



# Immunomodulation by the Commensal Microbiome During Immune-Targeted Interventions: Focus on Cancer Immune Checkpoint Inhibitor Therapy and Vaccination

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Emerging evidence in clinical and preclinical studies indicates that success of immunotherapies can be impacted by the state of the microbiome. Understanding the role of the microbiome during immune-targeted interventions could help us understand heterogeneity of treatment success, predict outcomes, and develop additional strategies to improve efficacy. In this review, we discuss key studies that reveal reciprocal interactions between the microbiome, the immune system, and the outcome of immune interventions. We focus on cancer immune checkpoint inhibitor treatment and vaccination as two crucial therapeutic areas with strong potential for immunomodulation by the microbiota. By juxtaposing studies across both therapeutic areas, we highlight three factors prominently involved in microbial immunomodulation: short-chain fatty acids, microbe-associate molecular patterns (MAMPs), and inflammatory cytokines. Continued interrogation of these models and pathways may reveal critical mechanistic synergies between the microbiome and the immune system, resulting in novel approaches designed to influence the efficacy of immune-targeted interventions.

**Keywords:** microbiome, immune checkpoint inhibitors, vaccines, innate immunity, immuno-oncology, adaptive immunity

## INTRODUCTION: THE MICROBIOME AND THE IMMUNE SYSTEM

Humans are colonized by trillions of microbes collectively termed the microbiome, consisting of bacteria, archaea, viruses, fungi, and protists (1). Together, these interrelated microbial communities represent a rich source of metabolites and ligands that broadly influence human biology, including nutrient digestion, tissue homeostasis, neuroendocrine signaling, and the development and maintenance of the immune system (reviewed in 2–4). It is becoming clear that the microbiome modulates diverse immune processes, from defense against infection (5–7) to antibody production (8, 9), and from inflammation (10–12) to autoimmunity and allergy (13–15).

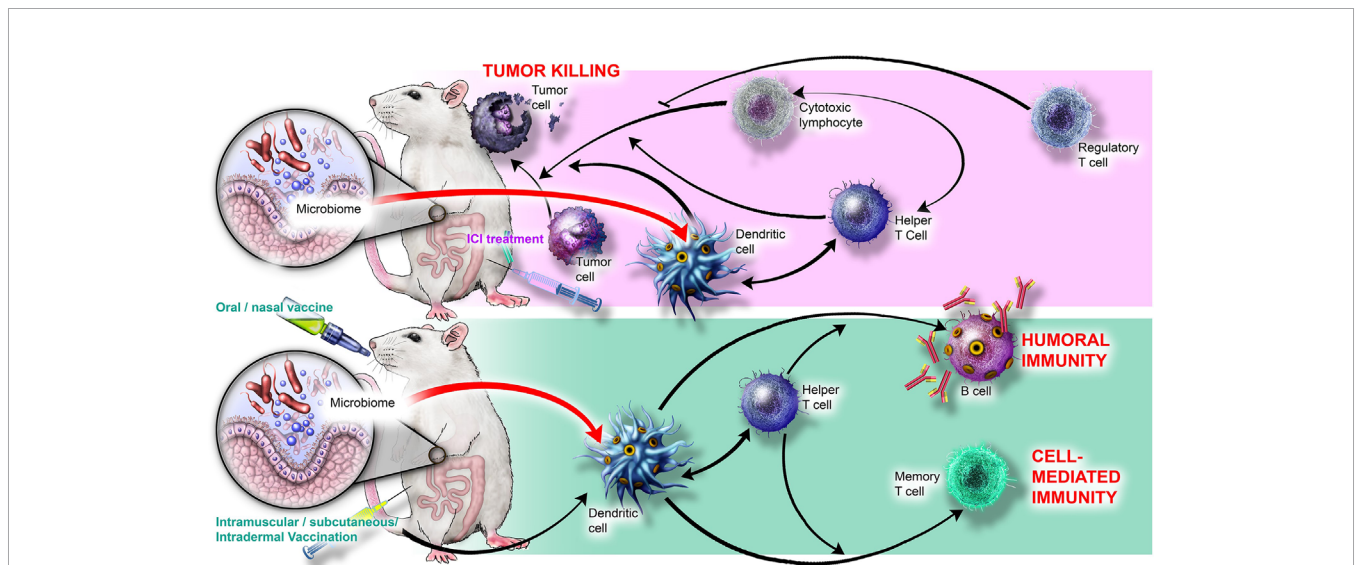
Immune-microbe interactions also directly regulate homeostasis and development of immune cells such as antigen-presenting cells (16–19) and T cells (20–24). Importantly, the microbiome significantly influences the host response to immune-targeted interventions (25–31), which harness the immune system to treat or prevent diseases including infections, allergy, autoimmunity, inflammatory disorders, and cancer. Two of the most prominent immune interventions currently employed in the clinic are cancer immune checkpoint inhibitors and vaccines, which together are the focus of this review; key cellular players and interactions involved in immune checkpoint inhibitor-mediated tumor killing and vaccine-induced immunity are presented in **Figure 1**.

In the past decade, immune checkpoint inhibitors (ICIs) have become an essential pillar of treatment for numerous cancers (32–34). Currently employed ICIs are monoclonal antibodies that block specific immune checkpoint receptors (CTLA-4, PD-1) or ligands (PD-L1) on the surface of lymphocytes or tumor cells, respectively (32, 34). Normally, immune checkpoint signaling prevents development of hyperactive immune responses and thus damage to healthy tissues (32). However, these checkpoints are exploited by tumor cells to evade immunosurveillance, block anti-tumor cytotoxic T lymphocytes (CTLs), and induce immunosuppressive regulatory T cells (Tregs) (35, 36). By blocking immune checkpoint receptor-ligand interactions, ICIs can restore endogenous anti-tumor immune responses and disrupt cancer progression. However, not all individuals respond to ICIs, and recent evidence suggests the microbiota may play a role in ICI responsiveness by modulating the immune system, particularly

the abundances and functions of NK cells, CTLs, and Tregs (37–43).

Equally consequential, vaccines have revolutionized our ability to prevent a myriad of infectious diseases and have had a long-standing impact on global human health (44, 45). During vaccination, individuals are exposed to a foreign antigen, sometimes in the presence of an exogenous adjuvant, to activate the immune system. Immunization leads to development of immune memory: molecules and cells that are able to recognize and eliminate the corresponding pathogen before infection can be established (46). Protection is often mediated by humoral production of antigen-specific antibodies produced by B cells, though cell-mediated protection by T cells is also important for protection elicited by certain vaccines (47). Orchestration of vaccine-specific humoral or cell-mediated immunity requires finely-tuned interactions between antigen-presenting cells (APCs), B cells, and T cells (48–51). However, these cellular interactions may be predisposed to particular responses, which subsequently influence vaccine outcome, depending on the underlying immune state. This “immune tone” comprises the cytokine milieu, basal expression of surface proteins that mediate cell-cell interactions, and antigen presentation by APCs, all of which can be influenced by the microbiome (4, 52–54).

In this review, we summarize key findings in the literature that demonstrate the effect of the microbiome on outcomes of immune interventions, with a focus on ICI treatment and vaccination as the most studied examples. We discuss evidence that immunotherapies can influence the microbiota and that age plays a role in the effects of the microbiota on the immune



**FIGURE 1** | Cellular players and interactions involved in immune checkpoint inhibitor-mediated tumor killing and vaccine-induced immunity. (Top) ICIs stimulate cytotoxic lymphocytes, Th1 helper T cells, and DCs to kill tumor cells; Tregs inhibit killing. MAMPs produced by bacteria such as *B. thetaiotaomicron* and *B. fragilis* may interact with TLR2 and TLR4 on DCs and stimulate Th1 polarization and synergize with ICI activity. Microbial metabolites such as inosine (produced by *B. pseudolongum*) may also contribute to ICI efficacy by stimulating T cell proliferation. (Bottom) Live, inactivated, or molecular vaccination ultimately activates DCs and helper T cells to yield humoral immunity (B cell antibodies) and cell-mediated immunity (memory T cells). MAMPs, such as flagellin and peptidoglycan, interact with PRRs to stimulate B cells and Tfh cells, thereby augmenting vaccine response. Microbially produced SCFAs may also stimulate DCs.

system, as well as discuss the effects of live biotherapeutic products and prebiotics. We highlight studies that identify cellular and molecular mechanisms by which the microbiome modulates the immune system during immune interventions. Taken together, these studies reveal common microbial and immune elements across both ICI treatment and vaccination that have the potential to shape immune responses across diverse therapeutic spheres.

