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Review article

Probiotic species in the modulation of the anticancer immune response

Luciana Marinelli[1], Gian Carlo Tenore[1], Ettore Novellino*

Department of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, 80131 Naples, Italy

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ABSTRACT

Mounting evidences are supporting a key role of distinct gut bacteria in the occurrence and progression of intestinal and extra-intestinal tumors. More importantly, it has been recently demonstrated that some gut bacteria strains synergize with largely-used anticancer drugs as alkylating or immune checkpoint blockade agents thus optimizing the immune response against multiple solid cancers. However, the exact role played by each gut bacterium in cancer occurrence and response to therapy is still in its infancy; and the current knowledge, although exciting, still needs to be transferred from mice models to human beings. Here, the advances in the understanding of how gut microbes and immune response shape each other in a cancer context are reviewed together with the implications of these finding for future antitumor therapy. Herein, the most important bacteria strains, able to boost the immune response triggered by anticancer drugs, together with their mechanism of action, whenever known, have been surveyed. It is reasonable to think that cocktails of beneficial bacteria together with an *ad hoc* diet or food supplements may be used as novel anticancer adjuvant agents in future therapeutic regimens.

1. The mammalian immune system and cancer

Cancer arises from the accumulation of a variable number of genetic alterations that reroute key pathways in the regulation of cell survival and death. In principle, adaptive immunity should be able to prevent cancer development, at least in immune-competent hosts [1]. However, for an effective anticancer immune response, a series of events has to be initiated and allowed to proceed and expand iteratively [2].

At first, tumor antigens originated from different sources, such as mutations associated with genetic alterations, oncogenic viruses, and abnormally expressed self proteins [3], are released and captured by dendritic cells (DCs) for processing [4]. Subsequently, DCs present the antigens, bound to major histocompatibility complex molecules (MHCI and MHCII), to T cells, resulting in the priming and activation of effector T cells (Teff) against the tumor-specific antigens. However, recognition of cancer-specific peptide-MHCI complexes by the T cell antigen receptor (TCR) cannot *per se* activate naïve T cells. Additional stimulatory signals are necessary and include proinflammatory cytokines (e.g., TNF-a, IL1, IFN-a) [5], factors released by dying tumor cells (HMGB1, High Mobility Group Box 1, CDN, a STING agonist) [6], or by the gut microbiota (Toll-Like Receptors (TLR) ligands). At this stage, the effectiveness of the immune response against tumor depends on the balance between T effector cells (Teff, tumor-cytotoxic) and T regulatory cells (Tregs, immunosoppresive cells) [7,8].

If Teff cells prevail, they infiltrate the tumor bed, recognize the transformed cells through the interaction between their TCR and the tumor-associated antigens, and kill them. The attack to the tumor expands itself iteratively, as the killing of the cancer cells releases additional tumor-associated antigens that increase the depth of the Teff response. Unfortunately, the above described immune response against cancer not always performs optimally thus allowing the cancer cells to escape the death. In fact, it can happen that T cells do not properly home to tumors, or are inhibited from infiltrating the growing mass. Moreover, problems may arise in the recognition of the cancer antigens by T cells. In fact, the TCR bind the antigen only in the context of MHC presentation. Unluckily, many tumors down-regulate MHC class I expression, thus masking their presence from TCR itself. It can also happen that DC and T cells treat tumor antigens as self rather than non-self, thereby creating Treg, immunosoppresive responses rather than effector responses. In fact, increase in Tregs has been observed in patients with head and neck, pancreatic, stomach, breast, and liver cancers [9]. However, even when the Teff correctly home to the tumor and infiltrate the cancer, the local microenvironment might suppress the effector responses through the activation of inhibitory signaling pathways such as those involving CTLA-4 (Cytotoxic T-Lymphocyte-associated Antigen-4) or PD-1, Programmed cell Death 1) [10–12].

