REVIEW ARTICLE
Iran J Allergy Asthma Immunol
February 2022; 21(1):1-11.
Doi: 10.18502/ijaai.v21i1.8607

Gut Microbiota Modulates the Efficiency of Programmed Cell Death Protein 1 Cancer Immunotherapies

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Received: 13 October 2020; Received in revised form: 19 July 2021; Accepted: 2 August 2021

ABSTRACT

Program cell death protein 1 (PD1) is considered as an inhibitory molecule that is expressed on the surface of activated T-cells and bound to PD-L1 and PD-L2 ligands. Several types of cancer cells express PD-L1 which can bind to PD1 on the surface of tumor-specific T-cells. PD1/PD-L1 ligation triggers a pathway to protect tumor cells from an effective response of tumor-specific T-cells. Different PD1/PD-L1 blocker antibodies are clinically used to promote the T-cell response against the cancer cells. Current studies suggest that the gut microbiome impacts the efficiency of PD1 blockade therapy in cancer patients. The association of several bacterial species with PD1 responder patients has been determined. The present study reviewed previous reports on the relation between the microbiome and immune checkpoint therapy (ICT). The results of studies were discussed considering adjuvant and molecular mimicry of microbial antigens by tumor-associated antigens and metabolic effects of microbial products on ICT.

Keywords: Gastrointestinal microbiome; Immunotherapy; Neoplasms; Programmed cell death 1 receptor

INTRODUCTION

The dynamic balance between the gut microbiome and corresponding immune responses plays a key role in the protection of intestinal health and the pathogenesis and progression of the diseases. Profiling microbiota based on methods such as bacterial- and archeal-16S ribosomal RNA amplicon sequences and aligning whole genomes provides the chance for directly identifying and classifying the microbiota without the need for culturing.[1]

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According to reports, the dysbiosis of the gut microbiome affects the initiation and progress of inflammatory bowel diseases (IBDs). Supportive evidence regarding the role of the microbiome was found by observing the therapeutic effects of antibiotics in IBD patients. The findings indicated that a particular batch of probiotics can improve health in IBD patients.[2,3] Although previous studies have widely evaluated gut microbiome in IBD patients, this correlation has also been investigated in other diseases such as non-alcoholic fatty liver disease,[4] autoimmune diseases,[5] type 2 diabetes,[6] and obesity.[7] Previous pieces of evidence demonstrated that the use of penicillin was associated with an elevated risk of gastrointestinal cancers such as oesophageal, gastric, and pancreatic cancers.[8]

For instance, reported the direct effect of specific antibiotics in carcinogenesis and the role of microbiome dysbiosis in cancer creation and progression.[9] The crosstalk between microbiome and cancer is defined at several levels[10] such as the association of the effectiveness of chemotherapy materials with cancer immunotherapy and the gut microbiome.[9] Among immunotherapy strategies, evaluating the role of the microbiome on the effectiveness of the immune checkpoint therapy (ICT) approaches has received extensive attention which might be due to the widespread use of immune checkpoint therapy for cancer treatment. In the ICT, materials such as monoclonal antibodies are used for blocking the inhibitory pathways in T-cells to enhance their function. Naive T lymphocytes require two main signals for activation, namely, through engaging the T-cell receptor (TCR) to the major histocompatibility complex (MHC) and its associated peptide and attaching co-stimulatory molecule (i.e., B7-1 and B7-2) to their counterpart receptor on the surface of the T-cells (i.e., CD28).[11] Unlike the B7/CD28 pathway, the negative signal is delivered to the T-cell through the binding of other receptors such as cytotoxic T lymphocytes antigen-4 (CTLA-4) and program cell death-1 protein to their ligands leads to the suppression of the T-cell functions including T-cell cytotoxicity.[12,13] PD1 is considered as a clinically important inhibitory molecule expressed on the surface of the activated T-cells and binds to ligands including PD-L1 and PD-L2.[14] Several types of cancer cells express PD-L1 which can bind to PD1 on the surface of activated T-cells that are specific for tumor cells. Triggering the PD1/PD-L1 pathway can protect the tumor cell from an effective response of the tumor-specific T-cells. Different antibodies are blocking the PD1/PD-L1 pathway which can promote the T-cell response against cancer cells.[11,15] In 2014, the U.S. Food and Drug Administration (FDA) approved nivolumab and pembrolizumab as, two monoclonal antibodies specific for the PD1, regarding treating patients with advanced melanoma who failed to respond to ipilimumab and BRAF inhibitors. One year later, nivolumab was approved to be used for melanoma patients with normal BRAFF. Further, pembrolizumab was approved as the first-line treatment for advanced melanoma.[15] In addition to PD1-specific antibodies, PD-L1 was targeted by monoclonal antibodies. Atezolizumab, as an antibody specific for PD-L1, is licensed for the treatment of patients with bladder tumors.[16] Nowadays, there are several PD1 and PD-L1 FDA-approved monoclonal antibodies used for the treatment of different types of cancers which have been well-reviewed in other studies.[15,17]

The present review discussed the effect of the microbiome on immune checkpoint therapy with a focus on the PD1/PD-L1 blockade. To this end, the relationship between the microbiome and the ICT was reviewed, followed by describing possible mechanisms in the two main contexts including direct microbial antigen mimicry and microbial adjuvant effects, as well as the indirect metabolic and neurologic effects of the microbiome on the effectiveness of the ICT.

