Microbiota Modulation of Myeloid Cells in Cancer Therapy
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Abstract

Myeloid cells represent a major component of the tumor microenvironment, where they play divergent dual roles. They can induce antitumor immune responses, but mostly they promote immune evasion, tumor progression, and metastasis formation. Thus, strategies aiming at reprogramming the tumor microenvironment represent a promising immunotherapy approach. Myeloid cells respond to environmental factors including signals derived from commensal microbes. In this Cancer Immunology at the Crossroads overview, we discuss recent advances on the effects of the commensal microbiota on myeloid-cell functions and how they affect the response to cancer therapy. Cancer Immunol Res; 3(2): 103–9. ©2015 AACR.

The Microbiota Modulates Inflammation and Immunity by Priming Myeloid-Cell Differentiation and Functions

Commensal microorganisms are abundant on all our epithelial barrier surfaces, where, directly or through released molecules, they interact with innate receptors and cytoplasmic sensors, thus regulating the development, tone, and maintenance of local inflammation and immunity (1). The interplay between the host immune system and the microbiota prevents tissue-damaging inflammatory responses to the commensals and controls the growth of indigenous pathogens while it sets the stage for immune responses against pathogenic infections (2–4). This homeostatic immune regulation may be disrupted by changes in the microbial community that alter the symbiotic relationship with the microbiota, and the resultant microbial imbalance is commonly referred to as dysbiosis (5). Many regulatory mechanisms involved in these local interactions have been elucidated (6).

In addition to local immunity, the commensal microbiota regulates systemic inflammation, innate resistance, and adaptive immunity, affecting both resistance to infection and autoimmunity (7–12). Maturation of the immune system is dependent on exposure to the microbiota following birth (13). In germ-free (GF) mice, which are protected from exposure to external microbes, spleens and peripheral lymph nodes are hypoplastic, mesenteric lymph nodes are mostly missing, whereas primary microbes, spleens and peripheral lymph nodes are hypoplastic, mesenteric lymph nodes are mostly missing, whereas primary

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Cancer Immunology at the Crossroads: Microbiology

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Mice deprived of commensal microbiota have impaired ability to respond to virus infection or virus-derived products. Antibiotic treatment diminishes the immune response to respiratory influenza virus because of lowered constitutive expression of genes encoding pro-II15 and pro-II18, and the inability of immune cells to produce and respond to IFN (8, 9). Intranasal or systemic administration of the Toll-like receptor 4 (TLR4) ligand lipopolysaccharide (LPS) to antibiotics-treated mice corrected the defective anti-influenza virus immune response (8). Dendritic cells (DC) from GF mice fail to respond to the TLR3-ligand poly(I:C) and to LPS with production of cytokines, such as type I IFN, II12, II6, and TNF, and to induce natural killer (NK)–cell activation. LPS-induced recruitment of IFN3 and NF-kB as well as Pol II to the promoter region of inflammatory genes, such as Ifnb1, II6, and Tnf, is reduced in DCs from GF or antibiotics-treated mice as compared with DCs from specific pathogen-free animals (26). DCs from GF mice also show a reduced association of histone H3K4me3 around the transcriptional start site of the same genes, suggesting that signals from the microbiota are required to epigenetically poise these genes for transcription (26). Thus, the microbiota regulates immune homeostasis both locally and systemically, acting primarily although not exclusively at the level of myeloid cells. Microbiota-primed myeloid cells have a heightened response to stimuli derived from pathogens or tissue damage, thus establishing a tonic threshold of inflammation that is required for both innate resistance (e.g., activation of NK cells and other innate lymphocytes) and adaptive immunity (12, 26).

The Microbiota and Cancer

Although cancer-cell proliferation and fitness are linked to genetic mutations and alterations, tumor progression and metastasis formation are highly regulated by the host inflammatory and immune responses (27–31). Inflammation also regulates cancer-predisposing conditions such as obesity (30). Cancer often originates in tissues inflamed due to infection or to physical, chemical, or genetic causes (30). Inflammation may directly induce or facilitate genetic instability and mutations, and it always creates a tumor-promoting environment that is associated with the production of growth and angiogenic factors, as well as tissue-remodeling enzymes (28, 30). Chemical or infectious carcinogens, such as oncogenic viruses and Helicobacter pylori infection, may have a direct mutagenic or cell-transforming ability, but they also induce inflammation required for tumor promotion. Established tumors are infiltrated with inflammatory cells, and this cancer-associated inflammation maintains the tumor-promoting and immunosuppressive microenvironment (32, 33). Cancer comorbidities, such as anorexia/cachexia, immunodeficiency, pain, and depression, are known to be modulated by inflammatory unbalance and are likely induced or affected by cancer-associated inflammation (30). The commensal microbiota, by setting the inflammatory/immune tone and modulating host response to oncogenic pathogens, cancer-associated inflammation, and tumor-induced tissue damage, is a major player affecting the outcome of carcinogenesis, tumor progression, cancer comorbidities, and response to therapy.

