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Microbiota, Cancer and Immune Therapy

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Abstract: The relationship between cancer and microbes is complex and not entirely known. The objective of this manuscript is to review the scientific evidence on the relationship between the microbiome, cancer and immunotherapy. A non-systematic literature review was done in the databases MEDLINE, COCHRANE, and DATABASE, and articles of greater scientific rigor, mainly reviews or prospective studies/randomized clinical trials published to date (May 2018), were selected. Terms used in the search included: microbiome, microbiota, cancer, immune checkpoint inhibitors, PD-L1, PD-1 and CTLA-4.

Key words: microbiota; cancer; immunotherapy; neoplasms

1. Introduction

Human beings are to some extent dominated by host microorganisms. [1] The relationship between cancer and microorganisms is complex and not entirely clear. Although cancer is often considered a disease with important genetic and environmental foundations, microorganisms account for approximately 20% of human malignant tumors. [2] Microorganisms present in different mucous membranes may be a part of the tumor microenvironment in the air digestive tract, and the microorganisms within the tumor may affect the growth and spread of cancer. [3, 4, 5, 6]

On the contrary, the gut microbiota can detoxify dietary components, reduce inflammation, and maintain a balance between host cell growth and proliferation. For over 100 years, the possibility of microbial based cancer treatment has been of interest, from Coley's toxin (one of the earliest forms of anti-cancer bacterial therapy) to the era of synthetic biology designing microorganisms and microbiota transplantation. Therefore, the relationship between microbiota and cancer requires a holistic perspective.

In recent years, immunotherapy for treating cancer has made significant progress and transformation, and is currently one of the fundamental pillars of its treatment. This progress is mainly represented by so-called immune checkpoint inhibitors. In typical clinical practice, there are mainly two types of drugs: CTLA-4 inhibitors (Cytotoxic T Lymphocyte Associated Protein 4) and PD-1 and/or PD-L1 inhibitors. The purpose of these drugs is to eliminate the braking of the immune system, allowing it to attack tumor cells.

I searched for microbiota and immunotherapy terms on PubMed as of October 1, 2018. Out of the 475 initial articles

appearing in the search, review and clinical trial filters were applied, resulting in a decrease of 204 articles. Finally, based on its scientific quality, a total of 50 articles were selected. Then, the relationship between gut microbiota, immune system, and the effectiveness of immunotherapy against cancer was reviewed and discussed.

2. The Importance of the Microbiome

In recent years, a large number of microbial populations have been discovered in the human gut and skin (estimated to be around 30 billion microorganisms per person), and changes in these microbial communities may lead to profound changes in health. Their functions include regulating the immune system. [8]

The human gut is the anatomical site of the largest and most complex collection of microscopic entities, known as the microbiome, which includes bacteria, archaea, microbial eukaryotes, and viruses. [7] The gut microbiome associated with healthy individuals is mainly dominated by bacterial species of the Bacteriodetes and Firmicutes phyla, with representation from additional less dominant phyla, such as Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia. [9]

The intestinal epithelium, through its antibacterial secretory peptides and the cells network of the innate and adaptive immune system, regulates intestinal immunity. Intestinal mucosal immune cells are specifically organized to form gut-associated lymphoid tissue, where immune cells are activated by bacterial antigens. This immune system protects us from infections by pathogens, while maintaining tolerance to dietary and environmental bacterial antigens. The mucus layer over the intestinal epithelium contains antimicrobial effectors and secretory immunoglobulin A, being the first intestinal defensive component. [10]

3. Microbiome and Immunotherapy

Although immunotherapy is effective in a large number of tumors, there is still a significant percentage of patients who do not respond to it. One of the possible factors that could influence the lack of efficacy of immunotherapy is the intestinal microbiome. [11] Then, a brief introduction was given to the research conducted so far linking gut microbiota with immune checkpoint inhibitor treatment response.