Gut Microbiome and PD1 Therapy

Regarding the numerous clinical trials of PD1-pathway blockade with promising results, several studies attempted to identify the role of the gut microbiome in the immune checkpoint therapy (ICT) response (Table 1). Based on these studies, heterogeneous and transient patients' responses to immune checkpoint therapies are affected by the host gut microbiome. The efficacy of experimental ICT relied on the gut microbiome in the mouse models.[9]

Sivan et al. used two mouse facilities (i.e., mice derived from two different mouse facilities), namely, Jackson Laboratory and Taconic Farms to provide distinct microbiome and found that the genetically similar mouse model significantly demonstrated a different growth rate in the induced tumor.[18] In addition, two distinct mouse fecal suspensions were transferred to the new cases to find the prophylactic population of the microbiome. Further, fecal microbiome transfer (FMT) led to significant differences in tumor infiltrated $CD8^+$T-cells and tumor growth in recipient mice and the results suggested that the gut microbiome can regulate tumor growth. The results further indicated the comparable antitumor efficiency of the microbiome when the prophylactic fecal suspension was co-administrated with anti-PD-L1 antibodies. Finally, using the 16S ribosomal RNA metagenomic studies, a positive association was observed between *Bifidobacterium* and the amount of antitumor T-cell response in the mice that were fed with the therapeutic microbiome.

Table 1. Responsive microbiota in program cell death protein 1 (PD1)/PD-L1 therapy

Target	Antibodies	Responsive Microbiome	Tumor	Study Cases	References
PD1	Clone 10f.9g2	*Bifidobacterium*	Melanoma	Mice	[18]
	Nivolumab	*Bacteroides caccae* *Streptococcus parasanguinis*	Melanoma	Human	[19]
	Combination of nivolumab and ipilimumab	*Bacteroides caccae* *Streptococcus parasanguinis* *Faecalibacterium praausnitzii* *Holdemaniafiliformis*	Melanoma	Human	[19]
	Pembrolizumab	*Bacteroides caccae* *Streptococcus parasanguinis* *Dorea formicigenerans*	Melanoma	Human	[19]
	Nivolumab Atezolizumab Durvalumab	*Akkermansia* *Muciniphila Enterococcus* *hirae*	NSCLC[a] UC[b] RCC [c] MCA-205 sarcoma[d] RET-melanoma[d]	Human and mice	[20]
	Not defined	Rumicoccaceae	Melanoma	Human and mice	[21]
	Clone 29F.1A12	Akkermansia	MSS-type[e]Colorectal cancer	Mice	[22]
	Camrelizumab	*Akkermansia muciniphila*, Ruminococcaceae spp	Hepatocellular carcinoma	Human	[23]
CTLA-4	Clone 9D9	*Bacteroides hetaiotaomicron* *Bacteroides fragilis*	Melanoma	Mice and Human Fecal microbiome transfer (FMT) to mice	[24]

(a) NSCLC: Non-small cell lung carcinoma; (b) UC: Urothelial carcinoma; (c) RCC: Renal cell carcinoma; (d) Two models of tumor-bearing mice, MCA: Methylcholanthrene; (e) Colorectal cancer with microsatellite stability

Although *Bifidobacterium* colonization in the intestine led to an increase in the anti-tumor activity of the T-cells in the mouse model, a different batch of bacteria was correlated with an increase in anti-tumor responses and tumor progression in human immune checkpoint therapy.[19]

Frankel et al, evaluated gut microbiome obtained from melanoma patients who were treated with the therapeutic dosage of antibodies specific for CTLA-4 (ipilimumab) and PD-1 (pembrolizumab and nivolumab) based on their tumor progression.[19] Four different ICT regimens were used in this study, including receiving ipilimumab (I), nivolumab with ipilimumab (NI) followed by nivolumab, pembrolizumab (P), or nivolumab (N). *Bacteroides caccae* and *Streptococcus parasanguinsis* were significantly enriched among all ICT-treated patients. The population of *Faecali bacterium praausnitzii* and *holdemania filiformis* in addition to these bacteria were enriched in responder patients treated only with NI while *Dorea formicigenerans* was the enriched bacteria in responder patients receiving only P. In this study, the screening of the metabolic pathways of the gut microbiome revealed that enzymes involved in the fatty

acid synthesis and inositol phosphate metabolism increased in all ICT-responder patients and Ipilimumab-receiving patients, respectively. Conversely, different human clinical trials failed to represent a similar microbial signal related to responder patients. In tumor patients, including non-small cell lung carcinoma, urothelial carcinoma, and renal cell carcinoma, another population of the microbiome (*Akkermansia muciniphila)* was detected in responder patients.[20] The FMT from responder patients or the replacement of *A. muciniphila* to germ-free mice increased the antitumor activity of anti-PD1 blocking efficacy.

In both human and mouse studies, different strains of bacteria in a distinct position of taxonomic classification were detected related to responder patients who received PD1 antibodies. Due to the lack of integrity in the detected bacteria and the huge diversity of the gut microbiome finding a direct specific therapeutic signal from the microbiome is difficult (Figure 1A). The possible mechanisms of microbiome effects on ICT are discussed as follows.

