Harnessing Microbiota to Improve Immunotherapy for Gastrointestinal Cancers

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ABSTRACT

Immune checkpoint blockade has revolutionized opportunities for therapeutic intervention in cancer but demonstrates a low frequency of response in most patients and in some common types of tumors. An emerging paradigm supports the notion that trillions of normally beneficial microbes inhabiting the gastrointestinal tract, termed the microbiota, critically impact the success or failure of antitumor immunity induced by immune checkpoint blockade. Here, we briefly summarize the current knowledge on how interactions between the microbiota and immune system are contributing to the outcome of cancer immunotherapy. We propose that this immune–microbiota dialogue is particularly important in gastrointestinal cancers that exhibit striking resistance to immune checkpoint blockade and inherently develop in a unique environment that is rich in both immune-cell networks and direct exposure to the microbiota. Finally, we focus on how future studies should determine whether microbiota can be harnessed as a strategy to boost antitumor immunity in these contexts and beyond.

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Introduction

Microbiota that colonize the gastrointestinal tract include trillions of microbes that live in complex communities, exhibit a symbiotic equilibrium with their host, and play key roles in orchestrating human health (1). Disruption of beneficial host–microbiota interactions has been implicated in a large range of human disorders, including gastrointestinal, autoimmune, neurologic, metabolic, and malignant diseases. It also is emerging that the microbiota profoundly influences responsiveness to various therapies, including immune checkpoint blockade (ICB) in the setting of cancer (2). Thus, the microbiota represents a tractable target to both improve clinical responses and reduce treatment-associated toxicities during ICB (2). Despite these recent discoveries, the specific pathways by which host–microbiota interactions shape the response to immunotherapies remain poorly defined. Furthermore, the relevance of these findings to specific types of cancer that exhibit high levels of therapy resistance and develop in immune- and microbiota-dense sites, such as gastrointestinal cancers, remains poorly understood. Here, we review the current knowledge and the mechanisms through which the microbiota modulates antitumor immunity during ICB, and further interrogate the relevance of this paradigm in gastrointestinal cancers.

Gut Microbiota Is a Major Determinant of Outcome of ICB

Emerging evidence indicates that the intestinal microbiota substantially shapes the outcome of numerous clinical therapies. In particular, recent seminal studies defined that the gut microbiota is required for efficient antitumor immunity in the context of therapeutic interventions such as ICB (3–14). ICB is a revolutionary cancer therapy based on targeting negative regulators of T-cell activation that are frequently "hijacked" by tumors and in chronic infectious or inflammatory contexts, thus promoting an immune-resistant microenvironment. To date, the most prevalent agents used for ICB are mAbs targeting programmed cell death protein 1 (PD-1), its ligand PD-L1, and/or cytotoxic T lymphocyte-associated protein 4 (CTLA-4). These mAbs block the interaction of T cells with their suppressive cognate ligands to elicit an antitumor immune response. Despite yielding impressive results in clinical trials across various types of cancer, the outcome of ICB remains heterogeneous between patients and some cancer types exhibit a striking resistance to such therapy (15). Response rates range from complete remission in a minority of patients, to significant life prolongation even in metastatic cancers, to complete lack of responsiveness in most patients, especially in certain tumor types like gastrointestinal cancers (16, 17). Therefore, understanding the mechanisms that shape antitumor immunity elicited by ICB currently represents a major area of interest with the potential to improve or extend the benefit of these therapies to intractable cancer types and resistant patient subgroups.

A large array of ICB resistance mechanisms have been uncovered, implicating low mutational burden, poor intrinsic antigenicity of tumor cells, absence of priming by potentially immunogenic pretreatment with chemo- or radiotherapy, defective antigen presentation during the priming phase, local immunosuppression by extracellular metabolites, functional exhaustion of tumor-infiltrating lymphocytes, and acquired resistance post treatment (15). In addition, studies originating in preclinical mouse models have revealed that the gut microbiota is a critical determinant that controls the outcome of cancer immunotherapies (**Fig. 1**). These seminal discoveries involved germ-free (GF) mice that harbor no living microorganisms or specific pathogen–free (SPF) animals treated with antibiotics. Antibiotics not only clear pathogenic infection but also markedly disrupt the gut microbiome composition and diversity, impairing a symbiotic dialogue with the immune system. With these approaches, a pioneering study demonstrated that GF animals or mice treated with broad-spectrum antibiotics were unresponsive to anti–CTLA-4 treatment as compared with SPF animals (4). Recolonization of these animals with

