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The spinal cord-gut-immune axis as a master regulator of health and neurological function after spinal cord injury

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Abstract

Most spinal cord injury (SCI) research programs focus only on the injured spinal cord with the goal of restoring locomotor function by overcoming mechanisms of cell death or axon regeneration failure. Given the importance of the spinal cord as a locomotor control center and the public perception that paralysis is the defining feature of SCI, this “spinal-centric” focus is logical. Unfortunately, such a focus likely will not yield new discoveries that reverse other devastating consequences of SCI including cardiovascular and metabolic disease, bladder/bowel dysfunction and infection. The current review considers how SCI changes the physiological interplay between the spinal cord, the gut and the immune system. A suspected culprit in causing many of the pathological manifestations of impaired spinal cord- gut-immune axis homeostasis is the gut microbiota. After SCI, the composition of the gut microbiota changes, creating a chronic state of gut “dysbiosis”. To date, much of what we know about gut dysbiosis was learned from 16S-based taxonomic profiling studies that reveal changes in the composition and abundance of various bacteria. However, this approach has limitations and creates taxonomic “blindspots”. Notably, only bacteria can be analyzed. Thus, in this review we also discuss how the application of emerging sequencing technologies can improve our understanding of how the broader ecosystem in the gut is affected by SCI. Specifically, metagenomics will provide researchers with a more comprehensive look at post-injury changes in the gut virome (and mycome). Metagenomics also allows changes in microbe population dynamics to be linked to specific microbial functions that can affect the development and progression of metabolic disease, immune dysfunction and affective disorders after SCI. As these new tools become more readily available and used across the research community, the development of an “ecogenomic” toolbox will facilitate an Eco-Systems Biology approach to study the complex interplay along the spinal cord-gut-immune axis after SCI.

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1. Introduction

Traumatic spinal cord injury (SCI) affects approximately 1.4 million people living in the United States. Loss of motor and sensory function are visible consequences of SCI. Less appreciated, although no less important or debilitating, are the “hidden” pathologies that manifest after SCI due to permanent dysfunction of the autonomic nervous system (ANS).

Many autonomic neurons, including all that comprise the sympathetic branch, are found in the spinal cord. When the spinal cord is injured, especially at higher spinal levels (above mid-thoracic spinal cord), axons that normally descend from brain/brainstem regions to control spinal sympathetic neurons are lost or damaged. The subsequent loss of normal sympathetic tone throughout the body leads to the development of some of the most devastating consequences of SCI including cardiovascular disease, bladder/bowel dysfunction and immune dysfunction. In this review, we highlight key changes in the spinal cord-gut-immune axis that converge to cause various co-morbidities and neurological complications after SCI (Fig. 1).

1.1 The spinal cord-gut axis and gut dysbiosis after spinal cord injury

The enteric nervous system of the gastrointestinal (GI) tract is innervated by the parasympathetic vagus nerve and sympathetic spinal nerves originating in the brainstem and spinal cord, respectively. After a SCI, an imbalance in autonomic tone develops because sympathetic control of the small bowel and colon is lost, causing acute and protracted GI dysfunction marked by impairments in gut motility, mucosal secretions, vascular tone and immune function. These physiological complications of a neurogenic bowel contribute to the constipation, bloating, nausea, impaired transit, incontinence and abdominal pain experienced by SCI individuals (Tate et al., 2016).

Spinal sympathetic nerves also innervate the gastrointestinal-associated lymphoid tissue (GALT), the immune system distributed throughout the GI tract. The GALT protects the body from microbes that attempt to invade the body through the mucosal surface of the GI tract (Hooper et al., 2012; Nicholson et al., 2012; Round and Mazmanian, (2009). This protective function of the GALT is remarkably specific and effective, especially if one considers that the mucosal surface of the gut is also home to trillions of microorganisms, i.e., the gut microbiota (Donaldson et al., 2016; Gu et al., 2013). The gut microbiota and their collective genomes (i.e., the *microbiome*), are critical for maintaining homeostasis both in the gut and throughout the body. In addition to exerting direct control over the GALT, the sympathetic nervous system also exerts indirect effects on the enteric microbiota through regulation of gut motility, mucosal secretions (e.g., mucin, acids) and epithelial permeability. In a neurogenic bowel, i.e., a functionally impaired bowel due to nerve injury, impaired intestinal transit limits the delivery of important nutrients to microbiota in the distal colon. Altered mucin production impairs production of the mucus layer, an important niche that is colonized by enteric microbiota creating a “biofilm”. Because of the distinct location of the biofilm relative to microorganisms in the gut lumen, these discrete ecological domains may be differentially affected by SCI (Fig. 1).