## LINKING THE MICROBIOME AND RESPONSE TO IMMUNE CHECKPOINT INHIBITOR TREATMENT

Immune checkpoint inhibitor (ICI) therapy can improve long-term outcomes in a number of different cancer types, such as melanoma, non-small cell lung cancer (NSCLC), and urothelial cancer. However, the majority of patients experience cancer recurrence or do not respond to treatment (55). A number of factors that are partially predictive of ICI responsiveness have been identified (reviewed in 56, 57). Recently, the gut microbiome, which is known to play a role in the development and function of the immune system, has also been suggested as a determinant of ICI efficacy (58–63). As a result, these studies have sparked interest in the gut microbiome as both a diagnostic and therapeutic target in the context of cancer immunotherapy. In the following sections, we review the body of clinical and preclinical studies that demonstrate a role for the gut microbiome in ICI responsiveness. We highlight potential microbial, molecular, and immune mechanisms by which the microbiome may influence response to ICI treatment, and discuss whether ICI treatment reciprocally modulates the microbiome.

### Clinical Findings

Given the role of the gut microbiota in the development and function of the immune system, it is unsurprising that the microbiome may influence ICI outcome measurements in the clinic. Several studies demonstrate that improved patient responses to ICI are associated with a “healthy” gut microbiome, as measured by higher diversity (61, 64). Similarly, antibiotic treatment either shortly before or during ICI therapy has been shown to influence outcomes (65–69). Conversely, other studies have found little to no impact of antibiotics on treatment outcome (70). It is likely that these discrepancies are due in large part to the wide range of antibiotic classes, cancer types, and treatment regimens captured by these clinical studies. Thus, additional work is required to validate these observations and better understand the impact of antibiotics and microbiome diversity on ICI efficacy.

A number of recent studies have identified unique microbial signatures that are associated with ICI treatment outcomes (Table 1). In particular, metastatic melanoma patients that respond to ICI therapy tend to have high abundances of *Faecalibacterium prausnitzii* (59–61, 64), which comprises a large proportion of the human gut microbiota and is known to

influence immune function *via* production of short-chain fatty acids (SCFAs) (72–74). In addition to *F. prausnitzii*, other bacteria have been associated with ICI responses in melanoma patients, including *Gemmiger formicilis* (63), *Dorea formicigenerans* (60), and *Ruminococcus bromii* (61), all within the order Clostridia, as well as *Bacteroides thetaiotaomicron* (60), *Holdemania filiformis* (60), and the Actinobacteria *Bifidobacterium longum* and *Collinsella aerofaciens* (62), which have been shown to promote CD4+ T helper cell 1 (Th1) polarization (75). In contrast to these studies in melanoma patients, a study in a cohort of patients with non-small cell lung carcinoma, renal cell carcinoma, and urothelial carcinoma found that ICI treatment outcome was influenced by abundance of *Akkermansia muciniphilia* and *Enterococcus hirae*, suggesting that beneficial microbial signatures could be cancer specific (58). It was recently demonstrated that *E. hirae* harbors a bacteriophage encoding a MHC class I-binding protein that induces a CD8+ T cell response and cross-reacts with cancer antigens (76), which could explain the influence of *E. hirae* on ICI treatment. The presence or absence of key microbial taxa may enable the stratification of patient populations and predict potential ICI treatment outcomes based on microbiome composition. Additionally, these findings suggest that the identification of key bacterial species may facilitate development of adjunct therapies to improve ICI outcomes, such as fecal matter transplants (FMT) or probiotics (77–81). Together, these approaches may accelerate development of treatment regimens to improve ICI therapeutic outcomes.

### Pre-Clinical Findings

The clinical observation that the microbiome can influence ICI treatment response is generally recapitulated in pre-clinical studies of microbiome disruption or supplementation with fecal material, individual microbes, or microbial metabolites (Table 2). Mice with different microbial communities, obtained from different vendors, have been shown to respond differently to ICI treatment (82). Microbiota depletion *via* administration of a cocktail of broad-spectrum of antibiotics containing ampicillin, streptomycin, and colistin reduces the efficacy of anti-PD-1 and anti-CTLA-4 mAbs, both alone and in combination (58, 83, 85). Furthermore, this effect was observed in multiple tumor types and corresponding models including melanoma (RET), renal cell carcinoma (RENCA), colon cancer (CT26, MC38), and fibrosarcoma (MCA-205), suggesting that the microbiota may contribute to treatment responsiveness in a broad array of cancers (58, 83, 85).

Fecal microbiome transplant (FMT) from patients into mice has revealed differential effects of microbiota from ICI responders and non-responders, demonstrating microbiome-mediated immunomodulation and impact on ICI outcome. For example, germ-free (GF) or antibiotic-treated mice receiving responder FMT accumulated Th1-polarized cells in the tumor microenvironment following ICI treatment (58). Another study found that GF mice receiving responder FMT had higher frequencies of CTLs, while those receiving non-responder FMT had higher frequencies of immunosuppressive Tregs and Th17 cells (61). Similarly, a third study found that mice receiving

**TABLE 1 |** Gut microbial composition is associated with the efficacy of immune checkpoint inhibitor therapy in patients.

Cancer Type (number of patients)	Immune checkpoint inhibitor	Identified factor	Key associations	Reference
Metastatic non-small-cell lung carcinoma (74)	anti-PD-1	Antibiotic prescription (within 3 months prior)	No association with progression-free survival	70
Metastatic renal cell (121) and non-small-cell lung (239) carcinomas	anti-PD-L1	Antibiotic usage (within 30 days prior)	Reduced ICI response <sup>a</sup>	65
Metastatic renal cell (67), non-small-cell lung (140), and urothelial carcinoma (42)	anti-PD-1 or anti-PD-L1	Bacteria: <i>Akkermansia muciniphila</i>	Enhanced ICI response <sup>a</sup>	58
Metastatic melanoma (26)	anti-CTLA-4	Bacteria: <i>Faecalibacterium</i> spp. and Firmicutes	Longer progression-free survival	59
Metastatic melanoma (39)	anti-CTLA-4	Bacteria: <i>Faecalibacterium prausnitzii</i> , <i>Bacteroides thetaioamicron</i> , <i>Holdemania filiformis</i>	Enhanced ICI response <sup>a</sup>	60
	anti-PD-1	Bacteria: <i>Dorea formicigenerans</i>	Enhanced ICI response <sup>a</sup>	
	anti-CTLA-4 or anti-PD-1	Xenobiotic: anacardic acid	Enhanced ICI response <sup>a</sup>	
Metastatic melanoma (43)	anti-PD-1	Bacteria: <i>Faecalibacterium prausnitzii</i> ; <i>Ruminococcus bromii</i> ; microbial diversity baseline	Enhanced ICI response <sup>a</sup>	61
Metastatic melanoma (42)	anti-PD-1; anti-CTLA-4	Bacteria: <i>Bifidobacterium longum</i> ; <i>Collinsella aerofaciens</i> ; <i>Enterococcus faecium</i>	Enhanced ICI response <sup>a</sup>	62
Metastatic melanoma (27)	anti-PD-1; anti-CTLA-4	Bacteria: Microbial community richness	Longer progression-free survival	64
Metastatic non-small-cell lung carcinoma (142)	anti-PD-1; anti-PD-L1	Antibiotic treatment (concomitant)	Shorter progression-free survival and overall survival	67
Metastatic renal cell carcinoma (69)	anti-PD-1	Antibiotic usage (within 2 months prior)	Shorter progression-free survival	66
Metastatic melanoma (568)	anti-PD-1; anti-CTLA-4	Antibiotic usage (within 3 months prior)	Shorter overall survival	68
Metastatic non-small-cell lung carcinoma (2208)	anti-PD-1; anti-CTLA-4	Antibiotic usage (within 3 months prior or concomitant)	Shorter median overall survival	69
Metastatic melanoma (50)	anti-CTLA-4	Bacteria: <i>Faecalibacterium</i> spp.; <i>Gemminger</i> spp	Longer progression-free survival	63
		Metabolite: fecal SCFA butyrate	Shorter progression-free survival	
Metastatic non-small cell lung carcinoma	anti-PD1; anti-PD-1	Bacterial delivery: <i>Clostridium butyricus</i>	Longer progression-free survival	71

<sup>a</sup>Response determined by biomarker-based disease progression criteria.

responder FMT displayed elevated levels of tumor-specific CTLs, but not Tregs (62). Together, these studies suggest that the microbiome from patient ICI-responders elicits anti-tumor immunity upon FMT, which in turn promote ICI response. FMT, as a critical research tool, not only demonstrated the direct modulatory effect of gut microbiome, but also opens the door to identify key microbes and/or their metabolites that stimulates the immune response.