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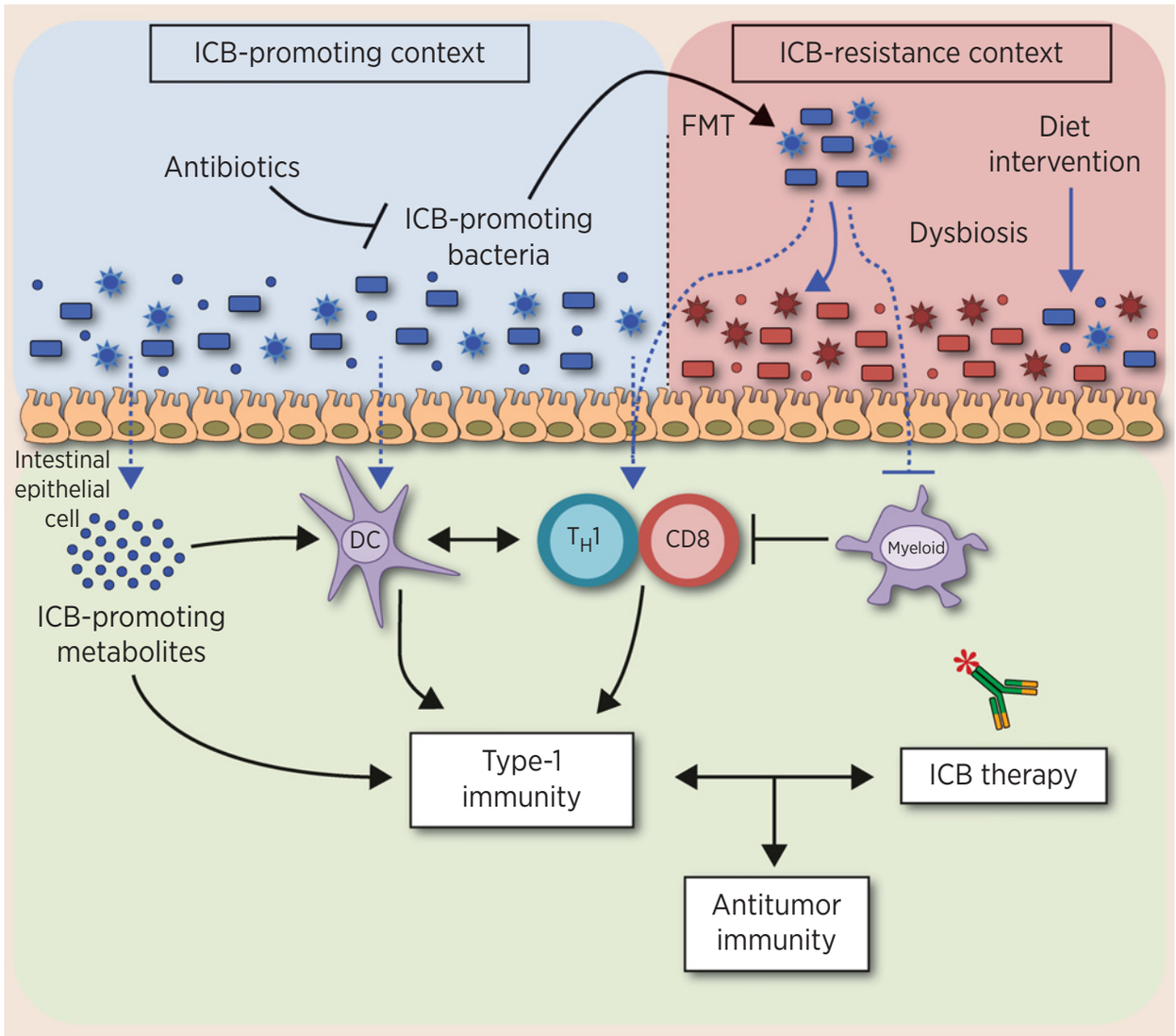

Figure 1.