One of the first publications to examine the relationship between the intestinal microbiota and immunotherapy was the work published by Vétizou et al. [12] specifically between commensal bacteria and treatment with anti-CTLA-4 antibodies. In a mouse model treated with antibiotics, the authors found that in the presence of specific pathogens and bacteria in mice, the efficacy of ipilimumab (antiserum CTLA-4) was significantly higher in intestinal colonization scale caused by two species of bacteria of the order Bacteroidales (phylum Bacteroidetes) and one species of the order Burkholderiales (phylum Proteobacteria). In addition, there is evidence to suggest that these two species (Bacteroidales and Burkholderiales) significantly reduce histopathological symptoms of colitis, which is an adverse event with anti-CTLA-4 antibodies that may become severe. [13] They also suggest that administering certain live bacterial cultures to patients before and after treatment with ipilimumab can improve their outcomes.

Sivan et al. [14] provided strong evidence that the effectiveness of PD-L1 blockade therapy can be improved by regulating the gut microbiota. SIY melanoma subcutaneous growth was examined in genetically similar C57BL/6 mice grown at The Jackson Laboratory (JAX) and Taconic Farms (TAC). They found that tumour growth was more aggressive in TAC mice compared to JAX mice, and that TAC mice had a significantly lower accumulation of intratumoural CD8+ T cells. They also conducted experiments in which they showed that this difference was due to the different gut microbiota of the mice. They showed that prophylactic transfer of faecal material from JAX mice to TAC mice was sufficient to delay tumour growth. To examine whether the microbial population was effective as a single therapy, they administered faecal material from JAX mice alone or in combination with anti-PD-L1 monoclonal antibodies (mAb) in TAC mice. They showed that faecal matter alone was sufficient to inhibit tumour growth and that combination treatment further improved

these results. To identify the responsible bacterial species, they used 16S ribosomal RNA (16S rRNA) sequencing and identified Bifidobacterium (B) species, particularly Bifidobacterium breve, Bifidobacterium longum and Bifidobacterium adolescentis as candidate species. The role of these Bifidobacterium species in enhancing protective anti-tumour immunity was investigated in melanoma xenograft TAC mice by oral gavage administration of a cocktail of Bifidobacterium species containing B. breve and B. longum. They also showed that greater anti-tumour control was obtained compared to mice that did not receive such treatment, with this difference being statistically significant. The authors explained this phenomenon by the effect of Bifidobacterium species on dendritic cell (DC) activation, which in turn enhances the effector function of tumour-specific CD8+ T cells. However, they were unable to explain the possible mechanisms by which Bifidobacterium species activate dendritic cells.

To better appreciate the functional biomolecular mechanism through bifidobacterial species generate an anti-tumour immune response, Spranger et al. [15] put Sivan's research into context. They suggest that certain DCs are clearly dependent on bifidobacterial species for priming and proliferation of effector CD8+ T cells. Such a statement would support other publications that recognize the immunomodulatory action of some bifidobacterium species. [16, 17]

Matson et al. [18] examined stool samples obtained from patients with metastatic melanoma prior to treatment with anti-PD-1 immunotherapy and found that B. longum, Collinsella aerofaciens and Enterococcus faecium were more abundant in patients who responded to treatment, supporting the potential benefit/synergy of certain microbiota with immunotherapy. Frankel et al. [19] showed that melanoma patients who responded to immune checkpoint treatment had a higher abundance of Bacteroides caccae. Wargo et al. [20] examined the human gut microbiota and metabolites of patients with metastatic melanoma who received anti-PD-1 therapy using 16S rRNA. They found that the bacterial diversity and composition in patients who responded to therapy were significantly different from those of non-responders. Responders had a higher diversity of bacteria and a higher abundance of Clostridiales, and non-responders had a higher abundance of Bacteroidales.

Gopalakrishnan et al. [21] compared the gut microbiota of patients with metastatic melanoma treated with anti-PD-1 therapy. They showed that responding patients had significantly higher bacterial diversity and abundance of the family Ruminococcaceae (belonging to the order Clostridiales) compared to patients who did not respond to therapy. In addition, they performed faecal microbiota transplantation experiments in germ-free mice, showing that transplantation of faecal samples from patients who responded to anti-PD-1 and anti-PD-L1 therapy resulted in a greater response with anti-PD-1 and anti-PD-L1 therapy, along with a higher density of CD8+ T-cells.