Gut microbiota contribute to host metabolism by breaking down complex molecules in food. During this process, commensal bacteria release metabolites that influence cellular function within and beyond the gut. In humans, correlations exist between metabolites in the blood and specific types of gut microbiota (Zhang et al., 2018). The range of cells that are known to be affected by gut-derived metabolites is extensive and includes intestinal epithelia, primary afferent neurons in the gut and immune cells in the GALT. These metabolites also diffuse into the circulation to influence cells in the liver, peripheral immune organs (e.g., bone marrow, spleen, lymph nodes) and the central nervous system (Clarke et al., 2014; Forsythe et al., 2014; Perez-Burgos et al., 2015; Tillisch, 2014; Wikoff et al., 2009; Yano et al., 2015). Changes in gut permeability brought on by chronic stress or trauma, can liberate commensal bacteria from the gut lumen, allowing the microbes themselves, not just the metabolites they produce, to enter the circulation. This increases the likelihood that gut-derived microbes will colonize and elicit inflammation in previously “sterile” tissues throughout the body.

In pre-clinical SCI models, intestinal epithelial cell permeability is increased and is associated with enhanced bacterial translocation and colonization of various organs including the lung (Kigerl et al., 2016). Whether bacteria or other gut-derived microbes can access the injured spinal cord is not known; however, it is possible since the permeability of the blood-spinal cord barrier and intestinal barrier increase at the same time after SCI (Noble and Wrathall, 1989; Popovich et al., 1996; Whetstone et al., 2003). Gut microbes and their metabolites may also influence the barrier functions of spinal cord endothelia, just as they do in the brain (Braniste et al., 2014).

Given the robust effects that the microbiota can have on host physiology, the changes that SCI causes in the ecological balance of microorganisms in the gut, i.e., gut dysbiosis, could broadly influence neurological function, overall health and quality of life. After SCI, gut dysbiosis has been documented in multiple clinical and preclinical studies (Gungor et al., 2016; Kigerl et al., 2016; Myers et al., 2019; O’Connor et al., 2018; Zhang et al., 2018). In most cases, 16s rRNA sequencing has been used to reveal the composition and relative abundance of bacteria in fecal samples or gut tissue samples after SCI. In mice, SCI causes robust and lasting gut dysbiosis and is characterized by inverse changes in the relative abundance of *Bacteroidales* and *Clostridiales*, the two most prevalent bacterial taxa in mouse gut: *Bacteroidales* decreases while *Clostridiales* increases after SCI (Eckburg et al., 2005; Kigerl et al., 2016; Krych et al., 2013). Minor taxa (*Anaeroplasmatales*, *Turicibacterales* and *Lactobacillales*) also are affected (Kigerl et al., 2016). The relative abundance of bacteria from the phylum *Proteobacteria* also increases in SCI mice (Myers et al., 2019). Chronic gut dysbiosis has also been described in rats after SCI, lasting at least 8 weeks post-injury (O’Connor et al., 2018). In humans, the abundance of butyrate-producing gut bacteria is reduced for at least 1 year after SCI (Gungor et al., 2016).

Much less appreciated than gut dysbiosis is the post-injury onset of urinary dysbiosis. In addition to developing a neurogenic bowel, SCI patients (and animals) also develop neurogenic bladders and the duration of neurogenic bladder (along with method and frequency of catheterization) can adversely affect the urinary microbiome. The pathophysiological effects of urinary dysbiosis are not known but there is diagnostic value in

performing 16s-profiling of the urinary microbiome. Clinical data indicate that at baseline the bacterial composition of urine from healthy men and women is different and that after SCI, compositional changes in bacteria can differentiate between asymptomatic bacteriuria and urinary tract infection (UTI). (Kreydin et al., 2018; Evans et al., 2011; Morton et al., 2002; Skelton et al., 2019).

