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COMMENTARY AND VIEWS

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Bugs in the system: bringing the human microbiome to bear in cancer immunotherapy

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ABSTRACT

The influence of the composition of the human microbiome on the efficacy of cancer directed immunotherapies, such as antibodies directed against the programmed cell death 1 protein (PD-1) or its ligand (PD-L1), has garnered increasing attention as the role of immunotherapies in the care of cancer has grown. Dysbiosis (altered microbiota) has recently been reported to adversely affect the efficacy of cancer directed immunotherapies, and correction of this dysbiosis has the potential to improve the efficacy of these treatments. However, the exact mechanisms underlying this relationship remains unknown. Current methods for characterizing the microbiome likely capture only a small portion of the highly complex interaction between the microbiome and the immune system. Here we discuss the recent reports of the influence of dysbiosis on cancer immunotherapy, methods to more fully characterize the interaction between the microbiome and the immune system, and methods of modulating the immune system to improve the efficacy of cancer immunotherapy.

Commentary

The role of the immune system in immune surveillance and immune editing of cancer has been described for decades, and has recently been drawn into sharper focus with the introduction of effective immunomodulatory treatments, such as antibodies directed against the programmed cell death 1 protein (PD-1) or its ligand (PD-L1).[1] Additionally, the human microbiome has recently emerged as an important factor in shaping patients' immune systems.[2] Recent reports suggest that dysbiosis may account for the immune dysfunction in some non-responders to anti-PD-1 therapy, and that correction of dysbiosis may improve treatment efficacy.[3,4]

Routy et al. analyzed the composition of the gut microbiome in 60 patients with non-small cell lung cancer (NSCLC), finding that the stool richness, as measured by metagenomic species level, correlated with patients' clinical response to anti-PD-1 immunotherapy.[3] In a similar study by Gopalakrishnan et al. of stool composition in patients treated with anti-PD-1 immunotherapy for metastatic melanoma, patients who responded to anti-PD-1 therapy were found to have higher alpha diversity (aka richness in species) in their fecal microbiome.[4] Both Routy and Gopalakrishnan then performed fecal microbial transplant (FMT) using stool from responding and non-responding patients into antibiotic treated and/or germ-free mice inoculated with sarcoma or melanoma cells. Mice receiving FMT from patients who had responded to anti-PD-1 immunotherapy and subsequently treated with anti-PD-1 immunotherapy demonstrated better response in both experiments. These experiments indicate that the fecal microbiome influences the efficacy of immune checkpoint inhibitors, and that beneficial effects of particular microbial compositions may be transferrable.

Given these pre-clinical results, immunomodulation via alteration of the gut microbiome appears to hold promise. However, several key issues need to be clarified or further addressed: the effects of antibiotic use on the microbiome and immunotherapy efficacy, obtaining a complete picture of the relevant interactions between the microbiome and the immune system, and the most

effective means of altering the microbiome to modulate the immune response.

Though antibiotics are known to significantly perturb microbial communities, whether administration of antibiotics can effectively alter the gut microbiome and adversely affect treatment efficacy of immunotherapies is still debatable.[5] Efforts to alter the gut microbiome with antibiotics to affect auto-immune and inflammatory conditions have been investigated and the mixed experience in this field is informative.[2] Treatment with clarithromycin, rifabutin, and clofazimine for 2 years in patients with Crohn's disease found greater maintenance of remission at 16 weeks, but long term remission was not maintained.[6] Studies of other antibiotics in patients with ulcerative colitis, including vancomycin, metronidazole and tobramycin, and ciprofloxacin did not demonstrate efficacy.[2] Close review of recently reported retrospectively studied cohorts of lung cancer patients treated with immunotherapy reveals similarly mixed results.