Abbreviations: TNF-α, tumor necrosis factor-alpha; IL1, interleukin-1; IFN-α, interferon-alpha; FOXP3, forkhead box P3; ROR, RAR-related orphan receptor gamma

* Corresponding author.
E-mail address: ettore.novellino@unina.it (E. Novellino).
[1] These Authors contributed equally to this work.

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Whenever in the tumor microenvironment PD-1 binds its ligand PD-L1, the T cell function is mitigated, so that T cell effector become unable to attack cancer cells, thus allowing tumor evasion. In fact, the recent success of anti-CTLA-4 and anti-PD-1 based immunotherapy has to be ascribed to their capability to remove cell intrinsic inhibitory pathways that block effective antitumor cell response [11,13–16]. However, a detailed description of the role of mammalian immune system in cancer is beyond the scope of this article and can be found in Reference section [17–20]. Instead, in this review, we briefly survey the up-to-date understanding of how gut microbiota can affect both spontaneous immune responses or immunotherapy against various cancer types.

2. The gut microbiota in immune homeostasis and cancer

Human intestine harbors hundred trillion organisms (mainly bacteria), representing the most densely populated ecosystem known to date [21]. The bacterial, fungal, and viral intestinal communities are commonly referred to as the gut microbiota and altogether their genomes are referred to as the gut microbiome. In humans, the latter may encode ~150 times more genes than human genomes themselves, thus the gut microenvironment may be regarded as a complex bioreactor replete with diverse biochemical activities [22]. The intricate interaction between the bacteria community and the human host is the result of half a billion years of co-evolution that has mutually influenced the repertoires of the intestinal consortia and the immune system, so that the gut microbiota in humans has become tolerated. At homeostasis, the microbiota benefits from the warm, nutrient-rich environment, while humans take advantages from a well functioning metabolic engine that increment our capability to harvest nutrients from food [23]. Besides a metabolic help, gut microbiota influences tissue development, inflammation, and immunity, thus, promoting either human health or disease [24–34]. Beneficial bacteria, that are those promoting the human health, are commonly known as “probiotics”, and are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001) [35].

As above stated, a mutualistic symbiosis between gut microbiota and host immunity exists. In fact, the immune system has to settle a proper balance between tolerance towards microbiota and surveillance against infectious agents. Gut homeostasis is maintained as an inflammatory tone, allowing a self-limiting response appropriate to infectious agents or stress [36]. The balance between tolerance and response to infections is the fruit of an extensive and co-operative crosstalk between the intestinal microbiota and host that involves both innate and adaptive immunity [37–39]. In fact, while the immune system shapes the gut microbiota composition, the latter regulates the immune systems responses [40]. Pathogen-associated molecular patterns (PAMPs), which are linked with microbial pathogens, as well as damage-associated molecular patterns (DAMPs), which are released during cell damage or death, are recognized in the gut by a number of innate immune cell pattern recognition receptors (PRRs) which mediate the recognition between bacterial ligands and the host [41]. On the other hand, Toll-Like Receptors (TLRs) and Nucleotide binding Oligomerization Domain proteins (NODs) both promotes tolerance and healthy inflammatory tone on the apical surface epithelium of the gut. An essential detector of bacterial cell wall component is the Nucleotide-binding Oligomerization Domain 2 (NOD2) which is able to activate the adaptive immune system by acting as an adjuvant receptor for antibody production, either directly or by enhancing the production of α-defensin or other immunoregulatory molecules such as type 1 interferon (IFN) [42].