In addition to sex, other covariates will likely affect the magnitude, duration and pathophysiological impact of SCI-induced gut dysbiosis. For example, age affects experimental and clinical outcomes after SCI. Since gut microbial composition is affected by age and the average age at time of injury has steadily increased since the 1970s (Level, 2018), age should, when possible, be included as a covariate in all pre-clinical and clinical SCI studies (Arumugam et al., 2013; Guo et al., 2014; Jašarević et al., 2016; Markle et al., 2013; Sheng et al., 2017; Stilling et al., 2015; Thevaranjan et al., 2017). The spinal level at which an injury occurs also is an important covariate with implications for gut dysbiosis. Injuries at higher spinal levels will cause greater imbalance in autonomic control over the GI tract than injuries affecting lower spinal levels (Holmes and Blanke, 2019). SPNs controlling the small and large intestines are located primarily in the intermediolateral cell column in thoracic segments T5–10 and T10-S4, respectively (Browning and Travagli, 2014; Levatte et al., 1998; Mabon et al., 1997). Therefore, although autonomic control of the gut will be adversely affected by SCI occurring at any spinal level, higher level injuries (above T5) will remove most or all bulbospinal control over spinal autonomic networks that innervate the gut. To date, there have been no controlled studies designed to evaluate level-dependent effects on gut microbiota; however, data in a recent clinical report indicated that cervical SCI caused changes in gut microbiota composition that were distinct from those found in individuals with a thoracic or lumbar SCI (Zhang et al., 2018).

Currently, the functional consequences of SCI-induced dysbiosis are unknown but significant effects can be inferred from experimental studies. For example, changes in the relative abundance of certain gut bacteria correlate with worse or better locomotor function and also with immune function (Kigerl et al., 2016). Also, changes in GALT immune cell composition occur coincident with increased or decreased production of cytokines (TNFα, IL-1β, TGF-β, IL-10) for up to one month post-SCI (Kigerl et al., 2016) A similar relationship exists in SCI rats; at 8 weeks post-SCI, increased production of inflammatory cytokines in the intestines correlates with the relative abundance of particular types of gut bacteria (O'Connor et al., 2018). Precisely how gut dysbiosis develops after SCI, whether it persists indefinitely, and how it affects structure/function in the CNS and other organ systems is unknown. But, if causal relationships could be proved, many comorbidities that affect individuals with SCI might be treatable by targeting the gut microbiota or the metabolites that they produce. In this context it is encouraging that readily available therapeutics that influence the gut microbiota, including *Lactobacillus-rich* VSL#3 probiotics and melatonin, can restore gut microbiota homeostasis and improve locomotor recovery (Jing et al., 2019; Kigerl et al., 2016). In the paragraphs that follow, several systemic comorbidities that may contribute to or exacerbate SCI-induced gut dysbiosis are discussed.

1.2. Immune dysfunction

SCI disrupts normal sympathetic nervous system control of all major immune organs (Brommer et al., 2016; Lucin et al., 2007; Zhang et al., 2013). This causes SCI-induced immune depression syndrome (SCI- IDS), a profound and lasting deficiency in the immune system's ability to fight infection. Indeed, SCI patients are at increased risk to develop infections and are 37x more likely to die of pneumonia than able-bodied individuals (DeVivo et al., 1993); the development of pneumonia and wound infections after SCI are associated with worse neurological recovery (Failli et al., 2012; Kopp et al., 2017). The higher incidence of infection after SCI may explain why survival rates have not improved for SCI patients over the past 30 years (Shavelle et al., 2015). What is needed to improve clinical care for SCI individuals is a better understanding of why infection develops more readily in this at-risk population. A likely culprit is a dysfunctional immune system that in turn alters the microbial ecology of gut commensals (Fig. 1).

SCI-IDS develops and persists indefinitely, in part, because maladaptive plasticity develops in the intraspinal circuitry located below the level of injury. This "new" circuitry, when activated by common recurring stimuli (e.g. bladder filling and other visceral/somatic input), triggers hyperactive neural-immune reflexes. As a result of excessive and uncontrolled reflex activity, leukocytes in peripheral immune organs are exposed to supra-physiological concentrations of hormones (e.g., glucocorticoids) and neurotransmitters (e.g., catecholamines) that elicit distinct intracellular signaling cascades that converge to kill immune cells (Lucin et al., 2007; Prüss et al., 2017; Ueno et al., 2016; Zhang et al., 2013). SCI-induced changes in autonomic tone to the GI tract may also cause or exacerbate SCI-IDS.

