched in *Bifidobacterium* species and the supplementation with *Bifidobacterium* restored antitumor efficacy of PD-1 blockade in mice that did not have these bacteria [247]. As for the anti-CTLA4 data discussed above, augmented dendritic cell function and enhanced CD8⁺ T cell priming and accumulation in the TME was shown to be associated with the efficacy of PD-1 blockade [247]. The relevance of these data in clinical setting was demonstrated by a follow-up study, showing that the bacterial genera *Akkermansia*, *Enterococcus*, *Bifidobacterium*, and *Faecalibacterium* were associated with a response to PD-1 blockade [9, 248, 249]. While our understanding on the mechanisms whereby the microbiota, and particular species of the microbiota, modulate the antitumor immune response is in its infancy, these data described suggest that targeting the microbiota may promote the efficacy of antitumor therapies. In this regard, it has been shown that mice treated with antibiotics do not respond to cisplatin, oxaliplatin, or cyclophosphamide drug treatment [250–252]. However, combining cisplatin with probiotics administration, *Lactobacilli*, improved mice response to therapy [250]. Accordingly, the oral administration of *Lactobacillus johnsonii* and *Enterococcus hirae* induced the differentiation of pro-inflammatory T helper cells which improved the efficacy of cyclophosphamide treatment in tumor-bearing mice [251].

In the light of the current knowledge, it is clear that the microbiota plays pivotal roles in cancer development, progression, and in the efficacy of classical and novel therapeutic approaches. As such, future studies should focus on the therapeutic potential of manipulating gut microbiota in cancer patients (by means of probiotic consumption, fecal microbial transplants, or design of specific diets) as adjunct approach to current or novel immunotherapeutic modalities.

Conclusion

Cancer is the second leading cause of death globally with an estimated death toll of 9.6 million people in 2018 alone. Recent developments in cancer therapy, including checkpoint blockade therapies, has brought significant improvements for patients in terms of survival and quality of life. Unfortunately, not all patients benefit for these novel approaches. As such, defining the factor that modulates cancer immune responses is key to improve current and novel therapeutic approaches. Genetics and functional studies have now demonstrated the dual role of the gut microbiome in cancer. While in some cases bacteria can overgrow during dysbiosis, triggering an inflammatory pro-cancerogenic environment, other bacteria and bacterial-derived metabolites protect the host. Adding to this knowledge is the recently identified gut microbiota species that contribute to improve the efficacy of immunotherapy in cancer immunotherapy responders. Together, these data highlight the potential of microbiota modulation as adjunct to current and novel immu-

notherapies. It is therefore critical to continue defining the therapeutic benefits derived from modulating the microbiota to further explore their potential as we move into the age of personalized medicine.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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