Another recent study by Routy et al. [22] investigated the effects of gut microbiota on anti-PD-1 therapy. In their study, they collected data from 140 patients with advanced non-small cell lung cancer, 67 patients with renal cell carcinoma and 42 patients with urothelial carcinoma. They found that in the 69 patients treated with anti-PD-1 who received antibiotics (two months before or one month after starting treatment), progression-free survival and overall survival were shorter. They analyzed the composition of the gut microbiota by sequencing and found a higher abundance of Akkermansia muciniphila in patients who responded to anti-PD-1 therapy. They confirmed these findings by transplanting stool samples from patients who had received antibiotics into specific pathogen-free mice or germ-free mice and observed tumour growth. They also showed that A. muciniphila alone was able to restore the anti-tumour effects of PD-1 blockade that had been inhibited by antibiotics. In parallel, they monitored the cellular response against A. muciniphila in blood, measuring IFN gamma generated by CD4+ and CD8+ T cells, with higher levels being associated with a better clinical outcome. However, they failed to decipher the underlying mechanism by which A. muciniphila improves immunotherapy outcomes. From the above studies, the question remains: at what concentration or in what situation does the gut microbiota

stimulate the immune system? Furthermore, other research has shown that certain species of bifidobacteria influence the development of autoimmune thyroid diseases [23] and allergic disorders in infants and children. [24] Further research is therefore needed to resolve the existing inconsistencies.

Taken together, the above studies allow us to conclude that the gut microbiome significantly influences the outcome of cancer immunotherapy treatment in both mice and humans. Modifying the body's immune system and transforming non-inflamed tumors into inflamed ones through the action of the gut microbiome could constitute a new therapeutic approach in the battle against cancer and the immune system. [25] However, prospective research studies aimed at understanding the functional properties of different species of gut microbiome and the mechanisms by which certain bacterial commensal communities interact with the immune system will allow us to better characterize and manipulate the human gut microbiome to improve the patient's response to immunotherapy. It is important to emphasize that extensive exposure studies were conducted in mouse models. Although these experiments are crucial as they allow for challenging experimental procedures in humans, data extrapolation is complex and controversial. There are significant differences in the anatomical structure of the gastrointestinal tract and the covering of the intestinal wall between humans and mice [26, 27, 28], and it has also been observed that 85% of the microorganisms that settle in the mouse intestine are not found in humans. [29]

Table 1 summarizes the different bacterial species that enhance the effectiveness of immunotherapy.

Table 1. Bacterial species that increase the efficacy of immunotherapy

Type of bacteria	Model	Methodology	Results
Bacteroidetes phylum, Proteobacteria phylum	Mouse	Pyrosequencing of 16S ribosomal RNA amplicons from stool.	The efficacy of ipilimumab was significantly higher in those cases of intestinal colonization by Bacteroidetes phylum and Proteobacteria phylum. These two species also significantly reduced histopathological signs of colitis [12].
Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium adolescentis	Mouse	Fecal transplantation; Microbial DNA analysis; Bacterial administration; Cell sorting; Gene expression profiling.	Some species of bifidobacteria enhance the effectiveness of anti PD-L1 therapy in vivo [14].
Fecalibacterium prausnitzii, Bacteroides thetaiotamicron, Holdemania filiformis, Dorea ormicogenerans	Human	Metagenomic sequencing; Intestinal metabolomic profiling.	Melanoma patients who responded to nivolumab (anti PD-1) were enriched with F. prausnitzii, B. thetaiotamicron and H. filiformis. Patients with melanoma who responded to pembrolizumab (anti PD-1), their gut microbiota were enriched with D. formicogenerans [19].
Clostridiales	Human	16S rRNA sequencing; Whole genome sequencing; Immunohistochemistry; Flow cytometry; Cytokine analysis; Gene expression profiling,	Melanoma patients who responded to anti-PD-1 therapy had higher bacterial diversity and higher abundance of Clostridiales [20].
Ruminococcaceae (belonging to the order Clostridiales)	Mouse/ Human	16S rRNA sequencing Whole genome sequencing; Immunohistochemistry; Flow cytometry; Cytokine analysis; Gene expression profiling; Fecal microbiota transplantation.	Melanoma patients who responded to anti-PD-1 therapy had higher bacterial diversity and higher abundance of Ruminococcaceae. Germ-free mice transplanted with fecal samples from patients treated with anti-PD-1 and anti-PD-L1 had significantly lower tumor growth and better response to anti-PD-1 and anti-PD-L1 therapy along with higher intratumoral CD8+ T-cell density [21].