Mechanisms by which the microbiota impacts the efficacy of ICB and opportunities for therapeutic intervention. Mechanisms of antitumor immune response driven by the microbiota during ICB-therapy. The gut microbiota and associated metabolites can promote dendritic cell (DC) activation and costimulatory functions, T_H1-cell polarization, cytotoxic $CD8^+$ T-cell functions, and maintain a strong antitumor immune fitness. Dysbiosis driven by treatment with antibiotics or intestinal inflammation can disrupt these pathways and inhibit the ability of the gut microbiota to promote ICB responsiveness. Therapeutic interventions like FMT or targeted diet are promising approaches to restore ICB-promoting microbiota in the gut, reduce tumor-associated immune suppression, and overcome ICB-resistance in patients with cancer.

specific bacterial isolates was sufficient to restore responsiveness to anti–CTLA-4 treatment (4), suggesting that a stable gut microbiota composition is a key determinant of response to ICB. Further research confirmed a negative impact of treatment with antibiotics prior to anti–PD-1, anti–PD-L1, or anti–CTLA-4 therapy in patients with cancers like melanoma, non–small cell lung carcinoma (NSCLC), renal cell carcinoma (RCC), and urothelial carcinoma (18), indicating that the disruption of a healthy gut microbiota composition, a situation referred to as gut dysbiosis, is a sufficient factor to significantly alter the therapeutic efficiency of ICB. A second pivotal study showed that basal tumor immunosurveillance against the B16-SIY subcutaneous tumor model was distinct in mice with similar genetic backgrounds (C57BL/6) but derived from two different animal facilities (Jackson Laboratory and Taconic Farms; ref. 5). The authors found that mice from the two facilities exhibit distinct microbiota compositions, with those from Jackson Laboratory harboring specific microbes such as *Bifidobacterium*, which track with enhanced antitumor immunity and synergistic effects when coupled with anti–PD-L1 treatment (5). Oral administration of *Bifidobacterium* to mice from Taconic Farms was sufficient to boost antitumor immunity comparable with that observed in mice from the Jackson Laboratory. This occurred by increasing dendritic cell functions, $CD8^+$ T-cell priming, and antitumor cytotoxic activity (5). Gut microbiota also shapes the antitumor immune response in subcutaneous tumors by inducing a

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type I IFN response via activation of the stimulator of IFN genes (STING) pathway in mononuclear phagocytes (19). GF or antibiotics-treated animals demonstrate impaired intratumoral type I IFN signaling, skewing mononuclear phagocytes from an immunostimulatory to a suppressive phenotype, a phenomenon that could also explain the inability of microbiota-devoid mice to respond to ICB (19). Altogether, these findings demonstrate that the gut microbiota is intimately linked with the antitumor fitness of the immune response and harbors immunostimulatory potential that is necessary to promote optimal ICB responsiveness.

Subsequent studies translated these findings to the human-derived microbiota. Three pivotal studies demonstrated in cohorts of metastatic melanoma (6, 7), patients with NSCLC or RCC (8) undergoing anti–PD-1 therapy, that "responder" and "nonresponder" patients harbored significantly distinct microbiota compositions. A favorable microbiota signature in responder patients was associated with higher α-diversity, enhanced systemic immunity, and intratumoral infiltration of adaptive immune cells. Astonishingly, fecal microbiota transplantation (FMT) from "responder" or "nonresponder" human donors was sufficient to transfer the phenotype of the respective donor to recipient GF or antibiotics-treated mice (6–8), indicating that the human gut microbiota composition is an independent determinant of responsiveness to ICB. Using a reverse approach, Tanoue and colleagues observed that the frequency of intestinal IFNγ$^+$ CD8$^+$ T cells in humans and mice was significantly associated with the composition of the gut microbiota (9). By mining the human gut microbiome, the authors identified a consortium of 11 bacterial strains associated with and that can transfer a strong intestinal IFNγ$^+$ CD8$^+$ T-cell response to GF recipient mice (9). Interestingly, this bacterial consortium also enhanced anti–PD-1 and anti–CTLA-4 responses in subcutaneous tumor models in a CD8$^+$ T cell–dependent manner, supporting the hypothesis that the gut microbiota not only shapes the local intestinal immune fitness, but also directly modulates the systemic antitumor immune response (9). In a more recent study, Andrews and colleagues investigated the influence of the gut microbiota in the context of combined anti–PD-1/anti–CTLA-4 therapy, and demonstrated significant microbial associations with treatment responses both in human melanoma patients and preclinical mouse models (20). Combined anti–PD-1/anti–CTLA-4 therapy is also associated with exacerbated immune-related adverse events, and Andrews and colleagues observed direct links between the gut microbiota composition and treatment toxicity, due to an enrichment of *Bacteroides intestinalis*, which drives enhanced *IL1B* expression and associated gut inflammation (20). These findings suggest that basal microbiota composition also contributes to toxicity associated with ICB therapy, which could serve both as a biomarker and a therapeutic target to respectively predict or reduce immune-related adverse events.