Sympathetic noradrenergic nerves innervate the vasculature and tissue parenchyma of the GALT, especially in regions of the GALT that are enriched with T and B lymphocytes (Straub et al., 2006). Generally, norepinephrine released by sympathetic post-ganglionic nerve terminals binds to beta-adrenergic receptors on innate and adaptive immune cells leading to suppression of their antimicrobial functions (Elenkov et al., 2000). Impaired sympathetic signaling may also alter the functional specialization of recently discovered macrophage networks found in the lamina propria and muscular layers of the intestine (Gabanyi et al., 2016). Overall, a break in immune homeostasis in GALT, caused by SCI-induced changes in sympathetic tone, will change how the mucosal immune system regulates the enteric microbiota.

After SCI, microbes that bypass the intestinal epithelial barrier could release peptides, metabolites and neurotransmitter-like molecules (e.g., quorum sensing molecules) that can directly activate vagal afferents located in the lamina propria. For those microbes that persist in the gut lumen, they also can elicit vago-vagal reflexes by signalling directly to enterochromaffin or enteroendocrine cells (EECs). EECs are sensor transducer cells that respond to nutrients, hormones, and microbe-derived factors produced in the gut lumen or the biofilm. In response to these stimuli, EECs release hormones, neuropeptides and neurotransmitters that activate neural networks via paracrine signaling. EECs also can directly activate sensory nerve terminals via neuropods, which are cytoplasmic extensions of

the EECs that form functional synaptic contacts with sensory nerves in the lamina propria (Bohórquez et al., 2015; Liddle, 2019).

Gut microbiota also influence systemic immunity through modulation of vago-vagal reflexes (Borovikova et al., 2000; van Westerloo, 2010) and monocyte egress from the bone marrow. Not surprisingly, gut microbes have been implicated as potent regulators of immune responses to respiratory infections and the development of atopic and autoimmune diseases (Hill et al., 2012; Ichinohe et al., 2011; Ochoa-Repáraz et al., 2010; Shi et al., 2011; Wu et al., 2010). Emerging data also implicate the gut microbiota in regulating CNS glial homeostasis and neuroinflammation. Indeed, metabolites (e.g., short-chain fatty acids, tryptophan metabolites) produced by gut microbiota affect the maturation and function of CNS resident microglia and cross-talk between microglia and astrocytes (Brown et al., 2019; Erny et al., 2015; Rothhammer et al., 2016, 2018).

SCI elicits a systemic autoimmune response, i.e., trauma-induced autoimmunity (TIA) (Ankeny et al., 2006, 2009; Arevalo-Martin et al., 2018; Davies et al., 2007; Hayes et al., 2002; Hergenroeder et al., 2016; Jones et al., 2002). TIA develops when cells of the immune system recognize and mount an immune response against “self” antigens including proteins, carbohydrates, lipids and nucleic acids (Ankeny et al.,2009). Since SCI causes profound immune suppression, the onset of TIA seems paradoxical. However, it may be useful to think of TIA as a continuum of a non-resolving inflammatory response, dominated by innate immune cells, that is set in motion by SCI (Schwab et al., 2014). TIA may also be a secondary consequence of gut dysbiosis and bacterial translocation from the gut. Bacteria within the gut, notably segmented filamentous bacteria, can activate Th17 cells in GALT and trigger systemic and CNS autoimmunity (Flannigan and Denning, 2018; Ivanov et al., 2009; Kriegel et al., 2011). Bacterial translocation has been implicated as a trigger for the onset of systemic and CNS autoimmune diseases (Lee et al., 2011; Manfredo Vieira et al., 2018).

1.3. Mental and cognitive health

People living with a SCI and without concurrent brain injury develop anxiety and major depressive disorder or depression-like symptoms at higher rates than able-bodied individuals. Although the relationship between SCI and reduced mental health has been recognized for decades (Cao et al., 2017; Frank et al., 1992; Krause et al., 2000; Shin et al., 2012), most have attributed changes in psychiatric and mental health to environmental stressors including sudden loss of independence, high health care costs and the unique physical challenges associated with living with a SCI. However, physiological changes also occur after SCI that can directly affect emotional and mental health. Magnetic resonance imaging of cortical volume in SCI patients has revealed changes in brain areas that are important for information processing and emotional affect (Hawasli et al., 2018; Nicotra et al., 2006). In preclinical SCI models, higher indices of depression, anxiety and cognitive impairment have been documented (Craig et al., 2017; Davidoff et al., 1992; Luedtke et al., 2014; Roth et al., 1989; Wu et al.,2010) indicating that mental and cognitive impairments after SCI are likely stereotypical consequences of SCI and are caused by factors other than environmental stressors.