Individual microbes have also been identified that promote ICI response by inducing Th1 polarization. Oral supplementation with *Bacteroides fragilis*, *B. thetaiotaomicron*, or *Burkholderia cepacia* restored ICI efficacy in antibiotic treated mice (83). In particular, *B. fragilis* potentiated ICI treatment by stimulating a Th1 response within the tumor-draining lymph nodes (83). This effect was mediated by stimulation of TLR2 and TLR4, which recognize microbe-associated molecular patterns (MAMPs), leading to the maturation of intratumoral dendritic cells (DCs). In a separate study, oral delivery of *A. muciniphila* and *E. hirae* to antibiotic-treated mice increased the incidence of central memory CD4<sup>+</sup> T cells within the tumor bed, mesenteric lymph nodes, and draining lymph nodes and also induced production of IL-12 by DCs, a Th1 cytokine that plays a role in ICI response (58). Another

study using mice with low baseline ICI response due to a unique microbial community found that administration of *Bifidobacterium* restored ICI response by stimulating antigen presentation by DCs and CTL activation (82). Additionally, a recent study found that *Bifidobacterium pseudolongum* was enriched in colon tumors of mice that responded to ICI treatment, and that colonization of germ-free mice with *B. pseudolongum* promoted ICI response (86). The authors found that this effect was mediated by stimulation of the adenosine A<sub>2A</sub> receptor on T cells *via* microbially-produced inosine. Oral delivery of inosine promoted clearance of colon, bladder, and melanoma tumor models in combination with anti-CTLA-4 and CpG treatment.

In addition to inosine, other microbial metabolites have also been shown to mediate the effect of microbes on ICI outcomes. In one study, delivery of a consortium of 11 bacterial species isolated from healthy human fecal material improved ICI response and induced a robust expansion of IFN- $\gamma$  producing CTLs (84). However, administration of the heat-killed consortium failed to recapitulate the effects, suggesting that active colonization is required. The authors found that mice receiving the live consortium displayed increased levels of mevalonate and

**TABLE 2** | Impact of gut microbiome on immune checkpoint inhibitor therapy: selected preclinical studies.

Tumor Cell Model (Cancer Type) <sup>a</sup>	Treatment	Immune checkpoint inhibitor	Key findings	Reference
B16.SIY (M)	Comparison of mice from different vendors and different microbial communities	anti-PD-L1	Differential tumor growth in mice from different vendors; Bifidobacterium promotes antitumor immunity and anti-PD-L1 efficacy	82
MCA-205 (FS) RET (M) MC38 (CRC)	Antibiotic cocktail: ampicillin, streptomycin, & colistin	anti-CTLA-4	<i>Bacteroides fragilis</i> promotes anti-CTLA-4 efficacy via TL2/TLR4 host signaling	83
RET (M) RENCA (RCC) MCA-205 (FS) MC38 (CRC)	Antibiotic cocktail: ampicillin, streptomycin, & colistin	anti-PD-1 ± anti-CTLA-4	Antibiotic exposure decreased ICI efficacy; oral supplementation with <i>Akkermansia muciniphila</i> restored the efficacy of ICI	58
<i>Bra<sup>N600E</sup>Pten<sup>-/-</sup></i> (mouse-derived melanoma)	Colonization with a consortium of 11 fecal strains	anti-PD-1 ± anti-CTLA-4	Colonization with an 11-strain consortium induces IFN-γ producing CD8 <sup>+</sup> T cells and increases ICI efficacy	84
CT26 (CRC)	Antibiotic: ampicillin, streptomycin, & colistin (cocktail); vancomycin; colistin	anti-PD-1	Antibiotics decreased efficacy of anti-PD-1 therapy and altered glycerophospholipid metabolism	85
MC38 (CRC) CT26 (CRC)	Prebiotic supplementation: butyrate	anti-CTLA-4	SCFA butyrate supplementation reduces efficacy of anti-CTLA-4	63
MC38 (CRC) MB49 (RCC) B16-F10 (M) Genetic CRC (Msh2)	Colonization with <i>Bifidobacterium pseudolongum</i> ; delivery of inosine and microbial ligands	anti-CTLA-4	Microbial-derived inosine activates anti-tumor T cell via the adenosine A <sub>2A</sub> receptor in combination with T cell costimulation by MAMPs	86

<sup>a</sup>M, melanoma; FS, fibrosarcoma; CRC, colorectal carcinoma; RCC, renal cell carcinoma.

dimethylglycine in both cecal contents and sera, which may increase CTL activation or expansion (87, 88). Interestingly, the effect of the consortium was independent of a number of key innate signaling pathways, but loss of CD103<sup>+</sup> DCs or MHC class Ia was sufficient to abrogate expansion of CTLs, suggesting that the consortium may interact with tissue-resident DCs in an MHC class Ia-dependent manner to promote ICI response (84).

Elevated levels of microbiota-derived short-chain fatty acids, which are known to induce anti-inflammatory Tregs (40), have also been associated with reduced survival in ICI treatment (63). DCs isolated from butyrate-supplemented mice receiving ICI therapy displayed reduced surface expression of costimulatory molecules on APCs, suggesting that SCFAs may interfere with ICI therapy by inhibiting DC maturation within the tumor-draining lymph nodes (63). Another study found that antibiotics reduced the efficacy of ICI therapy in mice and decreased plasma levels of microbiota-derived metabolites known to be involved in glycerophospholipid metabolism and glycosylphosphatidylinositol (GPI)-anchor biosynthesis (85). The authors also observed reduced levels of the inflammatory cytokines IL-2 and IFN-γ within the tumor microenvironment, leading to the hypothesis that impaired glycerophospholipid metabolism by the microbiota could dampen anticancer immune responses by muting production of proinflammatory cytokines.

Thus far, multiple studies have implicated the microbiome in modulating response to ICI treatment. Clinically, several studies have suggested that the overall diversity of the microbiome is a key determinant of ICI responsiveness (61, 89). However, other studies have identified unique microbial signatures of responders and non-responders, suggesting that the presence or absence of key taxa may be more predictive of response (58–61, 64, 66, 83).

Several bacteria identified in ICI responders have been shown to promote ICI efficacy in preclinical studies, indicating that supplementation with these strains could improve outcomes for patients undergoing ICI treatment. Recent molecular and cellular investigations have implicated several pathways by which the microbiota influence ICI treatment outcome, including SCFAs (63), inflammatory cytokines (58, 85), antigen presentation cell function (63, 82–84), and T cell polarization (58, 61, 83).

In addition to the established role of the gut microbiome in regulating immune function, there is also a growing body of evidence that bacteria present within the tumor microenvironment (collectively termed the tumor microbiome) may also impact anti-cancer responses. For example, *F. nucleatum* is known to form biofilm-like structures within tumor spheroids *in vitro* and has been shown to directly inhibit NK cells *via* engaging TIGIT (90–92). Furthermore, a recent analysis of human tumors found that different tumor types displayed unique microbial signatures and that responders to ICI had tumors containing elevated abundances of Clostridia (93). However, it is unclear what role the tumor microbiome plays as a determinant of ICI efficacy. Further investigations of the roles of these microbial communities and their impact on immune function may facilitate target-specific therapeutic approaches to promote ICI response in patients.

## Influence of Immune Checkpoint Inhibitors on the Microbiome

Immunomodulatory agents have been shown to change microbial composition, likely by modulating immune-mediated control of the microbiota (27, 94–98). Thus, an open question is whether cancer ICIs influence the microbial community.

Emerging evidence suggests that ICIs targeting different immune checkpoint proteins may differentially impact the microbiome depending on their propensity to cause gastrointestinal adverse events such as diarrhea or colitis. However, the immune-microbe interactions that mediate these distinct effects are largely unknown.

