Microbiome, Cancer and the Immune System

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The microbiome is the complex set of bacteria, viruses, and fungi which co-habit with the human. This concatenation of organisms generally are mutually beneficial. At time they may battle one another. Here we explore some recent work regarding the microbiome, the innate immune system and cancer.

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1 INTRODUCTION

It can be said that every mammal is a concatenation of a multiplicity of species generally living in harmony. A human exists side by side with commensurate bacteria, viruses, fungi, that help the human maintain normal homeostasis. They assist in balancing pH, in breaking down carbohydrates, and in interacting with the immune system. At the extreme there are exogeneous microorganisms which can be harmful to the prime organism and the resident microorganisms can become a part in some sense of the immune system of that organism. This would even include the collection of such cohabitating micro-organisms even controlling aberrant cell growth as seen in cancers.

The NIH has an extensive Microbiome Project[1]. As NIH states:

Microscopic study of the healthy human body has demonstrated that microbial cells outnumber human cells by about ten to one. Until recently though, this abundant community of human-associated microbes remained largely unstudied, leaving their influence upon human development, physiology, immunity, and nutrition almost entirely unknown. The NIH Common Fund Human Microbiome Project (HMP) was established with the mission of generating research resources enabling comprehensive characterization of the human microbiota and analysis of their role in human health and disease. The information generated by HMP is made available worldwide for use by investigators and others in efforts to understand and improve human health.

It appears that the NIH project has significant current limits. We shall explore dimensions outside of the NIH study, namely the interaction of the microbiome with cancer.

The microbiome is a terms to describe the collection of microbiological entities, bacteria, fungi, viruses, that inhabit the normal health individual and often play a key role in homeostasis. Soon after the discovery of bacteria, it was thought that any foreign microorganism may have deleterious effects. It soon changed since the colon is filled with microorganisms that assist in the digestion and utilization of foods. Recently the doctrine that urine was sterile and that anything the indicated a microorganism was present was a defect was overthrown (see Ainsworth).

Moreover a growth of studies showing that the microbiome is efficacious in fighting cancers has been developed. At one extreme is the importance of the microbiome in managing types of chemotherapy to the critical nature of the microbiome in facilitating the immune system early on in fighting cancer cells, and especially cancer stem cells. The microbiome may very well present an added tool to facilitating the body's own systems in fighting a variety of malignancies.

In this note we examine some of the recent research and

As Vogtmann and Goedert have recently noted:

[1] https://commonfund.nih.gov/hmp

Human microbiome research has garnered substantial attention, both by scientists and the media. The human microbiome refers to the collective genome of all bacteria, archaea, fungi, protists, and viruses residing in and on the human body. Made feasible by high throughput, next-generation deep sequencing of DNA, as well as expanding computational and bioinformatics support, the microbiome is a conceptual quantum leap from detection and identification of individual microbes to characterization of entire microbial communities, including both pathogenic and commensal microbes that have not yet been cultured or otherwise detected. Differences among individuals in our co-dependent relationship with the microbiota is postulated to modulate susceptibility to many malignancies via several pathways, including nutrition, detoxification, metabolism, hormonal homeostasis, immune tolerance, and especially inflammation

The above makes a significant point. Namely, the availability of new and improved measurement devices and methods allow us to look at the microbiome in significant detail.

As Eureka notes:

Enterococcus faecalis 2001 is a probiotic lactic acid bacterium and has been used as a biological response modifier (BRM). From physiological limitation of bacterial preservation in storage and safety, the live E. faecalis 2001 has been heat-treated and the BRM components containing high level of β-glucan, named EF-2001, were prepared. Method: The heat-treated EF-2001 has been examined for the antioxidative potential for radical scavenging and anti-tumor activities as well as immune-enhancing response in mice.

Lymphocyte versus polymorphonuclear leukocyte ratio was increased in mice upon treatment with EF-2001. The number of lymphocytes was increased in the EF-2001-treated group. In the mice bearing two different Ehrlich solid and Sarcoma-180 carcinomas, the treatment with EF-2001 resulted in anti-tumor action. Tumor-suppressive capacity upon treatment with EF-2001 was significantly increased compared to normal controls. Results: During the time interval administration of 5 weeks between the priming and secondary administration of EF-2001, the expression and production levels of TNF-α were also observed in the EF-2001administered mice. Additionally, anti-tumor activity examined with the intravenous administration of EF 2001 with a 34 time intervals was also observed, as the growth of Sarcoma180 cells was clearly inhibited by the EF-2001. Conclusion: From the results, it was suggested that the immune response is enhanced due to antioxidative activity caused by the EF-2001 and anti-tumor activity by NK cells and TNF-α.

The microbiome is the concatenation of organisms in the multiplicity of organism in the human body. The interaction of these microorganisms and the human cells, local and distant from their presence, presents an overwhelming complex system to be considered.

2 MICROBIOME

As we have noted, the microbiome is that collection of micro-organisms which can co-exist with the human organism and in the process not generate an immune response including inflammation as well as contribute towards a benign homeostasis. We have recently seen an increased interest in the microbiome as an adjunct in cancer therapy as well as a putative cause of many cancers.

As Cho and Blazer note:

Interest in the role of the microbiome in human health has burgeoned over the past decade with the advent of new technologies for interrogating complex microbial communities. The large-scale dynamics of the microbiome can be described by many of the tools and observations used in the study of population ecology. Deciphering the metagenome and its aggregate genetic information can also be used to understand the functional properties of the microbial community. Both the microbiome and metagenome probably have important functions in health and disease; their exploration is a frontier in human genetics.

Part of the issue is that the microbiome is generally neglected when examining cancers. Yet it has been found that it can in some cases facilitate the treatments and in others inhibit it. As Lloyd-Price et al note:

Microbiomes regularly show a large degree of interpersonal diversity even in the absence of disease. This complicates the identification of simple microbial constituents or imbalances that either cause disease or reflect a diseased state. An understanding of the properties of a healthy microbiome, and the many different microbial ecologies that are encountered in the absence of overt disease, is therefore a necessary first step to identifying and correcting microbial configurations that are implicated in disease. In this review, we use "healthy" to refer to the absence of any overt disease.

It is this diversity between people that makes it a difficult system to assess. In addition to person to person diversity is the temporal diversity in a single person. Furthermore is the diversity across organisms in the body not to mention the complexity of interactions between microorganisms and their responses. The authors continue;

Most available data describe the gut microbiome and so many of the findings discussed here are from this area, though most principles apply to microbial habitats throughout the body. Early research into the ecology of the microbiome sought to identify a "core" set of microbial taxa universally present in healthy individuals who lack overt disease phenotypes, under the hypothesis that the absence of such microbes would indicate dysbiosis; but studies of ecological diversity among healthy individuals revealed sufficient variation in the taxonomic composition of the microbiome to rapidly render such a hypothesis unlikely.

The microbiome is present in all organs; the gastro system, the oral cavity, the bladder and even the hematological system. As Thaiss et al state:

The intestinal microbiome is a signalling hub that integrates environmental inputs, such as diet, with genetic and immune signals to affect the host's metabolism, immunity and response to infection.

The haematopoietic and non-haematopoietic cells of the innate immune system are located strategically at the host–microbiome interface. These cells have the ability to sense microorganisms or their metabolic products and to translate the signals into host physiological responses and the regulation of microbial ecology. Aberrations in the communication between the innate immune system and the gut microbiota might contribute to complex diseases.

The innate immune system, with the collection of Pattern Recognition Receptors, such as the Toll Like Receptors, TLR, are integral in early recognition of and response to such microorganisms as those found in a microbiome. Then one may wonder why there is not a continual battle between the immune system and the microbiome. Why one may wonder is the mouth not in a continual inflammatory state with the ongoing release of chemokines and cytokines, why the intestinal system does not have a similar ongoing battle. Thaiss et al continue:

The past two decades witnessed a revolution in our understanding of host–microbial interactions that led to the concept of the mammalian holobiont — the result of co-evolution of the eukaryotic and prokaryotic parts of an organism. The revolution required two paradigm shifts that had a tremendous impact on their respective fields.

The first occurred during the late 1990s with the discovery of pattern recognition receptors (PRRs) in the innate immune system that sense microorganisms through conserved molecular structures. Several families of PRRs and their signalling pathways are now known, including the Toll-like receptors (TLRs), the nucleotide-binding oligomerization (NOD)-like receptors (NLRs), the RIG-I-like receptors, the C-type lectin receptors, the absent in melanoma 2 (AIM2)-like receptors and the OAS-like receptors1. These sensors are expressed by a variety of cellular compartments and constitute a continuous surveillance system for the presence of microorganisms in tissues.