The gut-brain axis is now recognized as a powerful physiological regulator of mood and mental health. When the gut is intentionally colonized with discrete types of bacteria, signaling networks in the brain can be activated that elicit anxiety-like behaviors, whereas anxiety-like behaviors are reduced in germ-free mice when compared to specific pathogen free control mice (Diaz Heijtz et al., 2011; Neufeld et al., 2011). Vagal afferent-microbe interactions in the gut are implicated in modulation of mental health. In rats, vagal afferents are stimulated by microbially-derived fatty acids and vagotomy makes mice resistant to microbially-induced anxiety-like behaviors (Bravo et al.,2011; Lal et al., 2001).

Gut dysbiosis can also impair mental health. When the ecology of fecal samples obtained from people with major depressive disorder was analyzed, large population shifts were noted in the types of bacteria that produce neuroactive metabolites (Zheng et al., 2016). When these fecal samples were transplanted into germ-free mice, recipient mice developed anxiety-like and depressive-like behaviors (Zheng et al., 2016). In rats and mice, SCI also affects mood and increases anxiety-like behaviors (Craig et al., 2017; Davidoff et al., 1992; Luedtke et al., 2014; Roth et al., 1989; Wu et al., 2014). Recent data suggest that post-SCI dysbiosis may be the reason why these behaviors develop. Specifically, a fecal transplant prepared from healthy rats, when delivered orally to SCI rats, reverses SCI-induced dysbiosis and has potent anxiolytic effects (Fouad, K; personal communication). The molecular basis for this treatment effect is unknown, even though fecal transplants are now being considered as treatments for depression in people (Kurokawa et al., 2018).

1.4. Metabolic disease

Emerging data indicate that after SCI, loss of sympathetic tone and the development of aberrant spinal autonomic reflex control over immune organs (e.g., spleen) and other major organs that control metabolism (e.g., liver, adrenal gland, muscle, adipose tissue and gut) causes immune dysfunction and multi-organ pathology. Since immune and metabolic processes are normally tightly coupled and are essential for life (Hotamisligil, 2017a, 2017b), most, if not all, co-morbidities that affect SCI individuals (e.g., spontaneous infections in lung or skin, impaired wound healing, non-alcoholic fatty liver disease, chronic depression, atherosclerosis, type 2 diabetes, fatigue and anxiety), could be explained by impaired immunometabolism, which is caused or exacerbated by gut dysbiosis.

Metabolic function is significantly impaired in both paraplegics and tetraplegics; they have higher body fat content compared to able-bodied individuals (Gater, 2007; Gorgey et al., 2011, 2014). SCI individuals also have higher levels of intramuscular fat that contributes to insulin resistance and impaired glucose sensitivity (Boettcher et al., 2009; Elder et al., 2004). Changes in Bacteriodetes and Firmicutes, two of the largest populations of gut bacteria found in both mice and humans (Eckburg et al., 2005; Krych et al., 2013), could cause or contribute to chronic metabolic disturbances after SCI (Baothman et al., 2016; Ley, 2010; Tilg and Kaser, 2011; Turnbaugh and Gordon, 2009). 16s rRNA sequencing of fecal samples from SCI mice revealed that the relative abundance of *Bacteroidales* decreased as a function of time post-injury with a corresponding increase in *Clostridiales,* a class of Firmicutes (Kigerl et al., 2016). In obese able-bodied individuals and rodents, a similar reduction in the Bacteroidetes:Firmicutes ratio occurs and the associated metabolic dysfunction can be

transmitted to naïve rats by colonizing their gut with fecal suspensions from obese animals or humans (Ley et al., 2006; Turnbaugh et al., 2006, 2008).