Anti-PD-1/PD-L1 drugs appear to have minimal impact on the gut microbiome, in line with a lower incidence of gastrointestinal adverse events compared to other immunotherapy drugs (99). A recent study examining the gut microbiota of melanoma patients receiving anti-PD-1 therapy found no significant differences in microbial diversity or composition (61). Similarly, anti-PD-1 therapy for either renal cell carcinoma or non-small cell lung cancer had no impact on microbiome diversity or gene content after one month treatment, despite of elevation after two months (58). In contrast, anti-CTLA-4 treatment, which is known to be associated with elevated incidence of gastrointestinal adverse events, has been found to increase the abundance of *Bacteroides* genus in mice and a cohort of metastatic melanoma patients (83). Another study also found that anti-CTLA-4 treatment was associated with a reduction of multiple Firmicute species and an enrichment of *Bacteroides*, but only in patients were experiencing acute immune-related colitis (59). Lastly, combinatorial therapy with anti-PD-1 and anti-CTLA-4 has been shown to induce limited changes in the gut microbiome (60). However, the changes observed (an increase in *Bacteroides stercoris* and a reduction in *Clostridium boltae*) are largely consistent with those seen in other studies examining anti-CTLA-4 alone (59, 60, 83).

Based on these findings, it appears that major changes to the microbial community after ICI treatment are likely the secondary result of immune-related colitis, and it is unlikely that ICIs have a direct impact on microbiome composition. As a result, regimens targeting CTLA-4 are more likely to induce microbiome shifts than those targeting PD-1 due to their greater incidence of gastrointestinal adverse events. However, there are a number of limitations to the existing studies that make it difficult to draw definitive conclusions. Several of the studies have very small sample sizes ( $n < 10$ ), making it challenging to detect potentially small changes in microbial composition. Additionally, these studies differ considerably in methodology of microbiome sequencing and analysis, which limits our capacity to compare microbial signatures across cohorts. Further complicating comparisons between studies are considerable differences between treatment regimens, ICI dosages, and cancer types. Therefore, these limitations highlight a need for additional well-controlled and thorough studies to understand the impact of ICIs on the gut microbiome and whether ICI-induced microbiome changes subsequently influence ICI treatment outcomes.

## Microbial Manipulation During ICI Treatment

Clinical and preclinical efforts suggest a link between the state of the microbiome and ICI treatment outcomes. As described above, preclinical studies in mice have found that fecal microbiota transplant, delivery of single or consortia of

microbes, or supplementation with microbial molecules can modulate ICI treatment outcomes. Whether microbiome manipulation improves ICI treatment outcomes in clinical patients is a crucial question for ongoing and future studies. In one study, fecal matter transplants (FMT) from donors that have achieved a complete response with anti-PD-1 therapy were shown to improve responsiveness to ICI in some metastatic melanoma patients (100). Additionally, FMT recipients were found to have increased expression of genes involved in the presentation of peptides on MHC-I molecules in APCs along with elevated IL-1-mediated signaling, suggesting a mechanistic link between FMT and improved ICI responsiveness in a clinical setting (100). Another recent study found that supplementation with the probiotic *Clostridium butyricum* prior to or during ICI therapy improved patient outcomes (71). Although *C. butyricum* was selectively given to patients based on symptoms of gastrointestinal upset, the effect was observed in patients with or without prior antibiotic treatment. This result suggests that promoting a normobiotic microbiome could not only relieve gastrointestinal side effects of ICI treatment as has been described (101), but may also feedback to enhance ICI treatment outcomes (102).

## LINKING THE MICROBIOME AND RESPONSE TO VACCINES

Vaccination confers protection against pathogens. However, the response to vaccination varies widely across individuals, which could greatly compromise individual and community protection (reviewed in 103, 104). Several factors that contribute to vaccine non-responsiveness in humans have been identified, including genetics (105, 106), advanced age (reviewed in 107–109), smoking (110), and comorbidities such as infection (111–113), obesity (114), malnutrition (115), kidney disease (116), and autoimmune disorders or allergy (117, 118).

Recently, clinical and preclinical studies have suggested microbial modulation of the immune system is directly responsible for the effects of the microbiome on vaccine response (summarized in **Table 3**). It is also possible the microbiome is the mediator of other factors associated with vaccine nonresponse, for example obesity or celiac disease, which are known to induce changes in the microbiome (147, 148). Thus, manipulation of the microbiota or direct targeting of microbially-regulated immune pathways could represent attractive strategies for promoting vaccine response in the broad healthy population or specific sub-populations with characteristically poor response (4, 147, 149–153).

In the following sections, we review key clinical and preclinical studies that link the microbiota and vaccine outcome, and we highlight putative immunomodulatory mechanisms by which the microbiome may influence vaccine responsiveness. We also discuss how the connections between the microbiome and vaccine response evolve over the course of a lifetime, and describe current approaches to harness the microbiota to promote vaccine response.

**TABLE 3** | Summary of preclinical studies linking microbiome and vaccine outcome.

Vaccine (route <sup>a</sup> )	Model/Treatment	Key Findings	Reference
<b>Studies in Germ-Free Models</b>			
Bovine gamma-globulin (SC)	Germ-free mice	Reduced serum IgG antibody response	119
<i>E. coli</i> O antigen (PO)	Germ-free pigs	Reduced IgA-positive cells in lamina propria	120
Sheep red blood cells (IP); Bovine serum albumin (IP) Heat-inactivated <i>E. coli</i> (PO)	Germ-free mice	Reduced serum IgG antibody response	121
	Germ-free chickens	Reduced intestinal and serum antibody (IgG, IgA) production	122
Sheep red blood cells (SC)	Germ-free mice	Reduced delayed-type hypersensitivity response; microbiota restoration restored response	123
Live attenuated Bacille Calmette–Guerin (IV)	Germ-free mice	Enhanced resistance to <i>Mycobacterium tuberculosis</i> challenge after immunization	124
Ovalbumin + complete Freund's adjuvant (SC)	Germ-free mice	Reduced ova-specific antibody response	125
Trivalent inactivated influenza (SC)	Germ-free mice	Reduced antigen-specific serum IgG	126
Attenuated human rotavirus (PO)	Germ-free mice	Enhanced antigen-specific antibody response	127
Human serum albumin + cholera toxin (PO or IN)	Germ-free mice	Reduced ova-specific plasma IgG	128
Human serum albumin + cholera toxin (PO)	Germ-free mice	Reduced ova-specific plasma IgG	129
<b>Studies of Antibiotic Treatment</b>			
Tetanus toxoid + alum (SC)	Antibiotics in mice: Clarithromycin or doxycycline (4 weeks)	Reduced vaccine-specific serum IgM antibody levels	130
Pneumococcal polysaccharides (SC)			
Hepatitis B surface antigen + alum (IP)			
Live-attenuated <i>S. typhi</i> Ty21A (IP)			
Ovalbumin + complete Freund's adjuvant (SC)	Antibiotic cocktail in mice: clindamycin, ampicillin, & streptomycin (maternal 5 days)	Reduced ova-specific antibody response in pups from antibiotic-treated dams	125
Live attenuated human rotavirus (PO)	Antibiotic cocktail in mice: Ampicillin & Neomycin (2 weeks)	Enhanced antigen-specific antibody response	127
Trivalent inactivated influenza (SC) <sup>b</sup>	Antibiotics in mice (4 weeks): cocktail of neomycin, ampicillin, Vancomycin, & metronidazole; vancomycin; polymixin B	Reduced antigen-specific serum IgG	126
Tetanus toxoid + diphtheria toxoid + acellular pertussis antigens + alum (SC)	Antibiotics in mice (4 weeks): cocktail of neomycin, ampicillin, Vancomycin, & metronidazole	No effect on antigen-specific IgG	126
HIV-gp140 + alum (SC)			
Live attenuated yellow fever YF-17D (SC)			
Human serum albumin + cholera toxin (PO; IN)	Antibiotic cocktail in mice: ampicillin, vancomycin, metronidazole, neomycin (3-4 weeks)	Reduced ova-specific plasma IgG	128
Live attenuated Bacille Calmette-Guerin (SC)	Antibiotic cocktail in mice: Ampicillin & neomycin (maternal 2-5 weeks)	Reduced vaccine-specific IgG titer	131
Pneumococcal polysaccharide-diphtheria toxoid conjugate + alum (IP)			
Meningococcal B surface proteins + outer membrane vesicles (IP)			
Meningococcal C polysaccharide-tetanus toxoid conjugate + alum (IP)			
Diphtheria toxoid + tetanus toxoid + pertussis toxoid + pertussis proteins + hepatitis B surface antigen + inactivated polioviruses + <i>Haemophilus influenzae</i> type b-polysaccharide + alum (IP)			
Trivalent inactivated influenza (SC) <sup>b</sup>			
Live attenuated Bacille Calmette-Guerin (SC)	Antibiotic cocktail in mice: ampicillin & neomycin (3 weeks)	No effect on vaccine-specific IgG titer	131
Pneumococcal polysaccharide-diphtheria toxoid conjugate + alum (IP)			
Ovalbumin + cholera toxin (PO)	Antibiotic cocktail in mice: Metronidazole, vancomycin, ampicillin, kanamycin (10 days)	Reduced ova-specific fecal IgA and serum IgG	132
Rabies vaccine iLBNSE (IM)	Antibiotic cocktail in mice: metronidazole, vancomycin, ampicillin, neomycin (4 weeks)	Reduced rabies-specific IgG, IgM, neutralizing antibodies; reduced Tfh cells, germinal center B cells, memory response	133
<b>Studies of Prebiotic Supplementation</b>			
Trivalent inactivated influenza (SC)	Prebiotic cocktail in mice: galacto- and fructo-oligosaccharides	Enhanced delayed-type hypersensitivity response; increased levels of Bifidobacteria and Lactobacilli	134
Trivalent inactivated influenza (SC)	Prebiotic cocktail in mice: fructo-oligosaccharides and inulin	No effect on delayed-type hypersensitivity response; increased levels of Bifidobacteria and Lactobacilli	134