Despite these fundamental advances, it should be noted that the key bacterial subgroups modulating the response to ICB therapy remain debated (13, 14). To date, diverse bacterial targets have been associated with clinical responses to ICB treatment between studies, including *Akkermansia muciniphila, Parabacteroides distasonis, Eubacterium rectale, Roseburia spp*., and more broadly *Clostridiales, Faecalibacterium, Alistipes*, and *Bifidobacterium* species. Furthermore, a higher microbiota diversity was initially reported to positively associate with ICB response (6, 8), but this has not been supported in recent studies (20–22). Therefore, the specific microbiota species associated with enhanced clinical response vary substantially between basic studies and clinical trials. Recent cross-cohort microbiome analyses have highlighted the limited reproducibility of microbiome-based signatures to predict ICB responses across human studies (13, 14). These studies suggest that single bacterial species are inconsistent biomarkers and that gut microbial communities may be a better predictor of clinical outcomes across different studies (13, 14). This might be explained by a collateral impact of certain bacterial strains favoring healthier intestinal bacterial communities or with stronger ability to stimulate an antitumor immune response, as recently suggested with *A. muciniphila* (23). Interestingly, unfavorable taxa to ICB response seem more consistently distributed across patients and cohorts than favorable taxa, suggesting that favorable bacterial species may be more difficult to identify and track between cohorts (14). Distinct geographically distributed intestinal microbial signatures also exist and have been associated with different ICB outcomes, which could also explain discrepancies between studies (14). Finally, these discrepancies may also reflect lack of consistencies and a need for adjustment in sampling, methodological, and analytical approaches across studies (13, 14). Further investigations are required to delineate unifying gut microbiota signatures that are associated with ICB responsiveness and to interrogate how host or environmental factors influence ICB-responsive microbiota across the world. Rather than relying exclusively on the taxonomy of specific microbes, one potential avenue to unify these findings is to focus on mechanistic studies that define functional host–microbiota interactions that coordinate the responses to ICB.

Microbiota Intervention Can Boost the Efficacy of Cancer Immunotherapies

Targeting the intestinal microbiome is now being investigated as a supportive approach to increase antitumor immunity and promote ICB efficacy. FMT that consists of the transfer of fecal material from an identified donor to a recipient is currently the gold standard approach to modulate the microbiota and represents an interesting therapeutic tool. FMT was initially used 1,700 years ago when Chinese medical doctor Ge Hong used "yellow soup," a slurry of stool from healthy individuals to treat severe diarrhea. More recently, FMT has demonstrated a remarkable rate of response in the treatment of patients with *Clostridium difficile* infection (24). FMT is also being investigated as a new method for treating inflammatory bowel disease (IBD), and has yielded encouraging results in a subset of patients (25). To date, FMT has demonstrated a reasonably high safety profile when used in the treatment of nonmalignant disease, and even among immune-compromised individuals. On the basis of this knowledge and recent findings on the impact of microbiota in modulating ICB response in preclinical mouse models (6–8), several groups have started to investigate the potential of FMT in patients with cancer refractory to ICB.

Two recent clinical trials reported that FMT administered to patients with metastatic ICB-refractory melanoma resulted in objective clinical response rates of ∼30% (11, 12). In both trials, microbiota from ICB-responder donors was transplanted into patients with metastatic melanoma that were refractory to anti–PD-1 therapy, followed by reintroduction of anti–PD-1 treatment (11, 12). Interestingly, a response to the combination of FMT with ICB was associated with favorable immune changes, higher number of dendritic cells in the gut lamina propria, and an increase of type I IFN pathways, antigen presenting cells, CD8$^+$ T cells, and T_H1 cells in the tumor microenvironment (11, 12, 19). Davar and colleagues also reported a decrease of IL-8–producing myeloid cells within tumors, suggesting that beyond its immunostimulatory potential, the microbiota could also be employed to decrease tumor-associated immunosuppression (11).