Signs of liver disease (e.g., nonalcoholic steatohepatitis), marked by an increase in liver adiposity and hepatic inflammation, also develop soon after SCI (Goodus et al., 2018; Sauerbeck et al., 2015). In SCI humans, liver adiposity is a prognostic indicator of metabolic disease (Rankin et al., 2017) and is associated with altered gut microbiota and increased bacterial translocation (Abu-Shanab and Quigley, 2010; Dumas et al., 2006). Similar to the GALT, the liver acts as a firewall between the gut and the body, filtering microbes that drain from the intestine into the hepatic portal vein (Balmer et al., 2014; Jenne and Kubes, 2013). In SCI animals and humans, an inflamed liver can limit hepatic filtration capacity, allowing gut microbes to pass through the liver and elicit systemic inflammation. These same microbes may also elicit or propagate liver inflammation.

1.5. Emerging topics and opportunities

Today, there is a growing appreciation in the field of SCI research and clinical care, that the gut microbiota represents an important but poorly understood biological variable that is capable of significantly affecting the health, recovery and well-being of SCI individuals. As a result, a growing number of pre-clinical and human subject research projects have been completed to better understand how SCI affects gut (and urine - see above) ecology. Below, we discuss technical aspects of gut microbiome research and emphasize the importance of understanding the limitations of commonly used techniques. We also introduce the gut virome as a novel and as yet understudied component of the gut microbiota, especially in the context of SCI.

Technical advances in microbiome science.—Recent technological advances have transformed the study of microbes throughout the life sciences. Early descriptions of the taxonomic profile, or enterotypes, of bacteria in the large intestine of mice were done using new (at the time) anaerobic culturing methods of fecal samples (Schaedler et al., 1965), where early studies characterized the relationship between mammalian gut commensal bacteria and development of the mammalian lymphatics (Bauer et al., 1963). With time, DNA-sequencing techniques improved and RNA experts transformed the study of microbial diversity. Now, rather than use culture-dependent techniques, they used gene markers (e.g., 16s rDNA) to survey naturally-occurring microbial communities (Lane et al., 1985; Woese and Fox, 1977). Over time, this evolution in technique revealed that culture-based methods missed most of the microbes that could be detected using molecular markers, often with far less than 1% of microbes being captured with culture-based techniques (Rappé and Giovannoni, 2003). Subsequently, large-scale screening of fecal and plasma samples from mammalian models, including humans and mice, provided comprehensive *“who is there?”* (compositional) databases using such molecular surveys rather than the less comprehensive *in vitro* culture-based methods. To date, 16S-based taxonomic profiling has enabled community-wide microbial surveys across diverse environments with unprecedented temporal and spatial scales. These emerging databases have transformed our understanding of such ecosystems, necessitating an *eco-systems biology* perspective to best understand the role of microbes in complex communities. For example, 16S studies in the human gut now

implicate specific bacteria in determining whether you are obese or lean (Turnbaugh et al., 2008, 2006) or whether you will be susceptible to inflammatory bowel disease (Imhann et al., 2018). The same tools have also revealed associations between the gut microbiota and other 'ecosystems' found in humans such as the oral cavity (Olsen and Yamazaki, 2019; Segata et al., 2012).

As sequencing costs have decreased, gene-targeted sequencing is rapidly being overtaken by metagenomic sequencing (i.e., shotgun sequencing or community genomic sequencing). In this approach, whole communities of organisms - including bacteria, archaea, fungi and their viruses - can be surveyed simultaneously. The 16S-based approach is commonly biased against archaea, and misses entirely fungi and viruses, as they lack this gene marker. Fungi can be targeted using 18S primer sets (Banos et al., 2018) but there are no universal gene markers to capture viruses (Sullivan, 2015). In many ecosystems, fungi remain virtually unstudied, but viruses are now credited with drastically impacting microbial communities through lysis, gene transfer and metabolic reprogramming during infection (Brum et al., 2015). Thus, to gain a fuller ecosystem perspective, it is critical to advance beyond 16S-based surveys so as to avoid known taxonomic blind spots in bacteria and archaea, while also capturing other entities such as viruses or fungi.