(Continued)

TABLE 3 | Continued

Vaccine (route <sup>a</sup> )	Model/Treatment	Key Findings	Reference
Live attenuated <i>Salmonella typhimurium</i> (oral)	Prebiotic cocktail in mice: fructo-oligosaccharides and inulin	Enhanced antigen-specific antibody titer, inflammatory cytokines, and survival after pathogen challenge	135
Trivalent inactivated influenza (SC)	Prebiotic cocktail in mice: Galacto- and fructo-oligosaccharides	Enhanced delayed-type hypersensitivity response; increased levels of Bifidobacteria and Lactobacilli	136
Ovalbumin + cholera toxin (oral)	Prebiotic cocktail: acetate and butyrate	Enhanced vaccine response and production of B-cell-activating factors in dendritic cells; <b>effect was dependent on SCFA-receptor GPR43</b>	132
Trivalent inactivated influenza (SC)	Prebiotic cocktail in mice: Galacto- and fructo-oligosaccharides 2'FL	Enhanced antigen-specific antibody titer and IL-6 production in male mice, increased levels of Actinobacteria	137
Cholera toxin + ovalbumin (oral)	Prebiotic cocktail: spirulina, amaranth, flaxseed, micronutrients	Enhanced antigen-specific antibody titer and germinal B cell frequency in mesenteric lymph nodes; effect was dependent on presence of particular microbes	138
<b>Studies of Microbial Delivery</b>			
Ovalbumin + complete Freund's adjuvant (SC)	Conventionalization of germ-free mice	Enhanced ova-specific antibody response	125
Live attenuated human rotavirus (PO)	Probiotic in gnotobiotic neonatal pigs: <i>Lactobacillus acidophilus</i>	Modulated balance of antigen-specific Th1 cells and Tregs in a dose-specific manner	139
Live attenuated human rotavirus (PO)	Probiotic cocktail in gnotobiotic neonatal pigs: <i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium animalis lactis</i> Bb12	Enhanced antigen-specific Th1 response and protection from rotavirus challenge	140
Live attenuated human rotavirus (PO)	Probiotic cocktail in gnotobiotic neonatal pigs: <i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium animalis lactis</i> Bb12	Enhanced intestinal antigen-specific antibody titers, cell responses, and protection from rotavirus challenge	141
Live attenuated human rotavirus (PO)	Probiotic in neonatal gnotobiotic pigs pre-colonized with human fecal material: <i>Lactobacillus rhamnosus</i> GG	No effect on protection from rotavirus challenge; modulated production of antigen-specific Th1 cells in a dose- and colonization-dependent manner	142
Trivalent inactivated influenza (SC)	Single strain in germ-free mice: flagellated or aflagellated <i>E. coli</i> Conventionalization of germ-free mice	Enhanced antigen-specific antibody response after conventionalization or colonization with flagellated, but not aflagellated, bacteria <b>MAMP flagellin functions as an endogenous adjuvant</b>	126
Live attenuated <i>Mycobacterium tuberculosis</i> Ad85A (IM)	Pathobiont in mice: <i>Helicobacter hepaticus</i> colonization	Reduced protection from <i>Mycobacterium tuberculosis</i> <b>IL10 production reduces vaccine response</b>	143
Live attenuated yellow fever virus YFV-17D (SC)	Pathobiont in mice: MHV86, MCMV, influenza WSN, & <i>Heligmosomoides polygyrus</i>	<b>Reduced pre-immunization production of inflammatory cytokines correlates with reduced anti-YFV antibody response</b>	144
Pneumococcal polysaccharide-diphtheria toxoid conjugate + alum (IP)	FMT in antibiotic-treated mice: fecal material from untreated mice	Enhanced vaccine-specific antibody titer	131
Keyhole limpet hemocyanin + alum (SC); NP conjugated to cholera toxin (PO)	Co-housing of aged mice with young mice FMT in aged mice: fecal material from young mice	No effect on antibody responses; improved germinal center reactions independent of vaccination	145
Cholera toxin + ovalbumin (PO)	FMT in germ-free mice: fecal material from undernourished children Co-housing of mice that received different FMT Consortium of five fecal strains: <i>Bacteroides acidifaciens</i> , <i>Bacteroides fragilis</i> , <i>Clostridioides difficile</i> , <i>Costridium innocuum</i> , <i>Fusobacterium mortiferum</i>	<b>Specific microbes mediate the pro-vaccine effects of prebiotics</b>	138
<b>Studies in Genetically Engineered Host Models</b>			
Trivalent influenza (SC) <sup>b</sup>	TLR5 <sup>-/-</sup> mice	Reduced antigen-specific serum IgG for nonadjuvanted vaccines	126
Inactivated poliovirus (SC)		<b>TLR5 mediates endogenous adjuvant response to vaccine</b>	
Live attenuated yellow fever YF-17D (SC)	TLR5 <sup>-/-</sup> mice	No effect on antigen-specific serum IgG	126
Hepatitis B sAg + alum (SC)			

(Continued)



TABLE 3 | Continued

Vaccine (route <sup>a</sup> )	Model/Treatment	Key Findings	Reference
Human serum albumin + cholera toxin (PO; IN)	Nod2 <sup>-/-</sup> mice Nod2-DC(CD11c) specific deletion	Reduced ova-specific plasma IgG <b>Nod2 in DCs mediates adjuvant activity of cholera toxin</b>	128
Human serum albumin + cholera toxin (PO; IN)	Myd88 <sup>-/-</sup> ; Ripk2 <sup>-/-</sup> ; Nod1 <sup>-/-</sup>	No effect on ova-specific plasma IgG	128
<i>Lactobacillus acidophilus</i> vaccine strain expressing HIV proteins (PO)	Nod2 <sup>-/-</sup> mice	Reduced antigen-specific IgG and IgA <b>Nod2 mediates response to vaccine strain</b>	146
Trivalent influenza (SC) <sup>b</sup>	TLR5 <sup>-/-</sup> mice	No effect on antigen-specific serum IgG	131
Human serum albumin + cholera toxin (PO)	Nod2 <sup>-/-</sup> mice IL1b <sup>-/-</sup> mice	Reduced ova-specific plasma IgG <b>IL-1β production via Nod2 is required for cholera toxin adjuvanticity</b>	129
Ovalbumin + cholera toxin (PO)	GPR43 <sup>-/-</sup> mice	Reduced ova-specific fecal IgA and serum IgG; <b>reduced pro-vaccine effect of SCFA prebiotics</b> <b>GPR43 mediates pro-vaccine effect of SCFAs</b>	132

<sup>a</sup>SC, subcutaneous; IP, intraperitoneal; PO, oral; IV, intravenous; IN, intranasal; IM, intramuscular; <sup>b</sup>Noted discrepancies with regard to effect of antibiotics and TLR5<sup>-/-</sup> on response to influenza vaccine could be attributed to different underlying microbial communities across study location; Key mechanistic insights are noted in **bold text**.