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Both studies reported favorable safety results (11, 12), and additional clinical trials employing the combination of FMT and ICB are ongoing (NCT04038619, NCT03819296, NCT04758507). Albeit limited by a small number of patients included in these studies (15 and 10 patients in references 11 and 12, respectively), these positive clinical results are a promising early indication that FMT holds great potential to break ICB resistance in a subset of refractory patients.

Beyond FMT, more recent studies have evaluated the role of extrinsic factors, including diet and probiotics, in shaping the gut microbiome composition and subsequently in modulating the response to ICB therapy. In two studies, it was observed that a high dietary fiber regimen significantly enhanced ICB clinical response in mice and a large cohort of human patients with melanoma (19, 21). In preclinical models, Lam and colleagues demonstrated that animals fed with a high-fiber diet were enriched in *A. muciniphila* and that FMT was sufficient to transfer the beneficial anticancer effects of the diet (19). Spencer and colleagues further observed that either a low-fiber diet or probiotics resulted in a lower frequency of IFNγ^+ tumor-infiltrating T cells and was associated with impaired responses to anti–PD-1 therapy (21). These exciting results provoke numerous new questions on how diet and probiotics are modulating the efficacy of ICB, and importantly, point to new approaches in applying targeted diets to modulate the ability of gut microbiota to overcome resistance to ICB therapy.

Microbiota and Immune Checkpoint Blockade Therapies in the Intriguing Case of Gastrointestinal Cancers

The pivotal role of the gut microbiota in ICB raises new questions about these approaches in gastrointestinal affections that severely impact the host–microbiota homeostasis. This occurs in diseases such as IBD or colorectal cancer, which are associated with chronic inflammation, dysbiosis, and a major dysregulation of the immune-microbiota dialogue. Furthermore, most research efforts so far have been dedicated to identifying microbiota targets promoting ICB clinical efficacy, few studies have investigated the role of specific bacterial targets in driving resistance to therapy. This is potentially important as most gastrointestinal cancers, including gastric, esophageal, and colorectal cancer, exhibit a notable resistance or limited efficacy to ICB (16, 17).

In a recent study, we investigated how microbiota dysbiosis in patients with IBD could impact the immune response to ICB in preclinical mouse models of subcutaneous MC38 colorectal cancer tumors (26). We observed that patients with IBD harbored a distinct gut microbiota composition from healthy control donors, and that FMT from these patients was sufficient to result in decreased type-1 T-cell immunity in the gut and tumor, as well as a significant resistance to anti–PD-1 therapy (26). ICB resistance was associated with the presence of specific *Bacteroidales* species and a substantial decrease of colonic group 3 innate lymphoid cells (ILC3). ILC3s are a recently appreciated population of innate lymphocytes that are enriched in the gastrointestinal tract and orchestrate a tolerogenic dialogue between the microbiota and the mucosal immune system. Similar findings of ICB resistance were also observed in mouse models of experimental intestinal inflammation and in mice with selectively impaired ILC3s (26). These data indicate that chronic inflammation in the gut, dysregulation of mucosal lymphocytes, and progressive dysbiosis could favor the rise of selective bacterial consortia that promote resistance to ICB therapies through unexplored mechanisms. This is particularly important as we also found that colorectal cancer is inherently associated with an impaired ILC3 response in the gut, and that this subsequently results in an altered microbiota composition, decreased type-1 immunity, and more aggressive and ICB-resistant tumors (26). These collective findings suggest that impaired ILC3 responses and microbiota dysregulation represent a tractable target during colorectal cancer development that contributes to ICB resistance in patients (**Fig. 2**).