Proposed Mechanisms

The impact of microbiota on the efficiency of cancer immunotherapy regimens, especially for immune checkpoint therapies is unknown. Naive T-cells receive three signals for their complete activation (Figure 1B). These signals include the attachment of TCR to MHC-bind peptide (refers to the specific signal), the binding of co-stimulatory receptors to their ligands (adjuvant effector co-stimulatory signal) for preparing the second signal, and finally, the types of produced-cytokines in the antigen-presenting microenvironment affecting the T-cell subtype differentiation (i.e., Th1, Th2, and Th17). From the three-signal point of view, the mechanisms for explaining the anti-tumor activity of the microbiome in immune checkpoint therapy (ICT) can be presupposed in the three contexts. In the first context, the mechanisms support the direct activation of T-cells against microbial peptides (first signal) which are similar to tumor-associated antigens (TAA). Additionally, the mechanisms can be presupposed through the binding of microbial antigens or pathogen-related molecular patterns (PAMPs) to pathogen recognition receptors (PRRs) on the antigen-presenting cells (APCs) leading to the APC activation and co-stimulatory signal preparation (second or adjuvant effect). In addition to the events that occurred in the immunological synapse, environmental changes in the tumor microenvironment (TME), including the metabolic alterations induced by a specific batch of microbial species affect the activation and function of T-cells, as well as the regulation of tumor cell growth. These three proposed mechanisms are further discussed in the following sections.

From another viewpoint, the second signal hypothesis refers to all microbiome effects which are beyond the antigenic mimicry leading to the bystander activation of immune responses.[9] The bystander activation created by microorganism infection can be performed by two different methods. Systemic inflammatory responses occurred in the infection can induce a defensive state in the body (the situation in the favor of antitumor immune responses) and microbe-induced tissue destruction may lead to antigen spreading and thus exposure of the hidden antigens which are not with their self-tolerance. In addition, the preparation of costimulatory signals in the activated dendritic cells due to microbial antigens or PAMPs completely activates naïve autoreactive T-cells. The initiation of the microbial bystander activation requires an inflammation or a pathological injury in the gut mucosa where immune responses failed to protect the submucosa from the invasion of microbes. However, microbial bystander activation may involve in the pathogenesis of autoimmune diseases.[25,26]

Adjuvant and Antigen Mimicry Effects

Some studies focused on the stimulatory interactions of bifidobacteria and immune responses.[27,28] For example, Sivan et al, reported that the oral administration of the heat-inactivated prophylactic genus of *bifidobacterium* abrogates the therapeutic effects of bacteria on tumor growth and T-cell responses.[18] Thus, their results supported the non-adjuvant mechanism of action at least for killed bacteria. The therapeutic effect of administrated *bifidobacterium* was not with the translocation of bacteria to immune organs around the gut mucosa such as mesenteric lymph nodes, spleen, and tumor. The lack of this translocation suggesting bacterial-derived anti-tumor effects differed from the chronic and acute intestinal inflammatory response to infection, the responses involved in diseases including inflammatory bowel disease (IBD).[29] However, Routy et al detected an adjuvant effect from *Akkermansia muciniphila*

(*A. Muciniphila)* and *Enterococcus hirae (E. hirae*) improving PD1 blockade therapy.[20] *A. muciniphila* and *E. hirae* induced dendritic cells to produce IL-12 which is a Th1 polarize cytokine (Figure 1A). Microbiome-induced IL-12 can change the efficacy of ICT since the T-cell fate is affected by the cytokines which are produced in the immunological synapse of the dendritic cell and T-cells. Vétizou et al demonstrated this effect for the anti-CTLA-4 therapy in tumor-bearing mice where IL-12 neutralizing antibodies abolished the antitumor activity of anti-CTLA-4 antibodies.[24] On the other hand, transferring the stool from responder patients led to an increase in the expression of PD-L1 on the splenic T-cell, leading to the tolerogenic function of T-cells.[20] IL-12-producing dendritic cells increased during the changes induced by antibiotics in the gut microbiome as well.[30] A systemic increase of IL-12-producing CD8+ dendritic was detected when the mice were treated with vancomycin, which was correlated with the amelioration of the anti-tumor activity of adoptive T-cell therapy. These findings of the role of IL-12 and positive effects of different populations of bacteria with a variety of expressing antigens on anti-PD1 therapy support adjuvant effects mechanism.

However, the exact mechanisms of adjuvant effect should be further evaluated since the microbiome-induced inflammatory condition is not considered as a diagnostic sign for the ICT. A previous study revealed the lack of replacing therapeutic bacteria from the mucosa into the submucosa.[18]

The cross-reactivity of immune responses between bacterial xenoantigens and molecular patterns in human cells is considered as another hypothesis for explaining the immunotherapeutic effect of the microbiome in the ICT. Abbas et al previously highlighted the role of microbial antigens in producing the autoantibodies in autoimmune diseases such as rheumatic fever and reported that the similarity of streptococcal protein to heart muscle proteins led to the production of cross-reactive antibodies.[13] Although no study has detected cross-reactive T-cells or antibodies in ICT-receiving patients, Vétizou et al, demonstrated the T-cells which were specific for microbiome peptide ameliorated anti-tumor activity.[24] The adoptive transfer of *Bacteroidesfragilis*-specific T-cells restored the efficacy of CTLA-4 blockade in the mouse model of MCA205 fibrosarcomas.[24] The alignment of the peptide sequence of T-cell epitopes and microbial peptides revealed several homologies as well. For example, mutated CSMD as an antigen in melanoma contains a peptide with the capability for eliciting a polyfunctional T-cell response and has 80% homology to a known *Burkholderia pseudomallei* antigen.[31] In general, further studies are needed to determine whether the microbiome can directly induce the immune response and direct it to an antitumor performance.