The second shift occurred fewer than 10 years later and was driven by the culture-independent characterization of the microbiome2 — the entirety of the microorganisms that colonize the human body and their genomes. Because of the enormous number of microorganisms that reside on the surface of the body — the skin and the gastrointestinal, respiratory and urogenital tracts — it seemed improbable that innate immune recognition of microorganisms could be coupled to the immediate initiation of immune responses against them without leading to overt, organism-wide inflammation and its damaging effects. It was therefore hypothesized that microbial sensing at the body surface needs to be tightly controlled to ensure a symbiotic relationship between the host and its indigenous commensal microorganisms3, while allowing for the initiation of a rapid, sterilizing immune response on penetration of microorganisms into non-colonized sites. This idea was developed further after the realization that host–microbiota mutualism is lost in the absence of innate immune recognition of commensal microorganisms, with detrimental consequences for health. The crosstalk between innate immunity and the microbiome is now known to extend far beyond the achievement of a careful balance between tolerance to commensal microorganisms and immunity to pathogens.

The innate immune system has often been looked upon as a poor cousin to the adaptive system Yet its relationship to and with the microbiome presents a complex system problem that may offer new dimensions to how to manage a stable biome while addressing the changes one sees in a malignant transformation. Thaiss et al conclude:

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Thus the stability between the microbiome and the innate system will be a key factor in comprehending its efficacy.

3 INTERACTION WITH IMMUNE SYSTEM

The microbiome, the benign and pathologic, are inherently all presenters of antigens to the immune system. In the case of the benign and common microbiome, some form of stasis is reached by various means such as physical isolation of antigens. The human body has a multiplicity of cells with pattern recognition receptors which are on a constant look-out for antigens to then be activated and provide an effective immune response. That would be counterproductive for that part of the microbiome which is part of the homeostatic system.

3.1 EXAMPLE OF INNATE SYSTEM WITH MICROBIOME

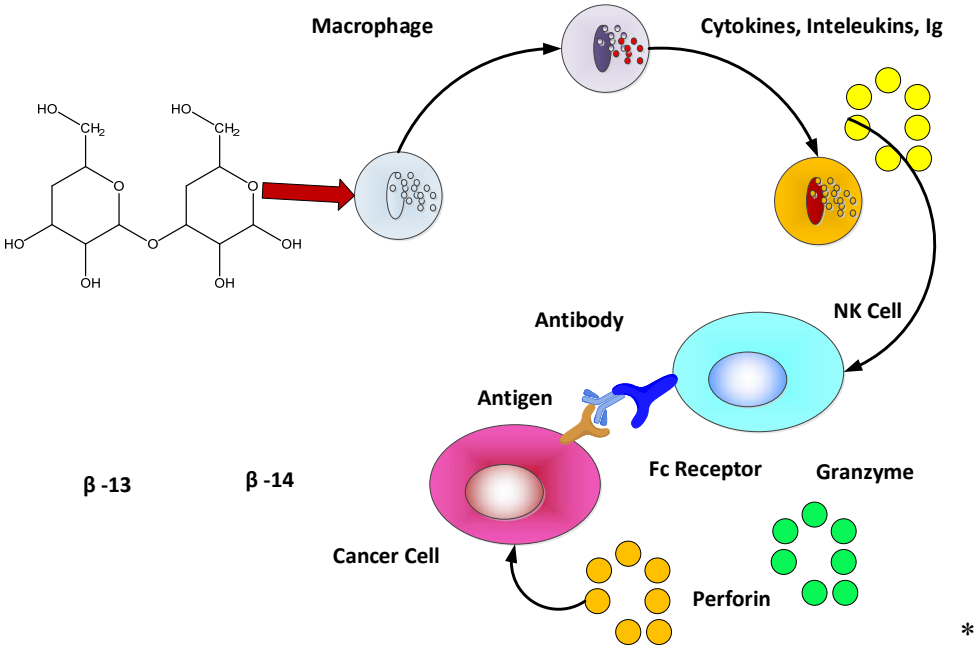
The interest in this topic as noted earlier was driven by some recent work in the intersection of the microbiome and the innate system. As Gu et al note:

Enterococcus faecalis 2001 is a probiotic lactic acid bacterium and has been used as a biological response modifier (BRM). From physiological limitation of bacterial preservation in storage and safety, the live E. faecalis 2001 has been heat-treated and the BRM components containing high level of β-glucan, named EF-2001, were prepared…The heat-treated EF-2001 has been examined for the antioxidative potential for radical scavenging and anti-tumor activities as well as immune-enhancing response in mice. Lymphocyte versus polymorphonuclear leukocyte ratio was increased in mice upon treatment with EF-2001. The number of lymphocytes was increased in the EF-2001-treated group.

In the mice bearing two different Ehrlich solid and Sarcoma-180 carcinomas, the treatment with EF-2001 resulted in anti-tumor action. Tumor-suppressive capacity upon treatment with EF-2001 was significantly increased compared to normal controls…During the time interval administration of 5 weeks between the priming and secondary administration of EF-2001, the expression and production levels of TNF-α were also observed in the EF- 2001-administered mice. Additionally, anti-tumor activity examined with the intravenous administration of EF 2001 with a 34 times interval was also observed, as the growth of Sarcoma180 cells was clearly inhibited by the EF-2001…

From the results, it was suggested that the immune response is enhanced due to antioxidative activity caused by the EF-2001 and anti-tumor activity by NK cells and TNF-α.

We can depict this effect as shown below. Here we have a definable mechanism wherein the activation of a product in the microbiome can then identify and attack an early stage malignant cell.



The above raises several interesting questions. First, we need the details of the interaction. Second, understanding the details can we manage to force this interaction, make it more aggressive, and facilitate it use in a cancer modulation process.

Zitvogel et al note:

The human gut microbiome modulates many host processes, including metabolism, inflammation, and immune and cellular responses. It is becoming increasingly apparent that the microbiome can also influence the development of cancer. In preclinical models, the host response to cancer treatment has been improved by modulating the gut microbiome; this is known to have an altered composition in many diseases, including cancer.

In addition, cancer treatment with microbial agents or their products has the potential to shrink tumours. However, the microbiome could also negatively influence cancer prognosis through the production of potentially oncogenic toxins and metabolites by bacteria. Thus, future antineoplastic treatments could combine the modulation of the microbiome and its products with immunotherapeutics and more conventional approaches that directly target malignant cells.

In fact, the microbiome not only modulates but it can initiate and facilitate the overall process of innate immune response.

3.2 INNATE LYMPHOID CELLS

We first consider the collection of cells called the Innate Lymphoid Cells, ILC. These cells, not fully understood, appear to play a critical role in many microbiome related processes. However their function and even their identity is still under study and for many their very existence is unknown.

From Abbas et al:

Innate lymphoid cells (ILCs)… are bone marrow–derived cells with lymphocyte morphology that were discovered as cells that produced cytokines similar to those made by T cells but lacked TCRs. We call them "lymphoid cells," not "lymphocytes," because they do not express clonally distributed diverse antigen receptors like the T lymphocytes they otherwise resemble.

There are different subsets of ILCs that arise from the same common lymphoid precursor that gives rise to B and T cells, but the precise steps in ILC development are not fully understood, especially in humans. It is clear that during their development, there are branch points giving rise to three different "helper" subsets of ILCs, which function mainly by secreting different types of cytokines, similar to CD4+ helper T cell subsets, and a separate branch giving rise to natural killer (NK) cells, which function as cytotoxic effectors in addition to secreting the cytokine interferon-γ, similar to CD8+ cytotoxic T lymphocytes…

Three subsets of innate lymphoid cells, called ILC1, ILC2, and ILC3, produce different cytokines and express different transcription factors, analogous to the Th1, Th2, and Th17 subsets of CD4+ T lymphocytes. The cytokines each subset produces determine the roles of these cells in defense, and the transcription factors are required for differentiation and function of each of the three subsets. ILC1s produce IFN-γ and express the transcription factor T-bet, like Th1 cells. ILC2s produce IL-5, IL-9, and IL-13, and express the transcription factor GATA-3, like Th2 cells. ILC3s produce IL-22 and/or IL-17 and express the transcription factor RORγt, like Th17 cells. Because ILCs do not express T cell receptors, they must be activated by different mechanisms than helper T cells to produce these cytokines. The best defined stimuli for ILC cytokine production are other cytokines, released in the context of innate responses to infections and tissue damage; each ILC subset is activated by different cytokines.