Impact of Commensal Microbiota on Cancer Therapy

The gut microbiota influences the response to cancer immunotherapy and chemotherapy by affecting the differentiation and functions of myeloid cells in the tumor microenvironment (Fig. 1). Intratumoral injection of CpG-oligodeoxynucleotide (CpG-ODN) combined with antibody neutralization of IL10 signaling is a very effective treatment of large transplanted subcutaneous tumors in conventional mice, but it is largely ineffective in GF or antibiotics-treated mice (34). Within hours following CpG-ODN and anti-II10R treatment, tumors undergo an extensive hemorrhagic necrosis that is dependent on TNF and nitric oxide production by tumor-infiltrating myeloid cells (35). DCs are then activated, and they migrate to the draining lymph nodes, where they induce a CD8 T-cell–mediated tumor-specific response required for tumor eradication. In antibiotics-treated or GF mice, tumor-infiltrating myeloid-derived cells fail to produce inflammatory cytokines, including TNF and II12, in response to CpG-ODN (34). Oral gavage of antibiotics-treated mice with LPS partially rescued the deficient response to CpG-ODN (34). Tumors from antibiotics-treated mice contained a lower number of monocyte-derived Ly6C+ MHC-class II+ macrophage-like cells, whereas the number of Ly6C+high MHC class II+ inflammatory monocytes was equivalent to that of control mice without antibiotics treatment (34). Tumor-associated myeloid-cell subsets have been shown to be mostly derived from circulatory inflammatory monocytes that differentiate in situ (36–38). Although inflammatory monocytes appear to infiltrate the tumors in equivalent numbers, their differentiation after reaching the tumor microenvironment is altered in the absence of gut microbiota, and this may affect their response to CpG-ODN (34). Thus, gut commensal bacteria-derived LPS (and possibly other innate receptor ligands) directly or indirectly primes tumor-infiltrating myeloid cells and/or their inflammatory monocyte precursors through the TLR4 receptor and enables them to respond preferentially.
to the TLR9 ligand CpG-ODN. The fecal microbiota composition in mice showing high and low TNF responses to CpG-ODN appeared distinct. In particular, the abundance of several individual gram-positive and gram-negative bacterial species positively correlated with the CpG-ODN response, whereas the presence of commensal Lactobacillus species decreased the response (34). In vivo association experiments confirmed that the gram-negative Alistipes shaii enhances the CpG-ODN response, whereas Lactobacillus fermentum attenuates it (34).

The effect of the microbiota on chemotherapy was analyzed utilizing platinum compounds (e.g., oxaliplatin and cisplatin; ref. 34). These compounds mediate genotoxicity by forming platinum DNA adducts followed by formation of intrastrand cross-links that inhibit protein synthesis and proliferation, and induce apoptosis in part downstream of the ATM kinase recruitment to DNA damage and p53 activation (39). In addition to their genotoxicity, oxaliplatin but not cisplatin induces immunogenic cell death that activates antitumor T-cell immunity (40). The therapeutic effect of oxaliplatin and cisplatin on mouse sterile subcutaneous transplanted tumors was dramatically reduced in antibiotics-treated or GF mice (34). In antibiotics-treated mice, platinum adducts to tumor-cell DNA were formed at a level comparable with that observed in control mice; however, already at 48 hours after treatment, DNA damage and cytotoxicity were significantly decreased. Antibiotics treatment of mice largely suppressed all the gene expression modifications induced in the tumor by oxaliplatin. In antibiotics-treated mice, tumor-infiltrating myeloid cells failed to produce reactive oxygen species (ROS) via the NADPH oxidase NOX2. ROS are needed for the oxaliplatin antitumor effect. Although the genotoxic effect of platinum compounds was known to require ROS and particularly H2O2 production (41), this requirement was mostly studied in tumor cell lines in vitro. In these conditions, ROS was endogenously produced in tumor cells. In the in vivo tumor microenvironment, however, most of the ROS required for oxaliplatin cytotoxicity is produced by tumor-associated myeloid cells. Thus, the microbiota affects oxaliplatin early tumor genotoxicity by systemically priming tumor-associated myeloid cells for ROS production.