Metabolic and Neural Circuit Effects

In other studies on a microbiome signal, metabolome profiling was evaluated and it was estimated that nearly the half amount of the metabolites in the human plasma are derived from the microbiome.[32] Several biochemical compounds were differentially defined between ICT-responded and non-responder patients and it was demonstrated that microbiome-derived products such as short-chain fatty acids (SCFAs) and inositol phosphates include immune-regulatory and anti-tumor activities.[33,34]

Oxidative phosphorylation is considered as a predominant metabolism in the naive and memory T-cells for generating energy (Figure 2). These cells utilize glucose, as well as fatty and amino acids to produce the adenosine triphosphate (ATP). A metabolic reprogramming occurs in the cells upon T-cell activation throughout the TCR to provide energy for their biological function such as cell growth and differentiation. Lipid oxidation decreases in the activated T-cells while the glycolysis of glucose and glutamine oxidation demonstrate an increase in these cells. Furthermore, activated T-cells enhance the synthesis of lipids, amino acids, and nucleotides to provide precursor molecules that are required for their growth and function. Interestingly, tumor cells use several metabolic programming similar to activated T-cells.[35] The lipid oxidation reverts during the differentiation of activated T-cells to memory cells as well.[36,37]

PD1 is regarded as a molecule that is expressed on the activated T-cells. Additionally, metabolic reprogramming occurs in the activated T-cells which are affected by the PD1 signal. These cells cannot use glucose glycolysis and amino acid metabolism while they increase the fatty acid β-oxidation of endogenous lipids.[37]A nutrient-deprivation condition may exist in the tumor microenvironment (TME) due to the high metabolic activity of the tumor cells and poor blood supply. In addition, the effector T-cell metabolism relies on the presence of sufficient nutrient ingredients