Akkermansia muciniphila	Mouse/ Human	Metagenomic sequencing; Fecal microbiota transplantation; Immunohistochemistry; Flow cytometry; Cytokine analysis.	27% of cancer patients who took antibiotics before or shortly after initiating anti-PD-1 therapy had shorter progression-free survival and overall survival. A. muciniphila was more abundant in those patients who responded to anti-PD-1 therapy. A. muciniphila alone was able to restore the antitumor effects of PD-1 blockade that had been inhibited by antibiotics [22].
Enterococcus faecium	Human	16S rRNA sequencing; Metagenomic sequencing; Species-specific quantitative PCR; Immunohistochemistry; Fecal transplantation.	Melanoma patients who responded to anti-PD-1 therapy had a higher proportion of B. longum, C. aerofaciens and E. Faecium. In germ-free mice transplanted with fecal material from the responding patients, better tumor control, increased T-cell activity and increased efficacy of anti-PD-L1 therapy were observed [18].

4. Probiotics and Immunotherapy

Although the initial definition of probiotics proposed in 1965 referred to substances secreted by microorganisms that stimulate the growth of others (as opposed to antibiotics), the term probiotics currently refers to a preparation or product that contains a sufficient number of live microbial strains to alter the microbial community of a host compartment (through implantation or colonization) and have beneficial effects on the host. [30] This definition includes products containing microorganisms (such as fermented milk) or microbial preparations (such as tablets or powders). [31] The World Health Organization (WHO) proposes a simpler definition and refers to live microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host. [32] The term prebiotic refers to non-digestible food ingredients that produce beneficial effects on the host by selectively stimulating the growth and/or activity of a type or limited number of bacteria in the colon. This definition partially overlaps with the definition of dietary fiber, although it adds the selectivity of prebiotics on particular microorganisms. [31]

It is postulated that probiotics improve intestinal microbial profiles by balancing and promoting microbiota homeostasis, avoiding situations that may trigger intestinal microbial or intestinal epithelial cell dysbiosis (intestinal barrier disruption). [33] It is postulated that probiotic administration positively influences local immune balance as well as local and extraintestinal physiology. [34]

Probiotics can modify biological response through multiple mechanisms, including: [35, 36]

- The competitive transfer of pathogens in the intestinal tract, epithelium, and intestinal mucosa.
- Synthesize antibacterial proteins that are toxic to pathogens (i.e. bacteria that can produce pathogenic activity against the host).
- Produce metabolic substrates that promote the maintenance of epithelial barrier, mucosal integrity, and regulation of immune function.

However, the mechanism by which probiotics exert beneficial health effects has not been fully elucidated, despite clinical and experimental data indicating that probiotics have immunomodulatory effects on the host. The interaction between human hosts and gut microbiota promotes immune tolerance and metabolic regulation/stability, which helps establish control over local and extraintestinal physiology of terminal organs (such as liver, kidney, and mucosal immunity). Therefore, the use of probiotics has opened up an experimental field in different fields, which can serve as both a monotherapy and an adjuvant for other drugs. [35]

Scientific evidence shows that probiotic administration can modulate both innate and adaptive immunity. Klein et al. [37] demonstrated in healthy young adults that daily probiotic supplementation significantly increased the proportion of

granulocytes and monocytes with phagocytic activity compared to placebo. These observations were confirmed by Gill's group, [38] demonstrating a significant increase in serum antibody responses to antigens (administered orally and systemically) in probiotic-treated mice.