As a notable fact, most patients with gastrointestinal cancer exhibit resistance to single-agent ICB (16, 17). This has been partially explained by mismatch repair status and tumor mutation load, with the notable example of "proficient" or "microsatellite stable" colorectal cancer tumors that harbor a low mutational burden being associated with poor responsiveness to ICB (27–29). The immune status of colorectal cancer tumors is another strong predictor of treatment response (30), and an intriguing paradox is that despite its poor sensitivity to ICB, colorectal cancer is considered an immunogenic cancer and patient outcome is strongly dictated by the tumor immune contexture (31). Thus, there is still a major lack of a full mechanistic understanding of why most patients with gastrointestinal cancer have no response to ICB (16, 17). To explore this paradox, we propose that gastrointestinal cancer development inherently drives microbiota dysbiosis and that this represents a major determinant modulating ICB clinical efficacy, independently of the original mutational or immune landscape of the cancer. Interestingly, in a recent study Oster and colleagues demonstrated that *Helicobacter pylori* seropositivity (a strong risk factor for gastric cancer) is associated with reduced effectiveness of ICB in mice and human patients (32). Mice colonized with the colorectal cancer-associated colibactin-producing *Escherichia coli* also exhibited increased resistance to anti–PD-1 therapy, demonstrating that opportunistic or disease-associated bacteria in gastrointestinal cancers can drive immunotherapy resistance (33). Consistent with this notion, recent metagenomic and metabolomic analyses across different cohorts of patients with colorectal cancer identified global and functional microbiota signatures associated with distinct steps of colorectal cancer development (34–36). Importantly, these studies established the presence of reproducible and disease-specific microbial alterations tractable in patients with colorectal cancer that could serve as new diagnostic and prognostic tools to detect and predict disease evolution. The impact of these signatures on therapy response remains currently unexplored and future studies are required to investigate whether and how the microbiota dynamic shifts occurring during colorectal cancer could influence ICB response.

Beyond microbial compositional changes, these metagenomic and metabolomic analyses also revealed major alterations in microbial-derived metabolites that may influence colorectal cancer progression (34–36). Investigations of the impact of these metabolite shifts in predicting ICB efficacy are currently in their infancy. In a recent study, Mager and colleagues identified that ICB-promoting effects of bacteria like *Bifidobacterium pseudolongum* or *A. muciniphila* can be induced through serum transfer from monocolonized to GF animals (10), thus demonstrating that soluble factors produced by these bacteria can modulate ICB therapy efficacy. Among these factors, the authors identified inosine as a key bacterial-derived metabolite that can promote T_H1-cell activation and ICB response through oral or systemic administration (10). Interestingly, the T_H1-cell promoting effects of inosine were context-dependent and reliant on the presence of costimulation, demonstrating that the ICB-promoting effect of the microbiota was intrinsically linked with the fitness of the immune reaction (10). In another study, Lam and colleagues demonstrated

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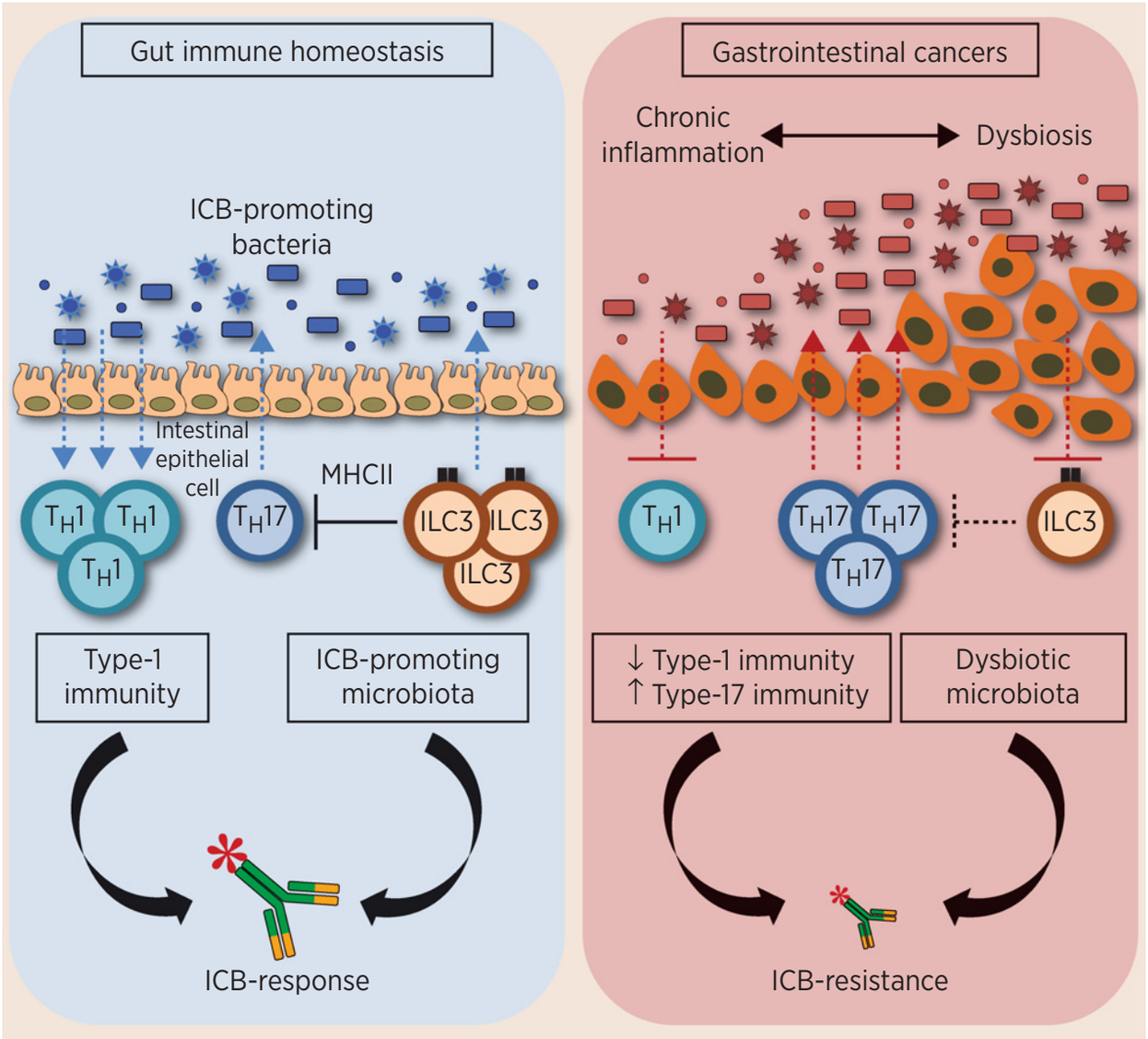