## Clinical Findings

Within the last two decades, observational clinical studies have revealed associations between microbiome community composition and host vaccine responses (recently reviewed in 152, 153). One common observation is that decreased vaccine response occurs in individuals with a disrupted microbiota (1). For example, several studies suggest that a normobiotic infant gut microbiome, replete with *Bifidobacterium*, promotes vaccine response, whereas a more dysbiotic microbiome harboring excess Proteobacteria interferes with vaccination outcome (154–159). Other studies have correspondingly shown that healthy human fecal material transplanted into neonatal gnotobiotic pigs or mice promotes strong responses to vaccines, while transplantation of dysbiotic human samples (harvested from individuals with intestinal enteropathy) dampens immune responses after vaccination (138, 160). Given the important role of the microbiota to educate the immune system during early development (161), it is possible that the dysbiotic infant microbiome could lead to immune deficiencies and reduced vaccine response. However, reducing the frequency of bacterial enteropathogens with azithromycin treatment prior to vaccination did not improve poliovirus vaccine response (162), suggesting that microbial dysbiosis may interfere with vaccine response *via* complex community changes or long-lasting immune effects.

Specific immunomodulatory bacteria have also been associated with vaccine response. In infants, two parallel studies found that a poor response to rotavirus vaccine was associated with increased *Bacteroides* and *Prevotella* spp., whereas a strong response was associated with higher levels of Proteobacteria and Firmicutes, particularly *Streptococcus bovis* (163, 164). Two additional studies have found that responders tend to have higher levels of Proteobacteria, though these observations were not statistically significant (165, 166). Nonetheless, a clinical study in adults found that vancomycin treatment, which recreates a similar microbial community comprising decreased *Bacteroides* and increased Proteobacteria, temporarily increased antibody levels in response to rotavirus (167). The authors speculate that in responders, highly immunostimulatory ligands such as LPS,

peptidoglycan, or flagellin could promote viral infection or act as endogenous adjuvants to promote vaccine response.

Other immunomodulatory bacteria have been identified in studies of vaccine response in adults. A study with oral typhoid vaccine found that the gut abundance of the Firmicutes Lachnospiraceae and Ruminococcaceae was associated with early cell-mediated response to antigen after vaccination (168). Presence of the Firmicutes *Lactobacillus* and *Streptococcus*, as well as the Bacteroidetes *Bacteroides* and *Prevotella*, in the nasal microbiome were associated with positive response to a nasal influenza vaccine (169). Furthermore, a recent meta-study analysis associated Actinobacteria and Firmicutes with positive vaccine responses, whereas Proteobacteria and Bacteroidetes were associated with poor vaccine outcomes (170). Though differences in study design, vaccine strategy, patient age, as well as other confounding variables complicates the interpretation of cross-study microbial associations, published studies together point toward an association of Firmicutes with successful vaccine outcome.

Interventional clinical studies including extensive immune characterization may facilitate more mechanistic explorations of the role of the microbiome during vaccination. A recent study in humans found that disruption of the microbiota by antibiotic treatment decreased response to influenza vaccination, specifically in individuals with low pre-existing immunity (171). Characterization of the metagenome and immune tone after antibiotic treatment implicated microbial bile acid metabolism, inflammasome signaling, and the underlying inflammatory state as key players that are influenced by the microbiota in the context of vaccination. We anticipate that similar investigational clinical studies may reveal crucial microbial and immune mechanisms that interact to either increase or decrease vaccine response in humans.

## Pre-Clinical Findings

Since the 1960s, germ-free mouse, chicken, and pig models have demonstrated that the microbiome influences vaccine response (119–124). More recently, microbiota disruption *via* antibiotic

treatment in mice has corroborated these early observations (125–127, 130, 131, 133). Multiple antibiotics from different classes and with different breadths of activity have been shown to decrease vaccine response (**Table 3**). This observation suggests that diverse species may play a role in vaccine response. It also appears that the microbiome influences response to multiple classes of vaccines, including live, inactivated, and molecular vaccines (**Table 3**). Nearly all studies employing molecular or inactivated vaccines have found that microbiome disruption decreases response; in contrast, studies of live vaccines have found the microbiome can either promote, reduce, or have no effect on response (124, 126, 127, 130). These differences raise the possibility that complex interactions between the microbiome and the immune system may influence the ability of a live vaccine to colonize the host (colonization resistance), and thus have clear implications for considering the impact of the microbiome during development of live-attenuated (155, 168, 169) and viral- or bacterial-vectored vaccines (172, 173) and could also influence cellular transfection by mRNA-based vaccines.

Notably, several studies have identified specific microbial products or host factors that mediate the effects of the microbiome on vaccine response. Broadly expressed microbe-associated molecular patterns (MAMPs) may mediate key effects of the microbiota on vaccine response. Indeed, a recent study demonstrated that signaling by the pattern recognition receptor (PRR) TLR5, stimulated by the MAMP flagellin, is important for vaccine adjuvanticity (126). Depletion of the microbiota compromised parenteral vaccine response, and this defect could be reversed by colonization with flagellated bacteria but not by colonization with an aflagellated isogenic strain. Further, loss of TLR5 specifically in B cells compromised response to several unadjuvanted vaccines but did not affect response to adjuvanted vaccines. Since deletion of TLR5 did not alter baseline plasma cell phenotypes (126), the authors hypothesized that gut-derived flagellin spreads systemically and functions as an endogenous adjuvant at the site of immunization (174). However, another group was unable to reproduce the requirement for TLR5 for vaccine response (131), suggesting that differences in the baseline microbial community likely dictate the mechanisms by which the microbiome affects vaccine response. Nonetheless, the data suggest that particular microbial communities may function as an endogenous adjuvant, or that steady-state intestinal MAMP-PRR signaling can alter the systemic immunophenotype and alter response upon subsequent vaccination.

Indeed, altered homeostatic cytokine production in response to microbes has been implicated in vaccine response and protection from infection. One study found that induction of the immunosuppressive cytokine IL-10 by oral introduction of *Helicobacter hepaticus* (*Hh*) into immunocompetent mice disrupted response to intramuscular vaccination with live-attenuated *Mycobacterium tuberculosis* (143). The authors found that, compared to animals with a normal microbiota, *Hh*-colonized animals exhibited lower antigen-specific cell-mediated responses and higher infection when nasally challenged with *M. tuberculosis*.

Infusion of IL-10-receptor-blocking antibodies restored vaccine response in *Hh*-colonized animals, demonstrating that the effect was mediated by increased IL-10 signaling. However, *Hh* colonization had no effect on IL-10 expression or Treg abundances in the lung but did increase IL-10 expression in the intestines, suggesting that intestinal IL-10 and other intermediate factors likely influence systemic immunity. Whether the effects of *Hh* on intestinal IL-10 production are direct as observed for *Hh* and T cells (175), or are due to *Hh*-mediated microbiome disruption (176) remains unknown. A second study also found that colonization of mice with viruses and a helminth disrupted response to vaccination with live-attenuated yellow fever virus, and observed that at the time of vaccination, colonized animals displayed altered expression of several inflammatory cytokines which could compromise vaccine response (144). Thus, microbially-induced changes in the steady-state cytokine milieu likely affect subsequent systemic immunity to vaccines.

Other studies suggest the microbiota are required to supply immune receptor ligands for the activity of mucosal adjuvants. Two independent groups have employed immunization with albumin and cholera toxin (CTx) to demonstrate that germ-free or antibiotic-treated mice have decreased antibody responses, which are rescued by supplementation with microbial molecules (128, 129, 132). Both groups demonstrated that CTx potentiates immune receptor signaling, which require the presence of microbial molecules for full signaling activation in response to CTx. One study found that microbial peptidoglycan signaled through NOD2 during vaccination with CTx, leading to increased IL-1 $\beta$  production and generation of T-follicular helper cells and plasma cells (128, 129). A second group found that microbe-derived short-chain fatty acids (SCFAs) synergized with CT to promote vaccine response, by driving DC-mediated production of B cell activators BAFF and retinoic acid (132). Notably, the authors also found that SCFAs increased vaccine response even in the absence of CTx through the same immune pathway, demonstrating that SCFAs can independently promote pro-vaccine immune tone, in addition to mediating the adjuvant effect of CTx. It is important to note that the two groups employed different antibiotic cocktails, which likely differentially affect the microbiome, which may account for the distinct mechanisms identified.