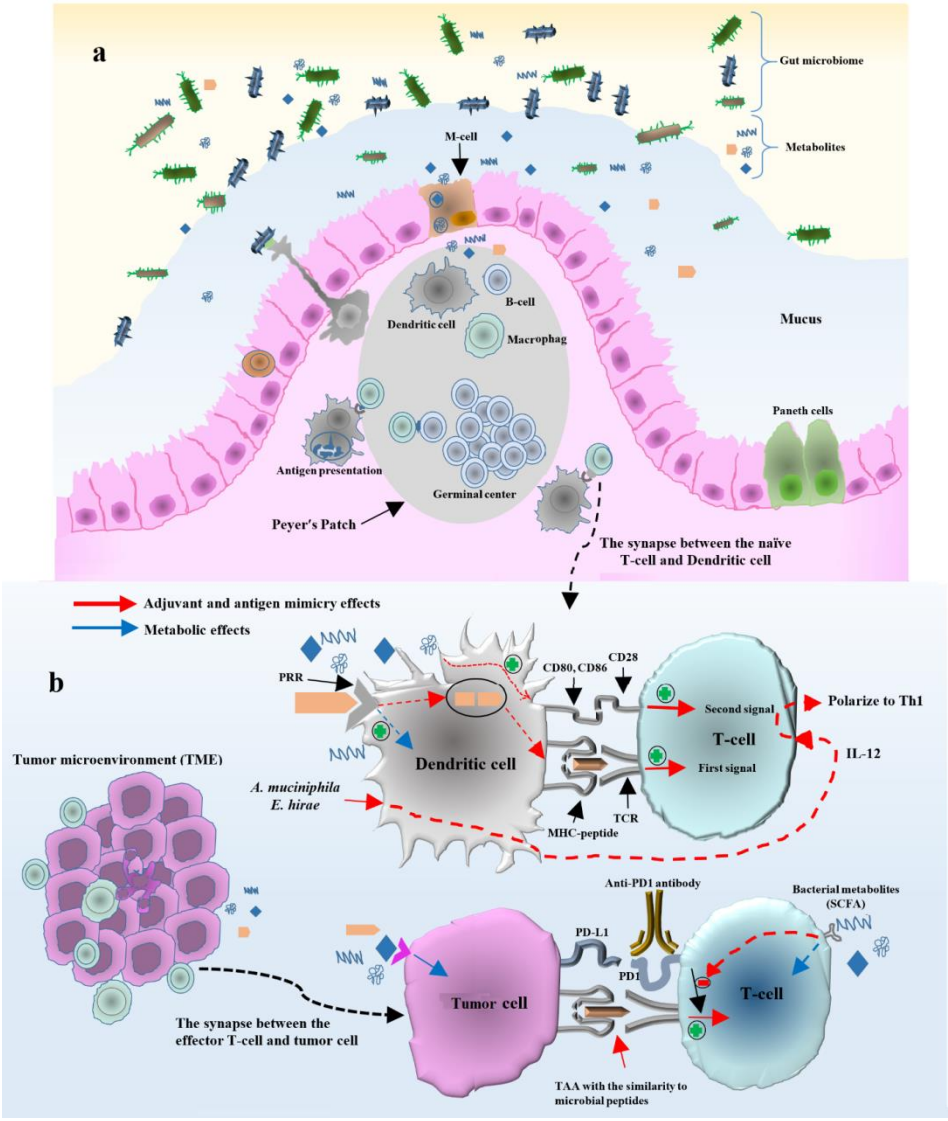

Figure 1. Schematic diagram of mechanisms for determining the impacts of the microbiota on the efficiency of the programmed cell death protein 1 (PD1)-immunotherapy of cancer. a) Microanatomy of the components of the immune system in the intestine. In the small bowel, defensin as an antimicrobial agent is produced constitutively by panteth cells located at the bottom of the epithelial crypts. Microbial antigens and metabolites can translocate through the gut epithelial using two methods, including passive diffusion, and active cell-mediated transfer. Microfold cells (M-cells) are located at a region of gut epithelium called dome epithelium and transport various substrates from the gut lumen to the underlying antigen-presenting cells. Future, some mucosal dendritic cells extend their membrane′s cytoplasmic processes between the epithelial cells into the intestine lumen to sample from the lumen antigens and then migrate to secondary lymphoid organs for presenting captured antigens. B cells located in the utter border of the dome of the peyer′s patch, recognize bacterial antigens and metabolites and then proliferate to form the germinal center of follicles in the peyer′s patch. b) Binding of microbial or pathogen-related molecular patterns (PAMPs) to pathogen recognition receptors (PRRs) on the antigen-presenting cells (APCs) leading to the APC activation and costimulatory signal preparation (second or adjuvant effect). For example, *A. muciniphila* and *E. hirae* induced dendritic cells to produce IL-12 which consider as a Th1 polarize cytokine. Microbial peptides which are similar to tumor-associated antigens (TAA) support antigen-mimicry effects of the microbiome on T-cells. The immunological synapse and environmental changes in the tumor microenvironment (TME) affect the activation and function of T-cells and the regulation of tumor cell growth.

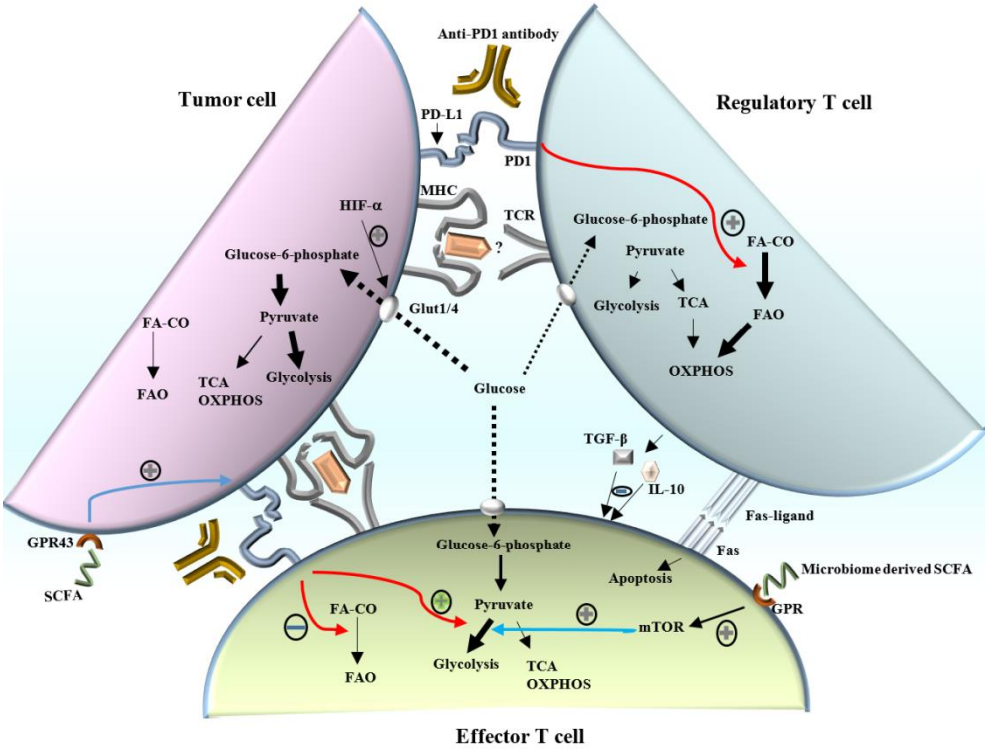

Figure 2. Schematic diagram of the impacts of the microbial-derived metabolites on the programmed cell death protein 1 (PD1)-immunotherapy of cancer. Metabolic pathways in the tumor cell, regulatory and effector T cells are affected by the PD1 signal. Glycolysis is the predominant metabolic pathway to provide energy in the effector T-cell and tumor cell while fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) are the predominant pathways in the regulatory T-cell. The SCFA (propionate) produced by *A. muciniphila* binds to G-protein coupled receptors 43 (GPR43) on the tumor cell and then delivers an apoptotic signal to the cell and can promote the expression of PD-L1 molecules as well. Microbiome-derived SCFA triggers the mTOR pathway in the effector T cell and the metabolic effect of the mTOR signal may revert metabolic reprogramming induced by the PD1 signal.