Kaderbhai et al. and Thompson et al. retrospectively reviewed patients treated with antibiotics immediately prior to the initiation of anti-PD-1 therapy for NSCLC, and Routy et al. reported on 140 patients with non-small cell lung cancer retrospectively reviewed as well as 239 patients with NSCLC in a validation cohort from another institution.[3,7,8] While Thompson et al. and Routy et al. identified a statistically significant association between antibiotic use and progression free survival and overall survival in their reviewed patients, Kaderbhai et al. did not. Intriguingly, Thompson et al. reported no significant difference in the response rate (partial response + complete response) in the antibiotic vs. no antibiotic groups, despite the reported difference in overall survival, suggesting confounding variables might have contributed to the analysis. Similarly, Routy et al. found no difference in progression free survival in the validation cohort, though the association between antibiotic use and shortened overall survival persisted. One explanation for these discordant results may be the varying inclusion criteria for antibiotic exposure. The studies variously defined recent antibiotic exposure as within 6 weeks before,[8] within 3 months before,[7] and within 2 months before or 1 month after first

administration of PD-1/PD-L1 therapy.[3] Another explanation may be that the patients receiving short courses of antibiotics shortly prior to the initiation of anti-PD-1 therapy represented a more ill cohort of patients compared to those who did not receive antibiotics, and therefore had shorter overall survival. Further study of these issues is warranted. In light of these considerations, caution with overuse of antibiotics in patients treated with immunomodulatory agents is indicated, though antibiotics should not be withheld given their established beneficial effects.

Since antibiotics kill all bacteria with particular features such as Gram positive or negative staining, they are not ideal to eliminate pathogens from the perspective of dysbiosis. A better approach would be to identify specific metabolic pathways utilized by pathobionts (disease causing bacteria) or symbionts (health promoting bacteria) and target them specifically. Utility of this approach has been shown in animal model of colitis. One example of the utility of such studies is a recent report from Winter's group.[9,10] Shotgun metagenomic sequencing was performed in dextran sodium sulphate (DSS) colitis model, an animal model of human IBD. They successfully identified that DSS induced colitis results in gut dysbiosis which is associated with enrichment of certain respiratory pathways utilizing formate as substrate. Interestingly, bacteria from Enterobacteriacae family such as *E. coli* can utilize this pathway which give them survival advantage over other commensal bacteria. Since this pathway is dependent on molybdenum as co factor, treating mice with tungsten, which specifically can block this pathway, results in the reduction of pathogenic bacteria and amelioration of disease. This is an excellent example of utility of shotgun metagenomic sequencing of microbiota because it can help in identifying the pathways responsible for pathogenicity. This approach would allow for precision targeting of the specific pathways to correct gut dysbiosis and associated pathologies.

A majority of studies of the human microbiome have focused primarily on characterizing the bacterial gut microbiome, owing to its accessibility and to its mass, accounting for 99% of the microbial mass in humans.[11] However, such analyses may miss other important aspects of the human microbiome.

In contrast to the bacterial gut microbiome, the contribution of the oral and respiratory epithelial microbiome, which accounts for the second greatest microbial mass in the microbiome, has been relatively underinvestigated.[11] To characterize the complex oro-pharyngeal microbiome, Huttenhower et al sampled 9 distinct sites: the saliva, keratinized gingiva, palate, tonsils, throat, tongue, supra- and sub gingival plaques, and buccal mucosa.[12] Microbial compositions along this tract have been associated with risk of multiple malignancies, including squamous cell cancer of the head and neck.[13] Additionally, significant differences in the respiratory microbiome have been identified between patients with and without lung cancer.[14] Though Gopalakrishnan et al. found no significant difference between the oral microbiomes of melanoma patients who responded or did not respond to anti-PD-1 therapies, the investigators sampled only the buccal mucosa for their study. Sampling of a single site of the oral/respiratory epithelial microbiome may not reflect important differences in the numerous niches along the respiratory tract.[4] Additionally, there may be a stronger relationship between other types of malignancies and these other microbial niches along the respiratory tract, such as lung cancers or squamous cell cancers of the head and neck. Thus, further investigation of these relationships may be more fruitful.

Beyond considering these other niches, analytic techniques other than 16S rRNA sequencing may be necessary to capture the full breadth of the interaction between microbial communities and the immune system. Products of microbial metabolism are known to modulate immune responses. Short chain fatty acids, produced by gut microbiota from insoluble fiber, are one such example. They have been shown to modulate pulmonary immune responses, affecting patients' allergic airway disease.[15] The regulatory T cell pool is modulated by short chain fatty acids via a G protein-coupled receptor mechanism, offering a molecular explanation for this association.[16] Characterization of the bacterial microbiome via 16S rRNA sequencing may fail to identify relevant variations in these and other bacterial metabolites.