The most widely studied probiotics in animal models and clinical trials are Lactobacillus and Bifidobacterium. Their immunomodulatory potential has been demonstrated through research on the prevention of allergic diseases. [39] Intestinal bacteria (pathogens or diners) interact with the lymphatic system of the intestinal mucosa through pattern recognition receptors expressed in specialized intestinal epithelial M cells and dendritic cells. Antigen presenting cells regulate the host's immune response through this interaction. [39, 40] These signaling pathways are crucial for maintaining the immune homeostasis in the gut and beyond, thereby protecting the host from intestinal pathogens and preventing immune overactivation by inducing tolerance responses. Almost all developments in the synergistic effect between probiotics and the immune system have occurred in the field of vaccines. Table 2 summarizes various probiotic and vaccine studies conducted in adults. [42]

Table 2. Clinical studies investigating the effects of probiotics on vaccine responses in adults

Probiotic	Methodology	Vaccine	Results
L. rhamnosus GG (LGG)	1×10^{10} CFU + 295 mg inulin every 12 h for 4 weeks	Nasal attenuated influenza virus	Protection against H1N1 strain similar for the placebo and probiotic groups. H3N2 strain showed an increased protective titer for the LGG group. Enhanced immunogenicity [43].
L. casei Shirota	1.3×10^{10} CFU per day during 176 days	Trivalent influenza vaccine	There is no statistical or clinical significance in preventing respiratory symptoms or improving serum protection rate. No enhanced immunogenicity [44].
B. animalis ssp. lactis BB-12 L. paracasei ssp. paracasei L. casei 431®	1×10^{9} CFU per day for 6 weeks	Trivalent parenteral influenza vaccine	The probiotic enhanced immunogenicity group showed a significant increase in vaccine specific IgG antibody titers and an increase in vaccine specific secretion of IgA antibodies [45].
L. plantarum CECT7315/7316	Group A: 5×10^{9} CFU per day for 12 weeks. Group B: 5 x 10^{8} CFU per day for 12 weeks.	Trivalent influenza vaccine	Probiotic consumption after vaccination increased influenza-specific IgA and IgH antibody levels. An increasing trend in IgM antibodies was also observed. Enhanced immunogenicity [46].
B. longum BB536	5×10^{10} CFU every 12h for 12 weeks.	Trivalent influenza vaccine	Increase in IgA in the probiotic group compared to placebo at week 16. Beneficial modification in the intestinal microbiome. Improved immunogenicity [47].
L. casei 431®	1×10^{9} CFU per day for 42 days	Trivalent influenza vaccine	No benefit of immune response in the probiotic group although shorter duration of expiratory symptoms (no difference in the incidence or severity of symptoms). No improved immunogenicity [48].
L. paracasei MCC1849	1×10^{9} CFUs per day, lasting 6 weeks.	Trivalent influenza vaccine	No significant differences in immune parameters between the groups. Partially improved immunogenicity [49].
Note	L: Lactobacillus; B: Bifidobacterium; CFU: Colony-forming units		

Finally, it is worth emphasizing that the small intestine contains intraepithelial CD4+CD8+lymphocytes (DP), which originate from intestinal CD4+ T cells and have regulatory functions through negative regulation of the transcription factor THPOK. These DP are missing in sterile mice, indicating that their differentiation depends on microbial factors. It was observed that the number of these immune regulatory cells significantly increased in mice with the presence of Lactobacillus Reuteri. [50] Due to the potential synergistic effect between immunotherapy and this bacterial strain, this pathway can be explored in the future.

5. Conclusion

The relationship between the microbiota and the immune system is crucial. There is sufficient preclinical and animal model scientific evidence to suggest that the type of microbiome is associated with the effectiveness of cancer immunotherapy. On the contrary, a large number of clinical trials have shown that using vaccines in conjunction with probiotics can enhance the immunogenicity of vaccines. Unfortunately, despite this, there have been no prospective studies evaluating the potential synergistic effects of probiotics and immunotherapy for cancer. Therefore, this is an exciting open research field where prior simple things such as selecting and administering certain bacterial strains to individuals can optimize and improve the anti-tumor effect of cancer immunotherapy, which is also a cheaper and safer strategy.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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