They continue:

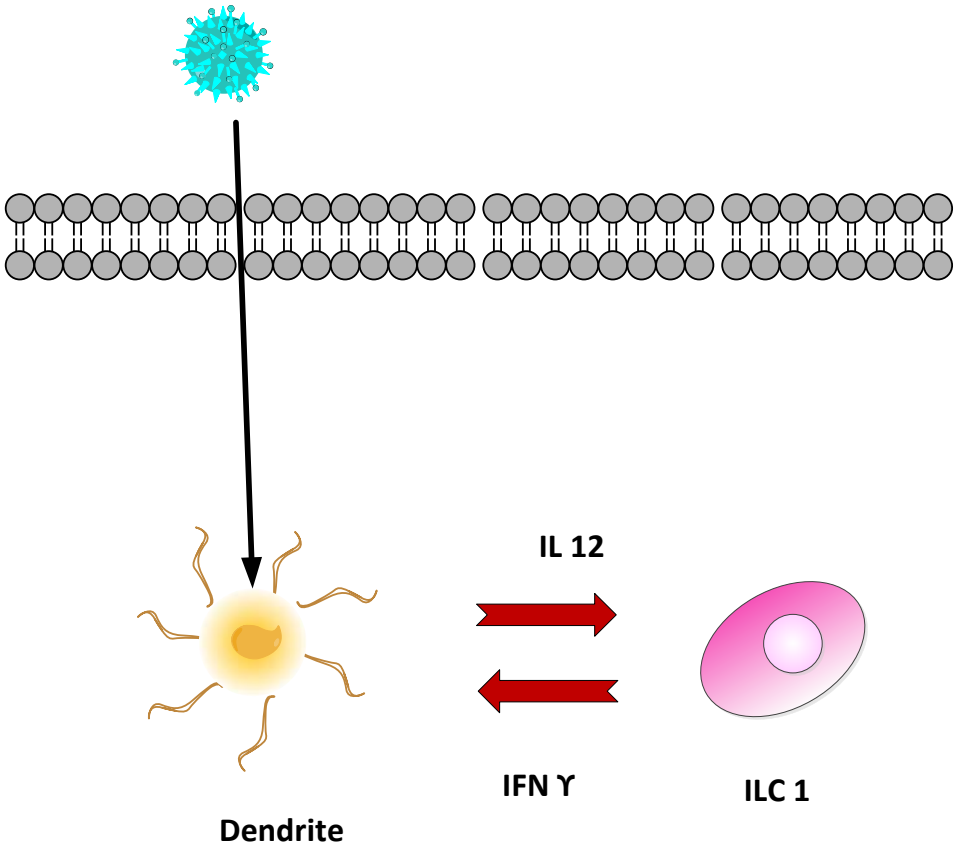
ILC subsets may participate in host defense against distinct pathogens and also may be involved in inflammatory disorders. ILC1s are likely important for defense against intracellular microbes. ILC2s are important for defense against helminthic parasites, and they also may contribute to allergic diseases. ILC3s are found at mucosal sites and participate in defense against extracellular fungi and bacteria, as well as in maintaining the integrity of epithelial barriers. Lymphoid tissue–inducer (LTi) cells are a subtype of ILC3s, which, in addition to secreting IL-17 and IL-22, also express the membrane molecule lymphotoxin-α and secrete TNF, both of which are required for the normal development of lymphoid organs.

The contribution of ILCs to host defense has been difficult to establish because it has not been possible to selectively eliminate these cells or their cytokines without impacting the analogous T lymphocytes as well. The feature of ILCs that makes them potentially important for early host defense is that they are always resident in epithelial barrier tissues, poised to react against microbes that breach those barriers. In contrast, T cells circulate through secondary lymphoid organs and migrate into tissues only after they are activated and differentiate into effector cells, a process that may take several days after encounter with a microbe. It is, therefore, possible that ILCs are early responders to microbes that colonize tissues, and over time this role is

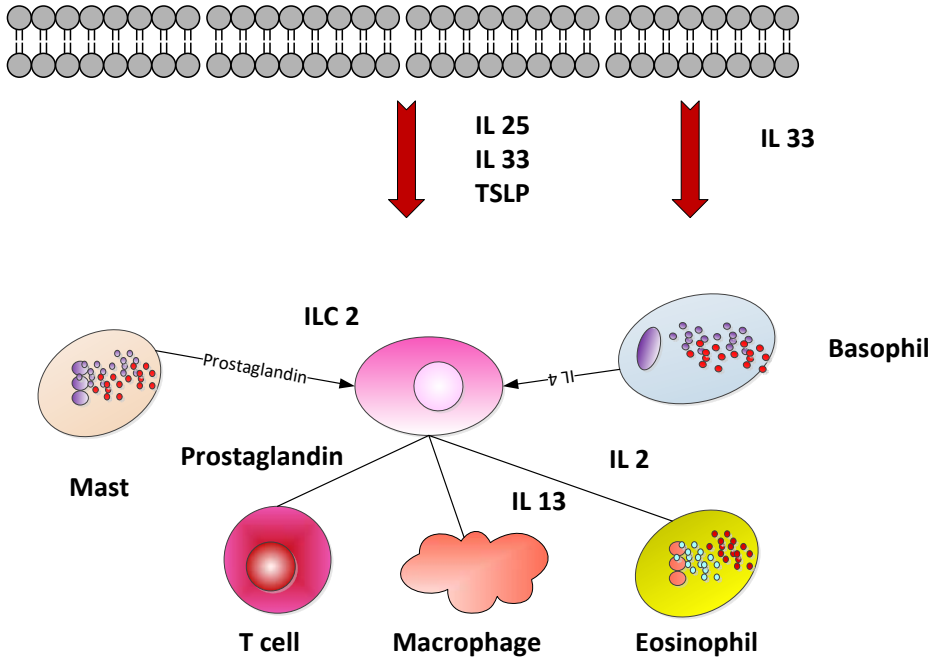
assumed by differentiated effector T cells, which are which are more specific and produce larger amounts of cytokines.

The three ILCs interact as follows:

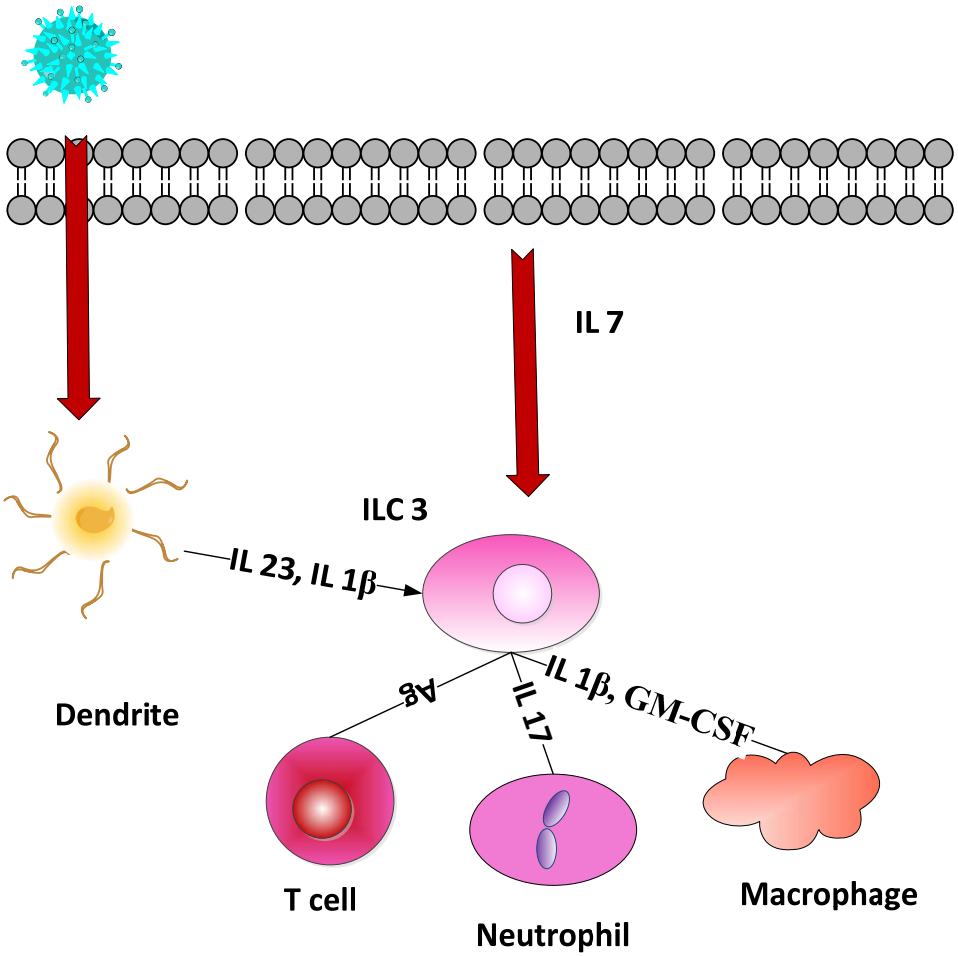
ILC 1 is activated by some organism through the cell wall via a dendrite. IL 12 activates the IL 1 and in turn the IL 1 produces a feedback IFN γ.