A number of microbial ligands stimulate activation of nuclear factor kappa B (NF-κB) and downstream proinflammatory cytokines such as tumor necrosis factor (TNF)-αor interleukin (IL)-1 [43]. Colonization of the intestine by commensal bacteria results in Paneth cell expression of the anti-microbial peptide, regenerating islet-derived 3 gamma (Reg IIIγ) [44,45]. More recent studies have recognized that these antimicrobial molecules are key mediators of homeostatic balance between host and the colonizing microbiota as well as of innate immune protection from enteric pathogens [46]. Moreover, the intestinal bacteria are involved in maintaining a balance of T-effector cell function. In germfree mice, natural killer (NK) T cells present in non-mucosal lymphoid organs cannot efficiently trigger anti-viral responses, because both DC cells and macrophages do not release type 1 interferon [47]. Besides the contribution of Paneth cells in tolerance towards the gut bacteria, a key role is played by Tregs that down-regulate pro-inflammatory response through the production of IL-10 and transforming growth factor (TGF)-β [48]. In fact, Treg cells, expressing both CD4 and Foxp3 proteins, comprise a high amount of the T cells of the lamina propria of the intestine [49–51]. The latters play an important role in maintaining immune tolerance not only towards the gut microbiota but also towards dietary antigens, and are also fundamental in suppressing tissue injure provoked by immune responses against pathogens (e.g. *Citrobacter rodentium*) [52–55]. The intestine contains both thymus-derived Treg (tTreg) cells and peripherally differentiated Treg (pTreg) cells. Since pTreg cells disappear under germ-free conditions, they are probably induced by the microbiota. Indeed, experiments to follow the destiny of immature T cells that express a transgenic T cells receptor (TCR) cloned from colonic Treg cells, have shown that the expansion and differentiation processes of T cells into Tregs take place in the colon in the presence of commensal microbiota rather than in the thymus [56,57]. A significant part of Tregs express IL-10 which, besides being important in maintaining homeostasis in the intestine, is even essential for the suppression of the abnormal activation of myeloid cells, TH17 cells and γδ T cells [58–62]. The role played by each member of the gut community in the accumulation and functional maturation of Tregs of the intestine is still in its infancy. Nonetheless, through two elegant studies, Atarashi et al. (2015) demonstrated that a mixture of *Clostridia* strains from the human microbiota is able to induce the accumulation, in the colon, of Tregs that, in turn, function in systemic immune regulation [49–63]. The same strains of *Clostridia* can also facilitate the expression of IL-10 and CTLA-4 by Treg cells although the exact mechanism through which *Clostridia* act is still elusive. Along the same lines, *Lactobacillus reuteri* and *L. murinus* led to an increase of the proportion of Treg cells in mice, while *Helicobacter hepaticus* induced IL-10-producing Tregs that inhibited the development of colitis and *Bacteroides fragilis* incremented the IL-10 production by Treg cells of the colon, an event which is mediated by polysaccharide A70 from the bacterium’s capsule [64–66,50].

In few words, homeostasis is maintained through a high number of microbial derived signals that govern the tolerance, and this issue has been extensively reviewed so far [23,67–69]. Pathogenic organisms or passenger commensals, antibiotics, anticancer drugs, dietary, or even smoking, may deeply alter the composition of the commensal microbiota so to brake the homeostasis status. In fact, the microbial imbalance in the gut, commonly referred to as dysbiosis, has been associated with a number of disease such as inflammatory bowel disease, asthma allergy, metabolic and cardiovascular disease as well as cancer [70–76]. Indeed, compelling evidences demonstrated that gut dysbiosis can lead to over-representation of certain bacterial species that are able to promote colon carcinogenesis by both favoring chronic inflammation or local immunosuppression [77–83].

Noteworthy, experimental alterations of the bowel microbiota even influence the occurrence and progression of extraintestinal tumors, such as breast and hepatocellular carcinoma, likely through inflammatory and metabolic circuitries [84,85]. In fact, prolonged exposure to a combination of metronidazole and ciprofloxacine antibiotics tripled the occurrence of breast cancer in protooncogene HER2/neu driven-transgenic mice [86]. Thus, the gut microbiota may influence oncogenesis and tumor progression both locally and systematically. Very recently, mounting evidences support a key role of distinct bacteria in cancer therapies outcomes. In this respect, a pioneer work by Iida et al. [87] has shown that disruption of the gut microbiota