The effects of microbiota deprivation in the response to CpG-ODN and platinum compounds were evident very early after treatment, suggesting that tumor-associated myeloid cells were primed for responsiveness to therapy by the preexisting microbiota composition. However, chemotherapy and radiotherapy, in addition to their antitumor effect, also induce damage of the intestinal mucosa, affecting mucosal permeability and inducing dysbiosis and bacterial transmucosal translocation. One of the most successful anticancer immune therapies is the adoptive transfer of tumor-specific cytotoxic CD8+ T cells (42). For best survival of the T cells and effectiveness of the transfer, lymphoblation and myeloablation are necessary (43). Both in human patients and in mice, total body irradiation (TBI) improves therapeutic efficacy by increasing DC activation and homeostatic cytokine levels (43). Because of the mucosal damage effect of TBI, commensal gut bacteria were found to infiltrate the mesenteric lymph nodes of irradiated animals, and elevated endotoxin levels were observed in their sera (43). The ability of TBI to improve tumor regression induced by adoptive T-cell transfer was reduced in animals treated with broad spectrum antibiotics, by neutralization of serum LPS using polymyxin B, or in mice genetically deficient in CD14 or TLR4 that are unable to respond to LPS. Administration of LPS or LPS-containing serum from irradiated animals to nonirradiated lymphopenic mice was able to enhance the number and function of transferred CD8+ T cells, leading to long-term cure of mice with large transplanted tumors (43).

Like oxaliplatin, cyclophosphamide is a member of a group of chemotherapy agents that induce immunogenic cell death. The ability of cyclophosphamide to induce adaptive antitumor responses is decreased in GF or antibiotics-treated tumor-bearing mice (44). Cyclophosphamide treatment of conventionally raised animals induces dysbiosis and mucositis. Because of the mucosal damage, gram-positive bacteria translocate into the mesenteric draining lymph nodes, prime pathogenic effector Th17 cells, and memory Th1 cells, all of which were not observed in microbiota-depleted mice (44). Thus, the activation of antigen-presenting cells and the subsequent induction of antitumor immune responses by chemotherapy-induced immunogenic cell death not only depend on the release of endogenous mediators of inflammation as previously shown (40), but also on the priming and/or activating effects mediated by commensal bacteria and/or by their products.

Clinical Perspectives

Inflammation and immunity are hallmarks of cancer, affecting tumor initiation, progression, dissemination, and response to therapy (45). The molecular pathways involved in this modulation of the neoplastic disease offer targets of therapeutic intervention for both cancer prevention and therapy. The power of the antitumor immune response present in patients but masked by tumor immunosuppressive mechanisms has been successfully harnessed in immunotherapy approaches (46). The most successful approaches have utilized either adoptive T-cell transfer or inhibition of immune checkpoints, such as CTLA-4 and PD-1/PD-L1/2, which free antitumor T cells from the immunologic brakes expressed by the tumor cells or by other cells in the tumor microenvironment (42, 46, 47). Therapeutic cancer vaccines have not been as successful yet because of the difficulty to permanently overcome the inadequacy of antigen-presenting cells in the tumor and the presence of immune checkpoints restricting both the natural and vaccine-induced immune responses (48, 49). The evidence discussed here adds a layer of complexity, indicating that the composition of commensal microbiota and its alteration by neoplastic disease or therapy significantly affect all stages of tumor development and therapeutic effectiveness via their local or systemic effects on inflammation, immunity, and metabolism. These regulatory mechanisms provide new targets and possibilities for therapeutic intervention.

Most of the studies examining the influence of commensal microbiota on cancer therapy have been performed in experimental animals, and the extent to which the microbiota regulates human myeloid-cell function and response to cancer therapy remains to be determined. Although some of the molecular mechanisms by which the microbiota locally affects inflammation, immunity, and cancer have been elucidated, our understanding of the systemic effects is still rudimentary. Nevertheless, it is tempting to speculate that the variable response to cancer therapy observed in patients may be in part due to different microbiota composition, affecting the inflammatory tone and myeloid-cell functions in the tumor microenvironment. This explanation would offer the possibility to improve the effectiveness of immune therapy, chemotherapy, and radiotherapy by altering the microbiota, targeting the pathways by which the...
microbiota communicates with inflammatory cells, or directly targeting the molecular mechanisms in myeloid cells and antigen-presenting cells that restrict their functions.