Taken together, these preclinical studies reveal several possible mechanisms by which the microbiota can promote immune response in the context of vaccine response: as an endogenous adjuvant, as ligands for immune receptors that are potentiated by exogenous adjuvants, and as regulators of systemic immune tone. Germ-free and antibiotic-treated mice represent crucial models to parse the mechanisms by which the microbiome affects vaccine response. Another relevant model for future studies is “dirty” mice, which receive microbes from pet store mice *via* cohousing or bedding transfer, and may prove a valuable system for understanding vaccine response (177). Given that there are multiple mechanisms by which the microbiome can affect vaccine response and that these effects can vary across different vaccines, additional preclinical studies are critically important to define the microbes and immune pathways involved.

## The Microbiome Modulates Vaccine Response in Infants and in the Elderly

Though infants and the elderly are profoundly at risk for severe infection, these populations also consistently fail to respond to vaccines (reviewed in 178–180). Lack of vaccine response can be attributed to maternal antibody interference (reviewed in 181, 182), early life immune immaturity (183–185), and immunosenescence in the elderly (186–192). However, emerging evidence suggests that microbiota may also influence vaccine response at the extremes of life, likely by modulating immune development and senescence (153).

The microbiome plays a key role in the proper maturation of the immune system (reviewed in 161), which is necessary for optimal vaccine response. Alterations in the neonatal microbiome are associated with defects in vaccine response in clinical studies (154, 163, 164) and in preclinical studies (125, 131). Although the mechanisms influencing vaccine response in neonates have not been clearly defined, related studies suggest that early-life microbial stimulation could promote vaccine response indirectly by modulating immune development and function (193–195), or directly by regulating adjuvant activity during vaccination (126). The possibility also exists that microbial dysbiosis during early life could interfere with vaccine response later in life, due to defects in microbial-immune imprinting (reviewed in 196), as has been observed for other immune-related disorders (9, 14, 15, 197). Although this idea has not yet been examined in clinical studies of vaccine response, a study in mice found that early life microbial dysfunction did not affect later vaccine response, provided the microbiome was repaired prior to vaccination (131). Thus, further studies are warranted to explore the immediate and long-term ramifications of early-life microbial dysbiosis on vaccine responses and relevant immune pathways; such discoveries could reveal novel therapeutic strategies to improve vaccine response in neonates by harnessing the microbiota.

In the elderly, emerging evidence suggests that age-induced changes in the microbiome contribute to immunosenescence (145, 198–200) and could thereby mediate changes in vaccine response (201). Indeed, defective intestinal germinal center reactions in aged mice were rescued by transplantation with fecal material from young mice (145), which could suggest an improved capacity for vaccine response after fecal transplantation. In humans, immunophenotypic similarities have been observed between elderly subjects and adults with antibiotic-induced microbial dysbiosis, consistent with the idea that age-mediated effects and antibiotic-mediated effects on vaccine response are both effected by the microbiota (171, 192). Others have speculated that immune senescence caused by changes in the microbiota could drive other pathological immune conditions such as asthma (202). Thus, in both elderly and neonatal individuals, the microbiome may mediate changes in vaccine response, and microbiome rehabilitation represents a promising approach to promote vaccine response in these populations.

## Microbial Manipulation to Improve Vaccine Response

Given the role of the microbiome in vaccine response in clinical and preclinical studies, a key question is whether manipulating

the microbiome can improve vaccine outcome, especially in populations at risk for poor vaccine response (182). Many clinical studies have evaluated vaccine response after dietary supplementation with probiotics, primarily species within *Lactobacillus* or *Bifidobacterium*. Recent reviews and meta-analyses of these studies highlight that there is significant heterogeneity in the ability of probiotic supplementation to increase antibody titers after vaccination (203–206). Effects are likely to be specific to particular bacterial strains, and may vary between different vaccines and adjuvants (149, 207). Several of these probiotic studies have also found correlates between probiotic supplementation and diverse measures of immune function, including serum levels of pro-inflammatory cytokines (208, 209), T cell responsiveness (210, 211), and innate immune cell activity (212, 213), although the mechanistic implications of these observations are unclear.

The effect of probiotics on vaccine response has also been evaluated in neonatal pigs, and recent studies have begun to elucidate the mechanisms by which *Lactobacillus* and *Bifidobacterium* strains promote vaccine response in this model. Early studies using gnotobiotic pigs revealed that colonization by probiotics prior to oral rotavirus immunization enhanced Th1 cellular immunity (139–141). However, prior colonization by human fecal material prevented subsequent *Lactobacillus* colonization and *Lactobacillus*-mediated effects on vaccine response (142), suggesting that an intact microbiota can obstruct the effects of probiotics and contribute to the heterogeneity of their effects across individuals.

Efforts are underway to use probiotic strains as vaccine vectors to capitalize on the vaccine-promoting and immunomodulatory effects of certain microbes, by engineering the expression of antigens to induce the desired immune responses including mucosal IgA (reviewed in 214, 215). In particular, *Lactobacilli* are known to activate the immune cell receptors NOD2, TLR2, TLR6, C-type lectin receptors, and the caspase-1 dependent inflammasome (reviewed in 216). Recently, a study demonstrated that activation of NOD2 was required for Th2 skewing and humoral immune responses to a *Lactobacillus* vaccine vector strain (146). Further work to elucidate the cell types and functional pathways modulated by *Lactobacillus* and other probiotics will contribute to understanding of key interactions between the microbiota and the immune system in the context of vaccines.

Several groups have also explored whether prebiotic supplementation improves vaccine outcome. Prebiotics are expected to promote a diverse microbiota and prevent expansion of dysbiosis-inducing microbes (149). Though animal studies suggest prebiotics can stabilize “normobiotic” microbes including *Lactobacillus* and *Bifidobacterium* and promote vaccine response (134–138), prebiotics in clinical studies have predominantly had no effect on vaccine outcome (217–222). Why prebiotics do not affect vaccine response remains unclear, as they have been shown to influence other immune-related conditions (reviewed in 149).

Taken together, these and previously discussed studies illustrate the potential of microbiome-modulating interventions

to promote vaccine response, but suggest that effective realization of such strategies is challenging. We note that the majority of studies have sought to enhance the abundance of Lactobacilli or Bifidobacteria, which are known to modulate the immune system (216, 223). These families belong respectively to the Firmicutes and Actinobacteria, which have been associated with vaccine response in a meta-analysis of clinical studies (170). It is possible that vaccine response could also be augmented by probiotic supplementation with other families within the Firmicutes, though this possibility has not been widely investigated. An outstanding question for probiotic approaches is whether it is necessary for probiotic strains to colonize within the context of an intact microbiome in order to promote vaccine response. Finally, an alternative strategy may be to leverage the pathways modulated by microbiome, either by delivery of live engineered organisms (146) or of bioactive molecules (132).

## CONCLUDING REMARKS: INTERACTIONS BETWEEN MICROBIOME AND THE IMMUNE SYSTEM HAVE THE POTENTIAL TO SHAPE RESPONSE TO IMMUNE INTERVENTIONS

Recent literature reveals that assorted microbes, metabolites, and immune factors interact to influence the patient response during cancer immunotherapy and immunization (summarized in **Tables 1–3**). To gain greater perspective into the most meaningful molecular and cellular mechanisms by which the microbiome modulates the immune system, we have juxtaposed two therapeutic spheres: ICIs and vaccines. Evidence from both therapeutic spheres highlights three elements of the microbiome that consistently play an immunomodulatory role: microbially-derived metabolites including short-chain fatty acids, microbe-associated molecular patterns (MAMPs), and inflammatory cytokines (**Figure 2**).