Figure 2.

Altered host–microbiota dialogue in gastrointestinal cancer impacts responsiveness to ICB. The development and progression of gastrointestinal cancers substantially impacts the gut microbiota and its ability to promote responses to ICB. The dysregulated immune response driven by gastrointestinal cancers alters the gut host–microbiota dialogue and exacerbates dysbiosis, disrupting the ICB-promoting microbiota or promoting the outgrowth of microbes that drive resistance to ICB. Cancer progression is also associated with alteration of key immune subsets needed for maintaining healthy gut immune homeostasis, like ILC3s. Disruption of the ILC3 population can exacerbate T_H17 cell–mediated inflammation, favoring the rise of microbial consortia that limit T_H1-cell responses, and orchestrate resistance to immunotherapies.

that *A. muciniphila* can produce the STING agonist cyclic di-AMP, which act as a systemic microbiota-derived product to induce type I IFN in the extra-intestinal tumor microenvironment (19). In addition, Peng and colleagues showed that short-chain fatty acid production by bacteria was positively associated with response to either anti–PD-1 or anti–PD-L1 treatment in patients with gastrointestinal cancers (22).

Altogether, these studies indicate that the microbiota composition and microbial-derived metabolites are major determinants of the efficacy of ICB. We suggest that these pathways are substantially altered during gastrointestinal diseases and represent an unappreciated mechanism driving ICB-resistance in patients with gastrointestinal cancer (**Fig. 2**). This creates an exciting opportunity to therapeutically target these pathways, restore host–microbiota homeostasis, and unleash the potential of ICB to combat these types of tumors.

Conclusion

The role of the gut microbiota in influencing ICB response is now clearly established and there is increasing interest in developing therapeutic strategies based on targeting specific bacterial consortia

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to stimulate antitumor immunity and enhance response to ICB. Novel associations between the gut microbiota and ICB interactions are routinely emerging, paving the way to a new era of "microbiota precision research." Exploration of these therapeutic strategies in gastrointestinal diseases like IBD or colorectal cancer is in its infancy, and a better understanding of how the local chronic inflammatory context and microbiota dysbiosis are impacting the success or failure of ICB is urgently needed. Another major field of interest consists of deciphering how the gut microbial metabolome evolves during these pathologic contexts or could be manipulated to improve therapeutic efficacy. Future mechanistic exploration may hold promising potential to design new therapeutic strategies aiming to overcome ICB-resistance in refractory gastrointestinal cancers, which can further be translated to the treatment of other types of extra-intestinal cancers exhibiting resistance to ICB therapy.

Authors' Disclosures

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