in the TME. T-cells that are inactivated by the PD1 signal can better adapt to this environment. The metabolites produced by the tumor cells such as lactate kynurenine and HIF-α impair the T-cell activity while promoting regulatory T-cell differentiation.[35] Effector T-cell and tumor cells compete for the glucose in the TME as well. Further, PD1-blocking antibodies induce the T-cells to maintain their cellular glycolysis and change the metabolic balance in favor of the effector T-cells in the TME.[38]

The metagenomics and metatranscriptomics of the microbial cDNA library are considered as the methods which provide the chance for the real-time monitoring of microbial functions.[1] Furthermore, Frankel et al and Xu et al used mass spectroscopy for profiling microbial metabolism.[19,22]

The intestinal microbiome produces a wide range of metabolites through the anaerobic fermentation of the undigested substance in food and compounds produced in the microbial and host cells. The intestinal microbiota metabolites can directly or indirectly affect the tumor progression through the immune system. Some studies evidenced the correlation between the gut microbiome-mediated changes in glycerophospholipid metabolic pathway and the efficacy of PD1 antibody immunotherapies.[22] Undigested carbohydrates are suitable substrates for microbial fermentation in the colon regarding making the SCFAs such as acetic, butyric, and propionic acids.[1] Two main molecular mechanisms are employed by the SCFAs which affect the local gut and systemic immune responses, including inhibiting histone deacetylase (HDAC), and are

considered as a ligand for G-protein coupled receptors (GPR). Among the immunoregulatory roles for the SCFAs, their anti-inflammatory effects, and the promotion of T-reg cells by increasing the FOXP3 transcription factor have received special attention.[39]

In another study, based on Routy et al, study, a hypothesis to explain how *Akkermansia Muciniphila* (*A.muciniphila*) regulates tumor progression was presented. In the proposed model, two types of cells (i.e., immune and cancer cells) were affected by SCFAs produced by *A.muciniphila*.[40] The propionate produced by *A. muciniphila* binds to G-protein coupled receptors (GPR43) on the tumor cells and then delivers an apoptosis signal to these cells. Additionally, the inhibition of HDAC6 and HDAC9 by the SCFAs in the mouse model leads to the activation of mTOR-S6K and STAT3 pathways, eventually stimulating the T-cell to differentiate to Th17, Th1, IL-10 producing T-cells, as well as anti-inflammatory IL-10+ regulatory T-cells.[40,41] The SCFAs induced T-cell to differentiate to the effector (i.e., Th1 and Th17) and regulatory (IL-10+ regulatory T-cells) cells, which are the T-cell subsets in the favor of immunogenic and tolerogenic responses, respectively. These experiences revealed that the final immunological activity of the SCFAs relies on the immunological milieu including the presence of the distinct batch of the cytokines.

An *in vivo* and *in vitro* study demonstrated that the expression of PD-L1 is precisely regulated by the Akt/mTOR pathway.[42] The microbiome-derived SCFAs can promote the PD1/PD-L1 signaling through the mTOR-mediated increase in the expression of the PD-L1 molecules. Several mechanisms can be proposed to explain the anti-tumor activity of the SCFAs in PD1 blockade therapy. According to the reported data, increased expression of PDL1 on splenic T cells restores the anti-tumor effects of PD1 blockade. Hence, SCFAs can promote tumor cell responsiveness to ICT through increased expression of PDL1. The mTOR pathway and aerobic glycolysis are essential for the functioning of Th1, Th2, and Th17 subsets while adenosine monophosphate kinase and lipid oxidation pathways are more active in T-reg and memory T-cells. Therefore, the effector T-cells are more affected compared to the T-reg when the mTOR signaling is enhanced by the SCFAs. However, it was determined that colonic T-reg cells express the GPR43, and their function and frequency increase when the GPR43 is engaged with the SCFA.[33] The mechanism can be hypothesized to explain the SCFA-induced anti-tumor activity based on the mTOR pathway, namely, the metabolic effect of the mTOR signal may revert metabolic reprogramming induced by the PD1 signal. The hypoxia-inducible factor-1α (HIF-1α), as a transcription factor under the control of the mTOR pathway, increases glycolysis and glutaminolysis such as metabolic pathways that are inactivated by the PD1 signal in the cells. Thus, the mTOR signal can maintain the glycolysis activity and then the functionality of T-cells to fight the tumor cells.

Metabolic profiling of the serum taken from cancer patients receiving PD1 antibodies revealed promising information related to the metabolic alteration in PD1 antibodies responder patients. A significant portion of metabolites in the human body derives from the gut microbiome. Xu et al demonstrated that tumor-bearing mice with the impaired gut microbiome did not respond to PD1 antibody therapy.[22] They found that the enrichment of Akkermansia bacteria in the gut was correlated with a positive response to the PD1 antibody therapy as well as the change in the glycerophospholipid metabolic pathway. Comparisons of pathway enrichment between responder and non-responder melanoma patients showed changes in metabolic functions, predictively, anabolic functions including amino acid biosynthesis enriched in responder whereas catabolic functions in non-responder patients.[21]

The effects of the microbiome on the immune system can be mediated by the nervous system. The microbiome has a role as the regulator of neuro-inflammation, brain damages, and neurogenesis. In addition, Fung et al. in their study on the intestinal-brain axis emphasized the important effect of the microbiome on immune responses and brain development.[43] Based on the evidence, several microglial abnormalities were found in germ-free mice or mice which lost their microbial flora due to the antibiotic administration. Such abnormalities included abnormal morphology, as well as a change in gene expression and impaired response to the stimuli. It should be noted that microglial abnormalities in germ-free mice were resolved through administering the SCFAs as the primary products of bacterial fermentation.[43,44]

Changing neurotransmitter production is considered as one of the pathways that microbiota can employ to regulate the immune system. Serotonin, gamma-

aminobutyric acid, norepinephrine, dopamine, and tryptamine are reported as neurotransmitters which the microbiome can affect their production.[45-47] Although some immune cells have receptors for neurotransmitters and can respond to them, further studies are needed to evaluate the antitumor effects of microbial-induced neuroimmune changes.