ILC 2 is depicted below. Here we have a basophil activation along with a complementary mast cell. ILC 2 then in turn turns up the T cells, Macrophages and eosinophils.



Finally we have the ILC 3 cells activated by a flagellin via a dendritic cell, and co-stimulated by IL 7 and then it activates the T cell, the macrophage and now a neutrophil.



We find that the ILCs are apparently facilitators of the microbiome and its interaction with the innate immune system. It is not at all clear what the temporal characteristics are but generally the innate system does have a near real time response mechanism. The ILCs may be activated via an activated microbiome. As we will also note later that Guglielmi discusses the complex interaction between the microbiome, phagosome, phage or viral elements, and in turn the immune system.

4 CANCER

It has become mora apparent that many infections and resulting inflammatory effects are a basis for the initiation and development of a multiplicity of cancers. Now infections are basically the introduction of and proliferation of exogenous pathogenic organisms in the human biome. They are organisms, bacterial, viral or fungal, that initiate some possible immune response. They may have a PRR, pattern recognition receptor such as a TLR, type response. Inflammation is the immune system response which may be the result of any infection or even a self-generate an immune response such as a self-immunity. Finally we separate obesity as a major cause of cancer separate and apart. Obesity establishes an environment which results in inflammatory like responses. It places significant stress upon the organisms, in this case the human body, which, over time, result in changes leading towards a malignancy.

The effects that the above induce may be the result of genetic changes, deletions, additions or translocations, or the impact may be epigenetic via some methylation change or acetylation change, or it may even be a miRNA induction or suppression. Clarity of cause and effect has yet to be established.

As Garrett notes:

Microbiota contribute to carcinogenesis, whether by enhancing or diminishing a host's risk, fall into three broad categories: (i) altering the balance of host cell proliferation and death, (ii) guiding immune system function, and (iii) influencing metabolism of host-produced factors, ingested foodstuffs, and pharmaceuticals (Fig. 1). Assigning microbial communities, their members, and aggregate biomolecular activities into these categories will require a substantial research commitment. ...

Bona fide oncomicrobes—microbes that trigger transformation events in host cells—are rare. Beyond the 10 IACR-designated microbes, there are a handful of other microorganisms with robust but fewer aggregate data supporting their role in human carcinogenesis. As many of these and their carcinogenic mechanisms have been recently reviewed, select activities representing common pathways by which microbes influence cancer will be highlighted. Human onco-viruses can drive carcinogenesis by integrating oncogenes into host genomes. Human papillomaviruses (HPV) express onco-proteins such as E6 and E7.

Data from recent genomic analyses of HPV+ cervical cancers suggest that viral integration also selectively triggers amplification of host genes in pathways with established roles in cancer. Microbes also drive transformation by affecting genomic stability, resistance to cell death, and proliferative signaling. Many bacteria have evolved mechanisms to damage DNA, so as to kill competitors and survive in the microbial world.

4.1 INFECTIONS

Infections and the impact of the microbiome has become a compelling area of study in cancer epidemiology. As Martel et al have concluded:

In view of the high mortality rate of infection associated cancers, the fraction of cancer deaths attributable to infections is probably higher than the 16·1% that our study generated. Although a full investigation of cancer death due to infection is beyond the scope of this report, we can estimate the mortality burden by applying the PAFs to the 7·5 million cancer deaths that occurred in 2008. These calculations suggest that 1·5 million cancer deaths were attributable to infectious agents, or roughly one in five deaths due to cancer worldwide

Simply they estimated that 20% of cancer deaths were related to some form of overt infection. This does not include microbiome distortions, those changes that result in DNA reconfiguration. Their analysis is somewhat dated, not due to the analytical approach but to what impact the microbiome truly has on cancer development.

4.2 INFLAMMATION

In contrast we also see that chronic inflammation, a process caused by some irritant not necessarily diagnosable as an overt infection has a dramatic effect. For example, as Thaiss et al note:

The idea that chronic inflammation drives carcinogenesis has been widely established in various tissues. For example, hepatocellular carcinomas arise in people with chronic hepatitis, colorectal cancer can occur in people with longstanding untreated IBD and Marjolin's ulcers develop on chronically inflamed skin. The presence of bacteria at tumour sites was first described more than a century ago, so it is surprising that the role of the microbiota in tumourigenesis has only recently been recognized. Colorectal carcinogenesis is triggered by a combination of microbiota- and host-dependent mechanisms. Certain bacteria promote carcinogenesis directly, through the secretion of substances that elicit DNA damage.

The colon is a likely place for various pathogens to flourish. In fact the microbiome of the colon is an ever changing environment and the presence of certain oncogenic products is well known as causal for malignancies. The classic Vogelstein model depicts how this may occur. The author continues:

Prominent examples include the excessive release of nitric oxide from immune cells that is triggered by Helicobacter hepaticus, the production of reactive oxygen species by Enterococcus faecalis and the secretion of an enterotoxin by Bacteroides fragilis, which activates the oncogene c-MYC. Other bacteria drive carcinogenesis indirectly by sustaining a proinflammatory microenvironment, such as the production by Fusobacterium nucleatum of the virulence factor FadA, which increases the paracellular permeability of colonic epithelial cells. Inflammation might also promote community-level alterations in the microbiome and facilitate bacterial translocation into neoplastic tissue, which further promotes the expression of inflammatory cytokines and leads to the increased growth of tumours. Dysbiosis that arises in the absence of NLRP6 promotes the development of cancer through IL-6-induced epithelial proliferation.

Reactive Oxygen Species, ROS, are well know instigators of DNA damage and in turn mutagenic effects leading to malignancies. However, these often occur at low levels in inflammatory conditions and here to we would see such damage. Continuing:

The influence of the microbiota on innate immunity has been shown to affect the host response to cancer therapy. For example, germ-free mice and mice that are treated with antibiotics both show a diminished response to immunotherapy by CpG oligonucleotides and chemotherapy owing to the impaired function of myeloid-derived cells in the tumour microenvironment. Furthermore, commensal Bifidobacterium enhances immunity to tumours through antibodies directed against programmed cell death 1 ligand 1 (PD-L1) through the augmentation of dendritic-cell function. These studies might open up a fascinating avenue of research to prevent cancer and develop cancer therapeutics through manipulation of the microbiota.

The PD-L1 effect is critical. Cancers have the ability to present PD-L1 and to block PD-1 from activation and inhibiting the immune system from destroying the cancer cell. There is a plethora of immunotherapy doing the same type of function done in the microbiome. The question is; why doe the microbiome achieve this for only a select types of malignant cells?

As Garrett notes:

Mechanisms by which microbes influence cancer development and progression.

(A) Bacterial toxins can directly damage host DNA. Bacteria also damage DNA indirectly via host produced reactive oxygen and nitrogen species. When DNA damage exceeds host cell repair capacity, cell death or cancer-enabling mutations occur.

(B) b-Catenin signaling alterations are a frequent target of cancer-associated microbes. Some microbes bind E-cadherin on colonic epithelial cells, with altered polarity or within a disrupted barrier, and trigger b-catenin activation. Other microbes inject effectors (e.g., CagA or AvrA) that activate b-catenin signaling, resulting in dysregulated cell growth, acquisition of stem cell–like qualities, and loss of cell polarity.

(C) Proinflammatory pathways are engaged upon mucosal barrier breach in an evolving tumor. Loss of boundaries between host and microbe engages pattern recognition receptors and their signaling cascades. Feedforward loops of chronic inflammation mediated by NF-kB and STAT3 signaling fuel carcinogenesis within both transforming and non-neoplastic cells within the tumors

Garrett note three factors. First is the DNA damage due to what bacterial elements release. This area clearly needs improved analysis. Second, b-catenin is a driver of E Cadherin, the protein that ties one cell to another. Break E cadherin and the cells start to migrate. We see this in melanoma, especially in melanoma in situ. Third, the activation of the NF-kB pathway, as we have discussed extensively before, is a major driver for proliferation and metastasis.

As Schwabe and Jobin note:

Microbiota and host form a complex 'super-organism' in which symbiotic relationships confer benefits to the host in many key aspects of life. However, defects in the regulatory circuits of the host that control bacterial sensing and homeostasis, or alterations of the microbiome, through environmental changes (infection, diet or lifestyle), may disturb this symbiotic relationship and promote disease. Increasing evidence indicates a key role for the bacterial microbiota in carcinogenesis.

In this Opinion article, we discuss links between the bacterial microbiota and cancer, with a particular focus on immune responses, dysbiosis, genotoxicity, metabolism and strategies to target the microbiome for cancer prevention.