Finally, because 16S rRNA sequencing has been the primary tool for characterization of the microbiome, the presence of other microorganisms, such as viruses and fungi, have not been well captured or characterized. Understanding of the human virome, the collection of all viruses in a human, is in its nascency compared to our understanding of the bacterial microbiome, though it may have a significant effect on cytotoxic T-cell immunity.[17] It is not known whether changes and diversity within these populations affect the host immune system, and whether they are adequately reflected in the analyses of the colonic bacterial microbial community.

Given the mixed effects of antibiotics and the myriad aspects of the interaction and the immune system discussed above, more profound and/or more precise means of altering the microbiome may be needed to achieve clinically significant immunomodulation. Phase 1 trials of FMT in patients refractory to treatment with anti-PD-1 agents utilizing stool from patients who successfully responded to anti-PD-1 treatment are currently proposed or underway (NCT03353402, NCT03341143). Development of standardized stool derived microbiota based suspension (RBX2660) and capsule based FMT (RBX7455, SER-109) are being investigated for treatment of recurrent *Clostridium difficile* infection, and represent possible avenues of effective alteration of the microbiome in cancer patients if these approaches are successful.

As the role of immunotherapy in the treatment of cancer continues to expand, progress in refinement and improvement of immunotherapy will require a full understanding of the complex interactions which shape human immunity. The human microbiome appears to play a prominent role in these interactions and offers an important avenue for therapeutic modification of human immunity.

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References

1. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33:1974–1982.
2. Shen N, Clemente JC. Engineering the microbiome: a novel approach to immunotherapy for allergic and immune diseases. Curr Allergy Asthma Rep. 2015;15:39.
3. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359:91–97.
4. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science. 2018;359:97–103.
5. Modi SR, Collins JJ, Relman DA. Antibiotics and the gut microbiota. J Clin Invest. 2014;124:4212–4218.
6. Selby W, Pavli P, Crotty B, Florin T, Radford-Smith G, Gibson P, Mitchell B, Connell W, Read R, Merrett M, et al. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. Gastroenterology. 2007;132:2313–2319.
7. Kaderbhai C, Richard C, Fumet JD, Aarnink A, Foucher P, Coudert B, Favier L, Lagrange A, Limagne E, Boidot R, et al. Antibiotic use does not appear to influence response to nivolumab. Anticancer Res. 2017;37:3195–3200.
8. Thompson J, Szabo A, Arce-Lara C, Menon S. Microbiome & immunotherapy: antibiotic use is associated with inferior survival for lung cancer patients receiving PD-1 inhibitors. J Thorac Oncol. 2017;12: S1998.
9. Zhu W, Winter MG, Byndloss MX, Spiga L, Duerkop BA, Hughes ER, Büttner L, de Lima Romão E, Behrendt CL, Lopez CA, et al. Precision editing of the gut microbiota ameliorates colitis. Nature. 2018;553:208–211.
10. Hughes ER, Winter MG, Duerkop BA, Spiga L, Furtado de Carvalho T, Zhu W, Gillis CC, Büttner L, Smoot MP, Behrendt CL, et al. Microbial respiration and formate oxidation as metabolic signatures of inflammation-associated dysbiosis. Cell Host Microbe. 2017;21:208–219.
11. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016;14:e1002533.
12. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, FitzGerald MG, Fulton RS, et al. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207–214.
13. Hayes RB, Ahn J, Fan X, Peters BA, Ma Y, Yang L, Agalliu I, Burk RD, Ganly I, Purdue MP, et al. Association of oral microbiome with risk for incident head and neck squamous cell cancer. JAMA Oncol. 2018;4:358–365.
14. Liu H-X, Tao -L-L, Zhang J, Zhu Y-G, Zheng Y, Liu D, Zhou M, Ke H, Shi -M-M, Qu J-M. Difference of lower airway microbiome in bilateral protected specimen brush between lung cancer patients with unilateral lobar masses and control subjects. Int J Cancer. 2018;142:769–778.
15. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20:159.
16. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341:569–573.
17. Zou S, Caler L, Colombini-Hatch S, Glynn S, Srinivas P. Research on the human virome: where are we and what is next. Microbiome. 2016;4:32.