Since Hippocrates' recognition that "all disease begins in the gut," there have been many attempts, most often not scientifically controlled, to modify the gut microbiota to correct dysbiosis and to promote health. Clinical therapeutic procedures, including the use of probiotics, diet modification, prebiotics, and fecal or defined microbiota transfer, have been utilized to enhance patients' response to cancer therapy (50). The major difficulties include our incomplete understanding of what constitutes a healthy microbiota, our current imperfect knowledge of which bacterial species affect which type of cancer therapy, and the fact that the little that we know is based on experimental animal data that may not reflect conditions in humans. Furthermore, the same bacterial species may have opposite effects in different types or at different stages of therapy. For example, in the experimental model of Cpg-ODN tumor therapy, the abundance in the gut or the oral administration of Lactobacillus species decreased the ability of the tumor-associated myeloid cells to produce effector cytokines, whereas bacteria of the same genus increased the ability of antigen-presenting cells to induce an effective antitumor immune response following cyclophosphamide-induced immunogenic cell death (34, 44, 51).

In the absence of precise indications of which specific bacterial species or their combination would offer the best possibility to prevent or cure disease, one approach has been to replace the disease-associated dysbiotic microbiota in patients with that from healthy subjects using fecal microbiota transplantation. However, fecal transplantation may have both anti-inflammatory and proinflammatory effects on mucosal inflammation as well as on systemic metabolism and immunity (52). Fecal transplants have been very successful in the treatment of Clostridium difficile infections, most likely by altering the dysbiosis permissive for colonization by this pathobiont (53). Although the composition of the commensal microbiota is defined by our genetics, nutrition, and environmental exposure, fecal transplants change the composition of the recipient gut microbiota to resemble that of the donor for at least several weeks (54). Fecal transplants have also been proposed as a treatment for inflammatory bowel diseases and metabolic disorders; transfer of intestinal microbiota from lean donors has been shown to increase sensitivity to insulin in patients with metabolic syndrome (55). However, the use of not well-characterized microbial mixture could increase or induce bowel inflammation elicited by the transplanted microbiota, resulting in bacteremia (56, 57). In theory, fecal transplants could treat dysbiosis that are associated with cancer comorbidities (30) and could contribute to optimal response to cancer therapy. There is, however, controversy on how and if fecal transplant should be regulated, and the FDA presently is considering feces as a drug that requires an investigational new drug approval (58). In any case, several safety and consistency concerns remain that suggest the usefulness of developing better defined and safer microbial replacement therapeutic procedures (52, 53, 55, 59).

An alternative to the use of bacterial preparations is to administer bacterial-derived or bacterial-induced products that modulate the immune system. Ligands for TLRs or other innate receptors are being developed for clinical use and could be used in combination cancer therapies (60). LPS for example has been shown to reestablish the resistance to influenza virus infection, the response to CpG-ODN therapy, and T-cell–adaptorive transfer in mice depleted of commensal microbiota (8, 34, 43). Short-chain fatty acids (SCFA) are bacterial products derived by fermentation of dietary fibers. SCFAs modulate mucosal immunity via G-protein–coupled receptors by inducing IL18 production by enterocytes and directly acting on the IL10-producing T-regulatory pool and regulating its size and functions (61–63). In ApoMin+/–Msh2–/– mice, antibiotics treatment or a diet reduced in carbohydrates, which changes the microbiota composition and results in lower SCFA production, decreased colon polyps incidence (64). Although these data show that it is possible to affect cancer by modifying the microbiota, they also caution that a clear understanding of the roles and functions of different microbial species in cancer is necessary because SCFAs have been shown in other experimental conditions to be protective against colon and mammary cancer (61, 65, 66).

The ideal therapeutic intervention would be to target directly the tumor-infiltrating myeloid cells and change their properties so that they could enhance and sustain therapeutic responses and antitumor immunity. In the tumor microenvironment, many factors are present that prevent anticancer immunity or response to therapy, including products of both classically and alternatively activated macrophages and other myeloid-cell subsets, T-regulatory cells, and the presence of anti-inflammatory factors such as TGFβ and IL10 (67–70). These mechanisms provide targets of intervention, and many of which have been tested experimentally and some clinically (71, 72). The ability of IL10 antagonism to enhance the response of tumor-associated myeloid cells to CpG-ODN indicates that it is possible to revert at least partially the unresponsive phenotype of those cells in vivo. However, even when IL10 signaling is blocked, myeloid cells still require commensal microbiota for their response, indicating that the unresponsive phenotype of these cells is controlled by multiple signals (34, 35). To identify new possibilities of precise targeting of myeloid cells in the treatment of cancer, it is essential to define the genetic and epigenetic pathways affected by the microbiota and their relevance in response to therapy (9, 26, 34).

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