The metaphor as a "super organism" may be apt. The question however may be; as humans change their consumption of or exposure to other organisms or substances that enable or suppress existing organisms, does the homeostatic balance we would expect get disturbed to result in a malignancy. Simply, we now know that cigarette smoking can lead to lung cancer, and transmission of papilloma virus leads to cervical cancers. Is this a result of such disturbances?

As Sfanos et al note:

Chronic inflammation promotes the development of several types of solid cancers and might contribute to prostate carcinogenesis. This hypothesis partly originates in the frequent observation of inflammatory cells in the prostate microenvironment of adult men. Inflammation is associated with putative prostate cancer precursor lesions, termed proliferative inflammatory atrophy. Inflammation might drive prostate carcinogenesis via oxidative stress and generation of reactive oxygen species that induce mutagenesis. Additionally, inflammatory stress might cause epigenetic alterations that promote neoplastic transformation. Proliferative inflammatory atrophy is enriched for proliferative luminal epithelial cells of intermediate phenotype that might be prone to genomic alterations leading to prostatic intraepithelial neoplasia and prostate cancer.

Studies in animals suggest that inflammatory changes in the prostate microenvironment contribute to reprogramming of prostate epithelial cells, a possible step in tumour initiation. Prostatic infection, concurrent with epithelial barrier disruption, might be a key driver of an inflammatory microenvironment; the discovery of a urinary microbiome indicates a potential source of frequent exposure of the prostate to a diverse number of microorganisms. Hence, current evidence suggests that inflammation and atrophy are involved in prostate carcinogenesis and suggests a role for the microbiome in establishing an inflammatory prostate microenvironment that might promote prostate cancer development and progression.

Now with some of the recent immunotherapy drugs the biome can be enhanced. As Leslie notes:

This team pinpointed members of the genus Bifidobacterium as an immune helper: Feeding mice a probiotic that contains several Bifidobacterium species increased the efficiency of a PD-L1–blocking antibody against tumors. The fact that the two teams implicated different bacterial groups doesn't worry microimmunologist Christian Jobin of the University of Florida College of Medicine in Gainesville. "Different drugs, different bugs, but the same endpoint," he says. Exactly how the microbiome bolsters the drugs remains unclear. Still, the discovery "opens up

novel ways to potentially augment therapy," says Cynthia Sears, an infectious disease specialist at Johns Hopkins School of Medicine in Baltimore, Maryland. Doctors could, for example, try to beef up antitumor responses with probiotics, although Zitvogel notes that regulatory agencies haven't approved their use for cancer patients

From Fulbright et al we have the following list of cancer related microbiome elements:

Intestinal bacteria	*Bacterial mechanism*	*Hallmark affected*
enterotoxigenic *Bacteroides fragilis* (ETBF)	*B. fragilis* toxin (BFT)	sustaining proliferative signaling
		genome instability and mutations
	unknown mechanism	tumor-promoting inflammation
Fusobacterium nucleatum	FadA adhesin	sustaining proliferative signaling
	Fap2 adhesin	avoiding immune destruction
pks+ Escherichia coli	colibactin	genome instability and mutations
		sustaining proliferative signaling
Enterococcus faecalis	unknown mechanism	genome instability and mutations
Alistipes spp.	unknown mechanism	tumor-promoting inflammation
Bifidobacterium spp.	unknown mechanism	inhibits avoiding immune destruction
Bacteroides thetaiotamicron and *B. fragilis*	unknown mechanism	inhibits avoiding immune destruction

The authors continue:

Normal tissues tightly regulate growth-promoting and death-inducing signals to maintain homeostatic cell densities, tissue architecture, and function. Dysregulation of these signaling pathways can lead to sustained cellular proliferation. The intercellular adhesion molecule, E-cadherin, is a common target engaged by intestinal bacteria that promotes epithelial proliferation by activating the Wnt/û-catenin pathway. For example, enterotoxigenic Bacteroides fragilis (ETBF), resident among the microbiota of some individuals, secretes B. fragilis toxin (BFT) that promotes cleavage of E-cadherin.

This enables the nuclear translocation of û-catenin, subsequent transcription of proto-oncogene c-Myc, and colonic epithelial hyperplasia. Through a similar mechanism, Fusobacterium nucleatum enhances epithelial proliferation through engagement of its adhesin FadA with E-cadherin. Neutralizing FadA abrogated the tumor-promoting activities of F. nucleatum in a murine xenograft cancer model, demonstrating the potential of targeting bacterial interactions with E-cadherin as a novel strategy in mitigating cancer progression.

Taken together, these studies demonstrate that the microbiota can be a source of activating signals for aberrant epithelial proliferation as an initiating step in cancer development.

The change in e-cadherin is critical. This is a binding protein and when broken the cell now has the ability to move about and this in many cancers is the first step towards an aggressive growth pattern.

From Vogtmann and Goedert they indicate the following possible list:

Pathogen	*Cancer Type/Organ*
H pylori	Gastric
H pylori	Hepatobilliary
Salmonella typhi	Hepatobiliary
Neisseria elongata	Pancreas
Streptococcus mittis	Pancreas
Porphyromonas gingivalis[2]	Pancreas
Mycobacterium tuberculosis	Lung
Spirochaetae	Lung
Bacteroides	Lung
Synergisters	Lung
Fusobacterium	Colorectal
Porphyromonas	Colorectal
Borellia burdorfii	Cutaneous B Cell Non Hodgkins Lymphoma
Chlamydophilia psittaci	MALT Lymphoma

These types of lists have been developed by many authors. Generally they lead to many similar and identical pathogens but frequently to an added new set.

4.3 OBESITY

We have discussed elsewhere that obesity is directly and as a result of its inflammatory nature is a putative cause of cancer. Obesity is a major epidemic throughout the world. It is insidious in that its effects are slow to develop and then once started are often near impossible to stop. It is a pandemic of a chronic and debilitating state, with disease sequellae. As Arnold et al note:

Worldwide, we estimated that 481,000 or 3·6% of all new cancer cases in 2012 were attributable to excess BMI. PAFs were greater in women compared with men (5·4% versus 1·9%). The burden was concentrated in countries with very high and high human development index (HDI, PAF: 5·3% and 4·8%) compared with countries with moderate and low HDI (PAF: 1·6% and 1·0%).

[2] See Thaiss et al, "*The microbiome and innate immune system also cooperate in the eradication of bacterial infection. Sometimes, neither innate immunity nor colonization resistance is sufficient to ensure the expulsion of pathogens. Instead, a combination of the two is required, as in the case of cooperation in the host defence against Citrobacter rodentium, a bacterium that can cause disease in mice. However, such combinatorial responses can be subverted by the pathogen. During infection with Salmonella Typhimurium, microbiota-induced IL-22 elicits a response that targets commensal bacteria and liberates a colonization niche for the pathogenic bacterium118. Porphyromonas gingivalis, an oral bacterium that is associated with periodontitis, evades the host by modulating the TLR2 pathway to support a niche for dysbiosis and subsequent inflammation*."

Corpus uteri, post-menopausal breast and colon cancers accounted for approximately two-thirds (64%) of excess BMI attributable cancers. One fourth (~118,000) of all cases related to excess BMI in 2012 could be attributed to the rising BMI since 1982.

Obesity has a massive amount of secondary effects. It generates a feeding ground for many microorganisms, provides nutrients, creates a multiplicity of reactive oxygen species, and the like.

4.4 BIOMES

We now examine two rather extreme cases. First the oral biome which we know is a complex and active biome. There has been extensive study of the relationship between that biome and head and neck cancers, including oral cancers. The lack of long term stability of the biome is often a problem in examining it for microorganism effects. The second is the bladder, an organ which has been generally assumed to be sterile. In reality there is a complex biome of microorganisms present but they generally cannot be studied using more classic techniques. We have one area of complex well known microorganisms and another presenting a new territory to explore.

We present two examples of these biomes. One is obvious, the oral cavity. The second is counter intuitive based on classic medical training, namely the bladder. We all assumed the mouth to be filled with bacteria. In fact we often wonder how the body manages to battle against this excess. In contrast we all assume urine is sterile. In fact there are commensurate bacteria in the bladder. These we discuss.

4.4.1 Oral

Lin et al have examined the oral biome. The following Table is a brief summary of the microbiome and this may very well be incomplete. Furthermore it may change from person to person and even with a single person there may be substantial temporal changes.