Triggering CD8+ T Cells in the Tumor Microenvironment

The type and number of tumor infiltrated cells can determine the score of immune responses against the tumor cells; active CTL, Th1, NK, and M1 macrophage give high immune score to the host, whereas exhausted CTL, Th2, M2 macrophage, and T-reg cells are related to the low anti-tumor immune score. The correlation between the gut microbiome and tumor infiltrated cells in patients receiving PD1 antibody therapy was also investigated in some studies. A statistically significant positive correlation between the infiltration of CD8+ T cell in the tumor-bearing mice and the *faecalibacterium genus* as well as the *ruminococcaceae* family was found in the study by Gopalakrishnan et al.[21] The high abundance of *faecalibacterium* in the gut also was correlated with higher levels of systemic circulating effector CD4+ and CD8+ T cells with a preserved cytokine response to anti-PD-1 therapy in melanoma patients[21] It was determined that Th1 response against *A. muciniphila* antigens correlated with the progression-free survival in patients receiving PD1 antibody therapy.[20] Sivan et al also confirmed the effect of the therapeutic microbiome on the intratumoral CD8+ T cell accumulation.[18] They found that tumor-specific CD8+ T cells were significantly higher in the mouse facility with therapeutic microbiome (Bifidobacterium). These accumulation data can indicate the pivotal role of CD8+ T cells in translating the effective signal from the gut microbiome to the cancer growth suppression. As proof of the hypothesis, it was demonstrated that the therapeutic effect of Bifidobacteriumfeeding was abrogated in CD8+ depleted mice.[18]

In conclusion, the present study evaluated the potential mechanisms associated with the effects of the microbiome on PD1 blockade therapy. Microbiome could be effective by adjuvant mechanisms such as the activation and regulation of inflammation, innate immunity, and T-cells. In addition, microbial antigen patterns which were similar to the tumor-associated antigen (TAA) could potentially stimulate the immune responses. It is noteworthy that adjuvant and antigen mimicry mechanisms are not specific for immune checkpoint therapy (ICT) and thus they can be implicated in all cancer T-cell therapies. The metabolic therapeutic effects of the microbiome can be mediated by bacterial metabolites or metabolic reprogramming induced by specific microbes in patients. Short-chain fatty acids, as one of the anaerobic fermentation metabolites, can perform both functions by binding to the G-protein coupled receptors and activating the mTOR pathway. Although the mTOR pathway is found to increase the PD-L1 expression, it can revert the inhibitory metabolic effects of PD1 signals.

Further, it is noteworthy to find whether the role of the microbiome for improving ICT is associated with an increase in autoimmune diseases. Accordingly, patients with ICT-related autoimmune disorders can be screened for finding a special microbiome. It is possible to detect a signal from a particular group of microorganisms that contribute to triggering the immune response against cancer and non-cancerous (autoimmunity) cells. On the other hand, autoimmune disorders can be screened in ICT responder patients with the therapeutic microbiome to determine whether the anti-tumor effects of the microbiome are a side or bystander effect of the created autoimmunity.

In the present study, several specific bacteria were identified in the responder patients although finding a precise microbial signal that can provide a therapeutic strategy has several complexities including the high number and amount, the complexity of gut microbiome and TAAs, as well as the differences of TAAs and microbiome in different individuals. The development of new approaches of personalized medicine and the entire sequencing of human cells and microbes increase the hopes for achieving a strategy regarding predicting the success of the ICT or the therapeutic use of the microbiome in the ICT.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study was funded by the Kashan University of Medical Sciences. The authors would like to thank Kashan University of Medical Sciences.