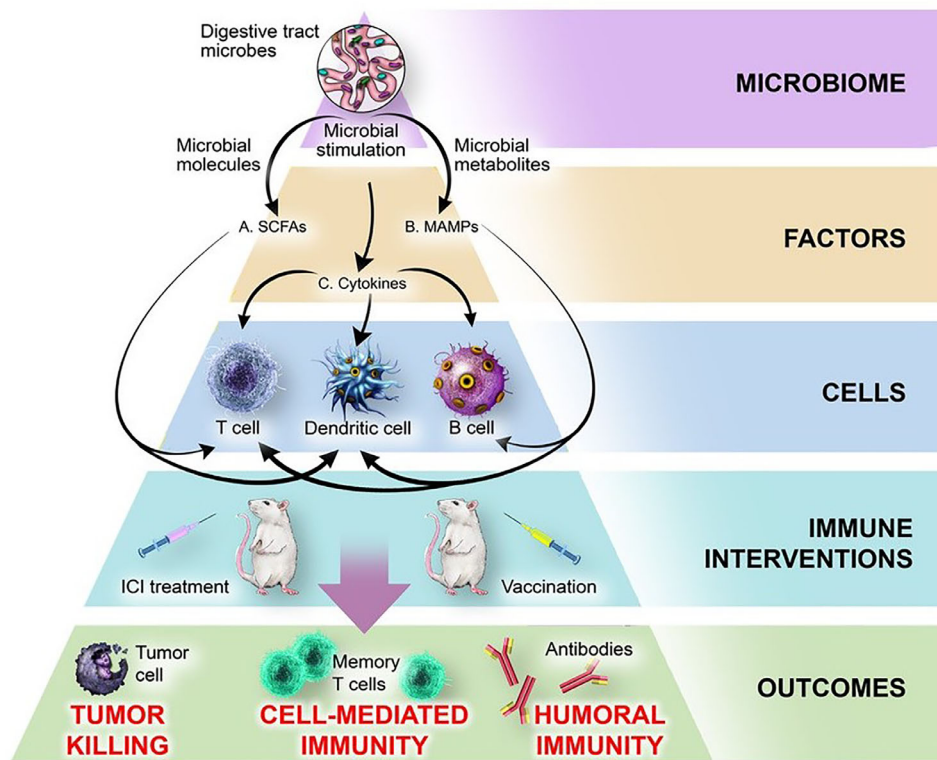
The microbiome is a rich source of diverse bioactive metabolites that can affect host biology and the immune system (reviewed in 150, 224, 225). One class of bacterial metabolites that influence response to both vaccination and ICI therapy are short-chain fatty acids (SCFAs). SCFAs are immunomodulatory metabolites and are produced by a subset of intestinal microbes (reviewed in 224, 226), some of which are implicated in clinical studies of immunotherapy and vaccine response. SCFAs such as butyrate and propionate appear to alter immunotherapy outcome by modulating CTL activation directly or indirectly by influencing DC maturation (37, 63, 227), whereas butyrate, acetate and some branched-chain fatty acids may alter vaccine response by modulating DC-mediated B cell activation (132, 228, 229). SCFAs are known to broadly influence the immune system as well as other host pathways, and have also been implicated in regulation of Tregs, allergic disease, resistance to infectious disease, obesity, intestinal barrier function, carcinogenesis, and microglia development in the central

nervous system (40, 224, 226, 230–235). However, future investigations must continue to define the biology of SCFAs, as well as other immunomodulatory microbial metabolites associated with outcomes of immune-targeted interventions (84, 85), including whether immunotherapies themselves influence the gut metabolome. Determining the unique effects of specific metabolites, including SCFAs, on immune pathways critical for ICI and vaccine response may identify key microbes and signaling pathways that could be harnessed to promote therapeutic success.

The outcomes of ICI and immunization are also influenced by MAMPs, including flagellin, unmethylated CpG DNA, peptidoglycan, muramyl dipeptide, and polysaccharides (6, 83, 126, 129). MAMPs directly and indirectly modulate activation and differentiation of immune cells (236–238), and synthetic MAMPs are already used as exogenous adjuvants during immunization to activate the immune system. In the context of microbial modulation of immunotherapy and vaccination outcomes, the cellular and molecular mechanisms by which commensal MAMPs affect B and T cells are incompletely defined. Commensal MAMPs can bind to host receptors and directly influence B cells during vaccination (126), possibly by promoting antibody production and regulating class-switching (239, 240). Similarly, endogenous MAMPs may also promote T cell function as costimulatory molecules (241–245). B and T cell functions are also indirectly influenced by MAMP-mediated modulation of APC maturation, leading to altered interactions and production of cytokines and chemokines (5, 6, 83, 129, 246, 247). By understanding the role endogenous MAMPs play in outcomes of ICI and vaccination, we may be able to identify key MAMPs and host pathways that promote immunotherapy response, which could lead to development of novel vaccine adjuvants or ICI co-therapies.

Inflammatory cytokines are emerging as a key host mediator of microbial immunomodulation (54). Both ICI and vaccine outcomes have been associated with microbial modulation of inflammatory cytokines, including IL-12, IL-1 $\beta$ , and IFN- $\gamma$  (6, 58, 83, 85, 129). These effects appear to be primarily mediated by MAMP stimulation of host receptors on APCs, but stimulation of epithelial cells with MAMPs or microbial metabolites can also alter epithelial cytokine production and impact immunity (143, 173, 248, 249). As the core communication toolbox of the immune system, cytokines represent a likely mediator through which the microbiome and intestinal immunity could influence systemic responses during ICI treatment and vaccination (58, 143, 247). Thus, microbiome-derived products could represent an indirect but powerful approach to control cytokine levels and broadly affect the immune system in diverse therapeutic contexts.

The effect of the microbiome on the outcome of immunotherapies certainly extends beyond ICI therapy and vaccination, as has been observed for non-ICI immunostimulatory treatments to control tumor growth (25, 26), anti-inflammatory treatment for irritable bowel syndrome



**FIGURE 2** | Mechanisms by which the microbiome influences response to ICI treatment and vaccination. The microbiome produces microbial factors and influences host factors and cells, thereby influencing the outcomes of immune interventions. **(A)** Microbiome-derived short-chain fatty acids (SCFAs) bind receptors including GPR43 on DCs and T cells, leading to changes in cytokine production, antigen presentation, cellular polarization, and interactions with other cell types. **(B)** Microbe-associated molecular patterns (MAMPs) including flagellin, polysaccharide A, fucosylated antigens, unmethylated CpG DNA, and peptidoglycan bind pattern recognition receptors on DCs (NOD2, TLR2, TLR3, TLR9, DC-SIGN) or B cells (TLR5) and modulate activation, cytokine production, and immune cell function. **(C)** Microbiome-dependent changes in production of cytokines (IL-1 $\beta$ , IL-12, IL-18, IFN- $\gamma$ , IL-10) produced by intestinal DCs or epithelial cells broadly affect immune cell function.

and rheumatoid arthritis (27–30), and antigen desensitization for allergy (31). It is also clear that the microbiome broadly affects the immune system, as has been well-documented in studies of infectious disease and immune function (5, 6, 230, 232, 235, 246, 247). Taken as a whole, the current literature argues that the microbiome likely plays a key role in the heterogeneity of the immune response across individuals. Though the majority of mechanistic studies to date have employed preclinical models, technological developments for *ex vivo* microbiome studies (58, 61, 138, 160), and microbiome manipulation of human subjects in the clinical setting (162, 167, 171) will facilitate our understanding of the role of the microbiota on the human immune system. As crucial immunomodulatory interactions continue to be identified, novel strategies also need to be developed to manipulate the microbiota and microbial regulation of immunity. Patient microbial profiles could be surveyed to anticipate therapeutic outcomes. Probiotics and prebiotics may play a role in repairing or supporting the microbiota (84, 138). Supplementation with immunomodulatory microbial metabolites or ligands (132), or direct targeting of microbially-regulated immune pathways (6, 247) could also bypass the effects of the microbiota on cytokine

production and cellular function. As we develop novel approaches to understand and influence the microbiome, we will also expand our ability to harness the immune system to treat or prevent disease.

## AUTHOR CONTRIBUTIONS

ALR, DJC, XL, JEN, and GS designed the work, collected and interpreted data, and approved the final work. ALR, DJC, and XL drafted the manuscript. GS, AGT, and DJH critically reviewed and approved the final work. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** All authors are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and may hold stock in Merck & Co., Inc. Kenilworth, NJ, USA.

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