Region	Microorganism
Tooth Surface	Streptococcus mutans, Actinomyces, Eubacterium, Peptostrepptococcus
Tonsil	Streptococcus viridans, Neisseria species, — Haemophilus influenzae, coagulase-negative Staphylococci
Tongue	Veillonella atypica, Porphyronas gingiva I is, Selenomonas species, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Capnocytophaga species, Streptococcus faecalis, Eikenella corrodens
Gingival Surface	Fusobacterium, Prevote I la, Porphyromonas
Oropharyngeal region	Streptococcus salivarius, Streptococcus mutans, Streptococcus anginosus, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenza, Haemophilus parainfluenzae
Dental Plaque	Actinomyces, Rothia, Kocuria, Arsenicicoccus, Microbacterium, Propionibacterium, Mycobacterium, Dietzia, Turicella, Corynebacterium, Bifidobacterium, Scardovia, Parascardovia

Lin et al then note:

Oral microbiome, by definition, is the collective genomes of microorganisms that reside in the oral cavity. Many researchers believe that the characterisation of oral microbiome is an essential step in understanding oral health and systemic diseases. The oral cavity has densely populated microbial communities and has the largest core of commonly shared microbes among unrelated individuals. As such, oral microbiome provides an ideal source for biomarker discoveries due to low inter- and intra- biological variations, in contrast to other tumour biomarkers originating from the host.

The oral cavity and associated nasopharyngeal regions are also an ideal environment for the growth of microorganisms. The average normal oral temperature is 37°C without significant fluctuation, providing bacteria a stable habitat to thrive. In addition, saliva maintains a stable pH of 6.5 to 7.5, the preferred pH for most bacteria species. Saliva also keeps bacteria hydrated and acts as a medium to facilitate the transportation of nutrients to microorganisms. As such, the

oral cavity harbors more than 700 bacterial species and is one of the most densely populated anatomical sites within the human body…

Studies have established that chronic inflammation is responsible for 25% of human malignancies and represents the seventh hallmark in the development of cancers. Chronic inflammatory mediators cause or facilitate increased cell proliferation, mutagenesis, oncogene activation, and angiogenesis that ultimately lead to the loss of normal growth control and cancer.

Bacterial infection is one of the major causes of chronic inflammation. The strongest link established between bacterial infection and the development of cancer due to chronic inflammation to date is the association between Helicobacter pylori (H. pylori) and adenocarcinoma of the stomach, while other known associations include Salmonella typhi and gallbladder cancer, Streptococcus bovis and colon cancer, Chlamydia pneumonia and lung cancer, and Bartonella species and vascular tumour formation. In general, studies have shown that bacteria alone are unable to induce cancer; the process is commonly accompanied by chronic inflammation and requires independent mutations in oncogenic signalling pathways

The study carried out by Schmidt et al., (2014) investigated the oral microbiome of five oral cancer patients and eight oral pre-cancer patients using 16s rRNA gene amplicon next-generation sequencing.

The biospecimens were collected using swabs on the oral lesion and a contralateral normal site. This study reported a significant decrease in abundance of Firmicutes and Actinobacteria in cancer patients. A significant decrease in these phyla were also confirmed in pre-cancer patients, suggesting that oral lesion-associated shifts in oral microbiome may occur early in oral cancer development and/or herald cancer progression.

The study from Guerrero-Preston et al., (2016) utilised oral rinse as biospecimens. The oral microbiome of 19 HNSCC patients and 25 normal healthy individuals were investigated using 16s rRNA gene amplicon next-generation sequencing and a decrease in microbial richness and diversity was reported in cancers. The enriched presence of Lactobacillus or the loss of Haemopilus, Neisseria, Gemellaceae or Aggregatibacter in saliva was reported as a potential biomarker for HNSCC. While HPV status did not have a significant impact on the oral microbiome, it is speculated that the small sample size may have influenced the outcomes.

The findings from both studies indicated that microbial diversity and taxonomic composition of the oral microbiome may be useful biomarkers for HNSCC as well as provide a solid framework for future oral microbiome research.

Thus the oral cavity may be an interesting area to examine for the development of microorganism based malignancies. However its extreme uniqueness and instability would make such an examination quite difficult.

4.4.2 Bladder

The bladder has for a long time been considered a sterile environment. It is often in contradistinction to the oral cavity. However recently its biome has been examined and is beginning to be ascertained. As noted in Ainsworth:

The dogma that urine, and by extension the bladder, must be sterile to be healthy has been overturned, and microbes are being discovered throughout the urinary system. Researchers are investigating potential roles for them in healthy bladders and in a range of conditions, including urge incontinence — where people experience a sudden need to urinate — and in some cancers. Burton's team has found traces of bacteria in cancerous kidneys, for example. Although still at the early discovery stage, research into the bladder's microbes promises to transform understanding of the urinary tract. "It's really grown and exploded rapidly," says Burton…

The potential link with chronic inflammation raises the question of whether repeated urinary tract infections might be involved in the development of bladder cancer. One of the largest epidemiological studies of bladder cancer conducted so far reported4 in 2015 that repeated, regular bouts of cystitis were associated with increased risk, but whether the association was causative is unclear.

Further studies will be needed to confirm any links, which remain "a little tenuous" at the moment, according to Burton. …., for example, a team in Japan reported5 that people with bladder cancer who drank a probiotic containing Lactobacillus casei (sold commercially as Yakult), while also receiving chemotherapy treatments infused into the bladder, had recurrence rates that were 15% lower than those of subjects receiving chemotherapy alone. Critics of the study said that the pattern of patient dropout and lack of blinding may have undermined its conclusions, although the authors disagreed.

Previous studies in animals, conducted by several research groups, also suggest that probiotics can have anticancer effects in the bladder. These studies suggest that probiotics deserve further investigation, says Burton.

Thus the biome of the bladder may have any combination of neutral, negative or positive effect on the potential for malignancies. In contrast to the oral cavity, the microorganisms in the bladder are complex and require sophisticated techniques to determine.

5 THERAPEUTICS

Cancer therapeutics, especially the explosive use of immunotherapy, has demonstrated an ability to attack cancer cells as one would attack any foreign body using the elements of the immune system. Many of the immune approaches use T cell methods and even uniquely targeted T cells developed using chimeric approaches. However, it must be remembered that the immune system has a plethora of attack mechanisms. In particular the innate immune system has a powerful set of near real time attack cells and molecules that can recognize an aberrant cell or collection thereof and commence its elimination. Equally, as we have discussed, the microbiome can enhance that effect. It likewise can, if improper, be the actual cause of the malignancy.

In the paper by Thaiss et al they note:

The influence of the microbiota on innate immunity has been shown to affect the host response to cancer therapy. For example, germ-free mice and mice that are treated with antibiotics both show a diminished response to immunotherapy by CpG oligonucleotides and chemotherapy owing to the impaired function of myeloid-derived cells in the tumour microenvironment58. Furthermore, commensal Bifidobacterium enhances immunity to tumours through antibodies directed against programmed cell death 1 ligand 1 (PD-L1) through the augmentation of dendritic-cell function.

These studies might open up a fascinating avenue of research to prevent cancer and develop cancer therapeutics through manipulation of the microbiota.

Thus it is now well known that there is a strong linkage between the microbiome and cancer therapeutics as well.

5.1 PUTATIVE MICROBIAL THERAPEUTICS

The following list is one which reflects those with some putative efficacy in humans (see Zitvogel et al):

Bacterial species[3]	*Cancer type*	*Interventions and outcomes*
Streptococcus pyogenes* and *Serratia marcescens	Osteosarcoma	Coley's toxins: injection of *S. pyogenes* and *S. marcescens* in patients with sarcoma, with some evidence of objective response
***Mycobacterium bovis* BCG**	Urothelial superficial cancers	Intravesical treatment of a live attenuated form of *M. bovis* reduces the risk of short- and long-term relapse
***Lactobacillus casei* str. Shirota (found in the fermented milk product Yakult)**	Superficial bladder cancer	Immune-mediated effects (by NK cells and macrophages) and decreased tumour recurrence (except with multiple secondary tumours)
IMM-101 (heat-killed *Mycobacterium obuense*; NCTC 13365) with gemcitabine	Melanoma and advanced pancreatic ductal adenocarcinoma	Activation of APCs, granulocytes and γδ T cells. Increased survival in metastatic disease in a randomized phase II trial
Live-attenuated *Listeria monocytogenes* expressing mesothelin (CRS-207) with GVAX-cyclophosphamide	Advanced pancreatic ductal adenocarcinoma	Priming of mesothelin-specific CTLs, loss of regulatory T cells and tertiary lymphoid organ formation, and increased overall survival
IL-13–PE: recombinant cytotoxin consisting of human IL-13 and PE	Adrenocortical carcinoma	Majority of patients produce neutralizing antibodies against IL-13–PE within 2–3 weeks
IL-4–PE: chimeric fusion protein composed of IL-4 and PE	Astrocytoma	Phase I trial: no systemic complications, median survival of 8.2 months and evidence of necrosis on MRI scans in several patients
Attenuated strain of *Salmonella enterica* subsp. *enterica* serovar Typhimurium: VNP20009	Metastatic melanoma and refractory solid tumours	Phase I trial of intravenous infusion of *S.* Typhimurium led to inflammation, DC and T cell activation and evidence of bacterial tumour colonization; however, there was no tumour regression
TAPET-CD: an attenuated *Salmonella* bacterium that expresses the *Escherichia coli* cytosine deaminase gene	Head and neck squamous cell carcinoma or adenocarcinoma of the oesophagus	Evidence of bacterial colonization and confirmation of the conversion of 5-FC to 5-FU in 2 out of 3 tumours
Genetically modified *Corynebacterium diphtheriae*: Tf-CRM107 is a conjugate of transferrin and a point mutant of diphtheria toxin	Malignant brain tumour	MRI scans showed regression of tumour volume in 9 out of 15 patients with no evidence of severe local or systemic complications at low dose