REFERENCES

1. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat RevImmunol. 2016;16(6):341-8.
2. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology. 2003;124(5):1202-9.
3. Sutherland L, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, et al. Double blind, placebo controlled trial of metronidazole in Crohn's disease. Gut. 1991;32(9):1071-5.
4. Safari Z, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). Cell Mol Life Sci. 2019;76(8):1541-58.
5. Dehner C, Fine R, Kriegel MA. The microbiome in systemic autoimmune disease: mechanistic insights from recent studies. Curr Opin Rheumatol. 2019;31(2):201-7.
6. Vallianou NG, Stratigou T, Tsagarakis S. Microbiome and diabetes: Where are we now? Diabetes Res Clin Prac. 2018;146:111-8
7. Tseng C-H, Wu C-Y. The gut microbiome in obesity. J Formos Med Assoc. 2019;118:S3-S9.
8. Boursi B, Mamtani R, Haynes K, Yang Y-X. Recurrent antibiotic exposure may promote cancer formation–another step in understanding the role of the human microbiota?. Eur J Cancer. 2015;51(17):2655-64.
9. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and anticancer immunosurveillance. Cell. 2016;165(2):276-87.
10. Daillère R, Vétizou M, Waldschmitt N, Yamazaki T, Isnard C, Poirier-Colame V, et al. Enterococcus hirae and Barnesiella intestinihominis facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. Immunity. 2016;45(4):931-43.
11. Khedri M, Rafatpanah H, Abnous K, Ramezani P, Ramezani M. Cancer immunotherapy via nucleic acid aptamers. Int immunopharmacol. 2015;29(2):926-36.
12. Capece D, Verzella D, Fischietti M, Zazzeroni F, Alesse E. Targeting costimulatory molecules to improve antitumor immunity. BioMed Res Int. 2012;2012.
13. Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology E-book. 8th ed. Elsevier Health Sciences. 2014.
14. Khedri M, Abnous K, Rafatpanah H, Ramezani M. An optimized protocol for the in vitro generation and functional analysis of human PD1/PD-L1 signal. J Recep Sig Transduct. 2018;38(1):31-6.
15. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. Int J Clin Oncol. 2016;21(3):462-73.
16. Inman BA, Longo TA, Ramalingam S, Harrison MR. Atezolizumab: A PD-L1–Blocking Antibody for Bladder Cancer. Clin Cancer Res. 2017;23(8):1886-90.
17. Wang Y, Ma R, Liu F, Lee SA, Zhang L. Modulation of gut microbiota: a novel paradigm of enhancing the efficacy of programmed death-1 and programmed death ligand-1 blockade therapy. Front Immunol. 2018;9:374.
18. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy. Science. 2015;350(6264):1084-9.
19. Frankel AE, Coughlin LA, Kim J, Froehlich TW, Xie Y, Frenkel EP, et al. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. Neoplasia. 2017;19(10):848-55.
20. Routy B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science. 2018;359(6371):91-7.
21. Gopalakrishnan V, Spencer C, Nezi L, Reuben A, Andrews M, Karpinets T, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97-103.
22. Xu X, Lv J, Guo F, Li J, Jia Y, Jiang D, et al. Gut microbiome influences the efficacy of PD-1 antibody immunotherapy on MSS-type colorectal cancer via metabolic pathway. Front Microb. 2020;11:814.
23. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, Tan D, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. J Immunother Cancer. 2019;7(1):1-7.
24. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350(6264):1079-84.
25. Christen U, von Herrath MG. Infections and autoimmunity—good or bad? J Immunol. 2005;174(12):7481-6.
26. Fujinami RS, von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev. 2006;19(1):80-94.

27. Ménard O, Butel M-J, Gaboriau-Routhiau V, Waligora-Dupriet A-J. Gnotobiotic mouse immune response induced by Bifidobacterium sp. strains isolated from infants. Appl Environ Microbiol. 2008;74(3):660-6.
28. Kawahara T, Takahashi T, Oishi K, Tanaka H, Masuda M, Takahashi S, et al. Consecutive oral administration of Bifidobacterium longum MM-2 improves the defense system against influenza virus infection by enhancing natural killer cell activity in a murine model. Microbiol and immunol. 2015;59(1):1-12.
29. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet. 2007;369(9573):1627-40.
30. Uribe-Herranz M, Bittinger K, Rafail S, Guedan S, Pierini S, Tanes C, et al. Gut microbiota modulates adoptive cell therapy via CD8α dendritic cells and IL-12. JCI Insight. 2018;3(4).
31. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N EnglJ Med. 2014;371(23):2189-99.
32. Martin FPJ, Dumas ME, Wang Y, Legido-Quigley C, Yap IK, Tang H, et al. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol Syst Biol. 2007;3(1):112.
33. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341(6145):569-73.
34. VUČENIK I, Stains J. Cancer preventive and therapeutic properties of IP6: efficacy and mechanisms. Period Biologorum. 2010;112(4):451-8.
35. Le Bourgeois T, Strauss L, Aksoylar H-I, Daneshmandi S, Seth P, Patsoukis N, et al. Targeting T cell metabolism for improvement of cancer immunotherapy. Front Oncology. 2018;8:237
36. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. Annu Rev Immunol. 2013;31(5):259-83.
37. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015;6:6692.
38. Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell. 2015;162(6):1229-41.
39. Tao R, De Zoeten EF, Özkaynak E, Chen C, Wang L, Porrett PM, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med. 2007;13(11):1299.
40. Santoni M, Piva F, Conti A, Santoni A, Cimadamore A, Scarpelli M, et al. Re: Gut Microbiome Influences Efficacy of PD-1-based Immunotherapy Against Epithelial Tumors. Eur Urol. 2018;74(4):521.
41. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. Mucosal Immunol. 2015;8(1):80.
42. Lastwika KJ, Wilson W, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of PD-L1 expression by oncogenic activation of the AKT–mTOR pathway in non–small cell lung cancer. Cancer Res. 2016;76(2):227-38.
43. Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017;20(2):145.
44. Erny D, de Angelis ALH, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965.
45. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci. 2011;108(38):16050-5.
46. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, et al. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Am J Phyiol Gastrointest Liver Physiol. 2012;303(11):G1288-G95.
47. Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M, Taketani M, et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. Cell Host Microbe. 2014;16(4):495-503.