Now the following list is one of putative efficacy yet to be fully vetted in humans (Zitvogel et al):

[3] Bacteria that have putative anticancer properties in humans, Zitvogel et al

Bacterial species[4]	*Cancer type*	*Interventions and biological effects*
Clostridium novyi **C. *novyi* non-toxic strain spores**	Orthotopic F98 rat glioma and dogs with spontaneous solid tumours	Intratumoural injections led to tumour haemorrhagic necrosis, lysis and regression
Lactobacillus casei	Orthotopic and transplantable bladder tumours and their metastases	Oral or intravesical injection of dead or alive bacteria increased the levels of IFNγ and the recruitment of neutrophils
Lactobacillus rhamnosus **GG**	Bladder tumours	Weekly intravesical instillations directed chemokine and/or cytokine release, recruitment of NK cells and direct cytotoxic effects on cell lines *ex vivo*
Alistipes shahii	MC38 colon cancer	Gavage after antibiotic treatment increased the production of TNF by intratumoural myeloid cells
Bacteroides fragilis **and** ***Burkholderia cepacia***	MCA205 sarcomas and MC38 and CT26 colon cancers	Oral gavage of *B. fragilis* stimulated the production of IL-12 by bone marrow-derived DCs *in vitro.* The mechanism of *B. cepacia* remains unknown
Prevotella **spp. and** ***Oscillibacter*** **spp.**	Subcutaneous hepatocellular carcinoma	Oral administration of Prohep, a probiotic mixture, altered the microbiota and reduced tumour growth
Enterococcus hirae **and** ***Barnesiella intestinihominis***	Sarcoma	Bacterial translocation: induction of TH1 cells and pathogenic TH17 cells, intratumoural regulation of Treg cells and IFNγ-producing γδ T cells, respectively
Bifidobacterium longum **and** ***Bifidobacterium breve***	Melanoma	Oral gavage led to the activation of DCs and an increased frequency of tumour-specific CTLs
Lactobacillus casei **str. Shirota**	MCA induced cancer	*L. casei* str. Shirota mixed into mouse diet delayed carcinogenesis through enhancement of NK cell cytotoxicity
Lactobacillus casei **ATCC334**	Colon cancer SW620 cells (Caco2 *in vitro*)	Secretion of ferrichrome, which induces JNK-associated induction of DNA damage-inducible transcript 3. Enhanced apoptosis of colon cancer cells
Lactobacillus casei **BL23**	DMH-associated colorectal cancer	Oral administration of *L. casei* BL23 led to differentiation of T cells towards a TH17-biased immune response (with the secretion of IL-6, IL-17, IL-10 and TGFβ)
Lactobacillus acidophilus	CRC *ApcMin*/+	Daily administration of yogurt formulation decreased overall intestinal inflammation
Bifidobacterium lactis **and RS**	Colorectal rat-azoxymethane model	The addition of RS to the diet andbacteria induced apoptosis in tumour cells at the time of cancer initiation
Antibiotic-induced loss of members of the Firmicutes and Bacteroidetes phyla; gain of members of the Proteobacteria	LLC and B16F10 lung metastases	Microbiota modifications following antibiotic treatment induced the loss of γδ T cells producing IL-17A

[4] Bacteria that have putative anticancer properties in experimental models, Zitvogel et al

Bacterial species[4]	*Cancer type*	*Interventions and biological effects*
***Bacillus polyfermenticus* and its culture medium**	HT-29, DLD-1, Caco2 human colon cancer in mice	Cyclin D1 expression required for ErbB-dependent cell transformation was decreased by culture medium injections near the tumour sites
Propionibacterium freudenreichii	Human colon adenocarcinoma HT-29 cells	Production of SCFAs, which induced pH-dependent differential cell death processes
L. acidophilus* and *L. casei	LS513 colorectal cancer cell line	Sensitization of colorectal cancer cells to 5-FU-induced apoptosis
***Enterococcus faecium* RM11 and *Lactobacillus fermentum* RM28**	Caco2 cell lines	Antiproliferative effects on CRC cells
***Lactobacillus delbrueckii* CU/22**	HT-29 cell line; probiotic supernatant	Apoptosis and necrosis through the production of bacterial hydrogen peroxide and superoxide radicals
***L. acidophilus* 606**	HT-29 colon cancer line	Cell-bound exopolysaccharides induced the activation of autophagic cell death promoted directly by the induction of beclin 1 and GRP78
***B. lactis* Bb12 and *L. rhamnosus* GG**	Caco2 cancer cell line	Induced apoptosis through the mitochondrial route
L. acidophilus* and *L. casei	LS513 colorectal cancer cell line	Sensitized colorectal cancer cells to 5-FU-induced apoptosis

5.2 MECHANISMS OF MICROBIALS

We now briefly examine the mechanisms which may be the basis for the therapeutic efficacy.

From Zitvogel et al:

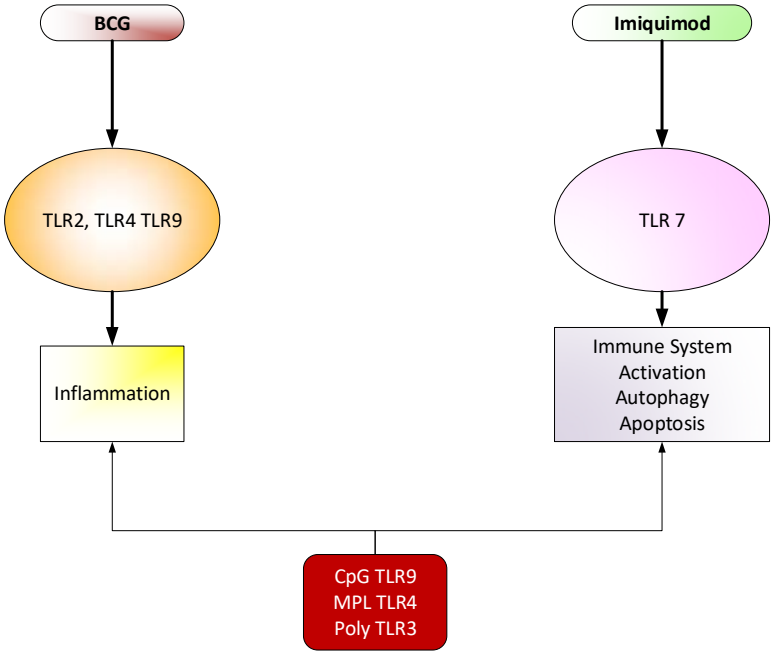
Bacteria produce various molecules that may affect the survival and growth of cancer cells, or that modulate anticancer immunosurveillance. These include bacterial toxins that have direct anticancer properties, ligands of PRRs that affect the Immune response and metabolites that affect host metabolism. There is no clear distinction between the latter two categories, as some metabolites can act on PRRs; this has been demonstrated for phenazines from Pseudomonas aeruginosa and phthiocol from Mycobacterium tuberculosis, which act on aryl hydrocarbon receptor (a PRR that functions as a transcription factor) and for N-acetylglucosamine (a sugar subunit of bacterial peptidoglycan), which acts on the hexokinase PRR to activate inflammation.

The authors procced to detail three specific mechanisms as follows:

1. Bacterial toxins. Bacteria produce different toxins and antibiotics, which allow them to compete with other microorganisms. Bacterial toxins may have direct anticancer effects, as illustrated for anthracyclines produced by Streptomyces spp. Indeed, anthracyclines, including doxorubicin, are widely used in anticancer chemotherapy and can induce immunogenic cell death, thereby stimulating anticancer immune responses78. However, it remains to be determined whether toxins are produced by intestinal bacteria at doses high enough to mediate such anticancer effects….

2. Ligands of PRRs. PRRs mostly recognize pathogen-associated molecular patterns (PAMPs), although they may also have endogenous ligands. One well-known PAMP is bacterial lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, which interacts with TLR4. LPS can stimulate inflammatory responses when bacteria enter the systemic circulation through breaches in the intestinal barrier. This can occur after cancer treatment with radiation therapy, and may improve the inhibition of tumour growth by activating T cells82. TLR4 is also thought to be fundamental for the anticancer effects of BCG83. PAMPs can be used as vaccine adjuvants to elicit an immune response against viruses that can cause cancer…..

We demonstrate this below:



It should be noted that TLRs, Toll Like Receptors have been shown clinically to be quite effective. TLR7 is a strong effector on antiviral activity. There are TLRs for many targets and when we combine these with specific microorganisms targets at cancer cells, we get a power set of immune tools in the innate immune system.

3. Bacterial metabolites. The microbiota has a key role in human metabolism; approximately 50% of metabolites in the plasma are estimated to have a bacterial origin. The gut microbiome synthesizes all SCFAs and secondary bile acids, polyamines and vitamins. Bacterial metabolites may affect cancer development and the efficacy of antineoplastic therapies.

6 OBSERVATIONS

The microbiome is now recognized as an essential element of homeostasis. Its changes are also recognized as putative causes of disease and specifically malignant changes. Furthermore the modulation of the microbiome may very well present opportunities to mitigate against various malignancies both directly as well as through secondary means. This paper is not intended to be a definitive statement on the efficacy of the microbiome as an adjunct in cancer care. Its intent is solely to attempt to identify the issue and lay forth several pathways for investigation. All too often the microbiome is not even recognized as an element of a balanced scale of health.

6.1 STATUS OF THE MICROBIOME

As Garrett noted:

Microbiota studies in cancer remain at an early stage. Information gathering and descriptive studies are still necessary, and many critical questions remain. What other mechanisms might microbes use to influence tumorigenesis?

If single microbes can compromise antitumor immunity or enhance susceptibility to oncomicrobes, are there configurations of the microbiota that do this, too (or are protective)? Are there microbes or microbiotas that enhance responsiveness to immunotherapies or other therapeutic interventions? To answer these questions, it is important to identify the key next steps in understanding how the human microbiota affects tumor growth and spread.

The understanding of the microbiome as part of the immune system and in terms of cancer mitigation is just beginning to be explored. The main challenges are twofold.

First is necessary to have the tools to be able to explore the consequences of the interaction of the microbiome and the normal cell.

Second, is the challenge of dealing with a temporally and spatially varying microbiome. This can be a real challenge. It presents such a complex environment that the modelling tools are far from adequate.

6.2 CLASSIC CARCINOGENESIS VS MICROBIOME MODULATION

As Vogtmann and Goedert conclude

There is epidemiologic evidence for associations between the human microbiome and cancer, particularly gastric and colorectal cancer. However, epidemiologic studies of this association have thus far been very limited, typically with small sample sizes and cross-sectional designs with single-time sampling. Although case–control studies can provide initial insights into microbial associations with cancer, reverse causation is of great concern.

In a case–control study, it is not possible to determine whether the carcinogenic process changes the local environment and creates a new niche for microbes or whether alterations in the microbial population or its functions contribute to carcinogenesis. New studies that incorporate repeated, prospectively collected oral, faecal, tissue, and other samples will be important to elucidate the temporal nature of microbial associations with cancer. Future studies should also incorporate the study of fungi, protists, and viruses, in addition to bacteria and archaea, to fully characterise the human microbiome and its relationship with cancer risk. In addition, standardised methods for the collection of samples, preparation and handling of samples, and bioinformatic processing of data are needed and work is ongoing in this area (e.g., www.mbqc.org). …

Finally, there is a need to explore postulated microbe-mediated carcinogenic mechanisms through transcriptomics, proteomics, metabolomics, and novel immunologic assays. Microbiome associations with cancer may differ across many host factors, including sex, age, smoking, alcohol consumption, diet, obesity, physical inactivity, and polymorphisms in major human oncogenes. Explicit consideration of these host factors may yield clear stratification of microbiome associations with the various malignancies.

Ultimately, across the identified strata, microbiome associations should be translated into practical applications in order to accelerate the diagnosis of cancer or precancer, to increase efficacy and reduce toxicity of cancer therapy… and ideally to prevent cancer by interrupting a microbial carcinogenic pathway.

Microbiome modulation as discussed above is a challenge in ascertaining causal relationships. As we discussed previously this challenge is drastically different from a normal causal relationship we normally attempt to define.

6.3 MICROBIOME WITHIN MICROBIOME

There is now another layer to this complex environment. Namely the interaction of phages, viruses, with bacteria and then in the microbiome, As Guglielmi has noted:

Though where the viruses end up is unclear, those data and other recent studies have scientists wondering whether a sea of phages within the body—a "phageome"—might influence our physiology, perhaps by regulating our immune systems. "Basic biology teaching says that phages don't interact with eukaryotic cells," says phage researcher Jeremy Barr of Monash University in Melbourne, Australia, who led the study published this week in mBio. He's now convinced "that's complete BS." For decades, most medical research on phages focused on turning these bacterial parasites into antibiotics.

There have been some compelling success stories, but phage therapy has struggled to become a dependable treatment. Yet Barr's earlier research showed that phages might naturally help protect us from pathogens. Studying animals ranging from corals to humans, he found that phages are more than four times as abundant in mucus layers, like the ones that protect our gums and gut, as they are in the adjacent environment. The protein shell of a phage, it turned out, can bind mucins, large secreted molecules that together with water make up mucus. This

works out well for both phages and mucus making animals. Sticking to mucus enables the phages to encounter more of their bacterial prey. And as a result, Barr showed in a series of in vitro studies, the viruses protect the underlying cells from potential bacteria pathogens, providing an additional layer of immunity.

This it is possible to use a complex sat of the microbiome elements, one against the other, in the world of microbiome therapeutics.

7 GLOSSARY[5]

The following is a collection of some useful definitions relating to the topics discussed.

1. Adaptive immune responses: As opposed to innate immunity, adaptive immune responses are specific to the type of pathogen that is encountered, thereby providing a tailored (albeit slower) immune response. This acquired response is typically mediated by B and T cells with the subsequent generation of memory cells.

2. Bacteriocins : Antimicrobial peptides released by bacteria to inhibit growth of similar or closely related microorganisms.

3. Commensalism: A relationship between two organisms in which one organism benefits, whereas the other does not.

4. Dysbiosis : A state of microbial composition that is characterized by an unbalanced proportion of bacteria compared with the proportion in a healthy state.

5. Eubiosis : A state of microbial composition in which population abundances are found in normal proportions and typically associated with healthy individuals.

6. Facultative anaerobic bacteria : Bacteria that are able to generate energy (ATP) through aerobic respiration (electron transport chain) or through fermentation, depending on the amount of oxygen or fermentable products available.

7. Germ-free animals : Animals born and raised in a sterile environment; they lack any microorganisms (except endogenous viruses).

8. Gnotobiotic: Describes an animal with a defined microbial population. These animals are born germ-free and then known microorganisms are introduced; this requires that the animals are housed in isolation, to maintain their defined microbial status.

9. Horizontal gene transfer: The movement of genetic material from one organism to another, without the need for cell division.

10. Innate immunity: An immune response that recognizes conserved microbial structures, typically through the action of pattern recognition receptors expressed on host cells.

11. Metagenome: The collection of genomes from members of a specific microbiota.

12. Microorganism-associated molecular patterns: (MAMPs). Conserved structural components such as lipopolysaccharide, flagellin and nucleic acids derived from microorganisms that are detected by the host innate immune system.

[5] From Schwabe and Jobin

13. Muramyl dipeptide: A peptidoglycan derivative that is common to both Gram-positive and Gram-negative bacterial cell walls and that triggers an innate immune response.

14. Mutualism: A relationship between two organisms, in which both organisms benefit.

15. Obligate anaerobic bacteria: Bacteria that grow without the need for oxygen.

16. Parasitism: A relationship in which one organism (pathogen) benefits at the expense of another organism.

17. Pathobionts : Normally innocuous microorganisms that can behave like pathogens if their abundance increases and/or their environmental conditions change.

18. Stratum corneum :The outermost layer of the epidermis that forms the protective layer of the skin.

19. Toll-like receptor :(TLR). A family of evolutionarily conserved receptors that recognize microorganism-associated molecular patterns such as flagellin, lipopolysaccharide or nucleic acids. These receptors have an essential role in innate immune responses.

20. Tumour tolerance :A state of immune hyporesponsiveness, in which tumour antigens induce T cell tolerance (a process that allows tumour immune evasion).

21. Virulence factors: Molecules expressed by pathogenic microorganisms that help them to gain a growth advantage in a specific ecosystem. These molecules are often responsible for disease manifestation in the host.

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