Fortunately, concomitant with the rise of new sequencing technologies that have enabled metagenomic data generation, legions of researchers have developed analytics and community-available tools to interpret these large-scale datasets (Fig. 2; Table 1). Beyond expanding our abilities to answer *"who is there?"* to non-bacteria, metagenomics also enables functional analyses to help answer *"what are they doing, and with whom*?". Such advances are gaining recent attention in human microbiome studies (e.g., (Quince et al., 2017)), but they have long been transforming our understanding of the oceans (DeLong et al., 2006) and other complex systems (Tringe et al., 2005; Tyson et al., 2004). For the oceans in particular, global scale surveys have now been conducted for viruses (Brum et al., 2015; Gregory et al., 2019b; Roux et al., 2016), prokaryotes (Sunagawa et al., 2015) and microbial eukaryotes(de Vargas et al., 2015). Such surveys have become the basis for analytics that have revealed global "interactomes" (the hypothesized interactions between hundreds of thousands of organisms)(Lima-Mendez et al., 2015), as well as genes-to-ecosystems based modeling approaches that take such data and identify those that best predict a feature of interest (Guidi et al., 2016). In oceans, this feature of interest was carbon flux and surprisingly identified viruses as the best predictor (Guidi et al., 2016). Still, the scalable analytical approach is generalizable to any large-scale dataset, such that it can be used to identify which tens of thousands of different microbes best predict disease. Given the importance of microbes and their viruses to 'ecosystem properties' in so many other environments, it is now well-accepted that anywhere microbes are abundant they are likely major players. Here, we will briefly consider what is known about the human gut virome in health and various disease states, as well as the potential role for the human gut virome in SCI.

The Virome.—In contrast to pathogenic eukaryotic viruses, the gut virome is dominated by viruses that infect bacteria (bacteriophages, or phages). Within the human gut, most known bacteriophages are in the order Caudovirales (containing the families myoviridae,

podoviridae, and siphoviridae) or the floating family, microviridae. While there are highly conserved phages across individuals (Dutilh et al., 2014; Yutin et al., 2018), the gut virome is largely unique between individuals and within an individual and is largely stable over time (Manrique et al., 2017; Reyes et al., 2010). Further, it has been shown that the composition of the gut virome can be altered by development, the immune system, diet, cohabitation, pharmacologic factors and disease (Abeles et al., 2015; Ly et al., 2016; Manrique et al., 2017; Minot et al., 2011; Scarpellini et al., 2015). Bacteriophages, like eukaryotic viruses, reproduce using a lytic (causing death of host bacteria) or lysogenic (non-lytic) life cycle, and metagenomic sequence data suggest that most phages in the human gut are lysogenic (Minot et al., 2011; Monaco et al., 2016). While the dynamics of the human gut virome are not well characterized, a system dominated by lysogenic vs lytic phages allows persistence of individual bacteria over longer time spans. Additionally, lysogenic viruses can be activated by stimuli, such as changes in diet, to rapidly alter gut bacterial community structure (Duerkop et al., 2018; Minot et al., 2011).

The human gut virome has now been examined in a handful of diseases: colorectal cancer, HIV, inflammatory bowel disease, hypertension, obesity, type 1 diabetes, *Clostridium difficile* infection, and autism. Though this is a nascent field of study, new data are revealing the dynamics of the microbiome in response to disease, viral interactions with the host immune system, and the classificatory power of the enteric virome. In obesity, mouse models have demonstrated an expansion of Caudovirales phages (Kim and Bae, 2016). In HIV, enteric eukaryotic viruses, including adenovirus and anelloviruses, can be found in blood, suggesting immune dysfunction and translocation across a compromised gut epithelium. At the same time, abundance of these viruses in the gut increases, while bacterial diversity decreases (Handley et al., 2012; Monaco et al., 2016). In inflammatory bowel disease, patient gut viral composition was sufficient to distinguish patients with ulcerative colitis from those with Crohn's disease. Further, as in HIV, it was observed that viral diversity increased as bacterial diversity decreased (Norman et al., 2015). While viral and bacterial diversity are calculated using different methods that are not directly comparable, it is unusual that they would move in opposite directions, raising the possibility that gut virome dynamics are not simply mirroring expansion or reduction in bacterial communities.

The role of phages is not limited to interactions with their host bacteria. In ulcerative colitis, there is evidence that phages directly interact with the immune system through stimulation of dendritic TLR9, inducing IFN-gamma production by CD4+ T-cells and exacerbating symptoms (Gogokhia et al., 2019). Phages also have high bio-marker potential: in colorectal cancer, differences in the gut virome and bacteriome could be used to distinguish human patients with cancer from healthy controls (Handley and Devkota, 2019; Hannigan et al., 2018). In hypertension, it has been shown that gut viruses have higher discriminatory power than gut bacteria in distinguishing between healthy, prehypertension and hypertension samples (Han et al., 2018). Likewise, in children with type 1 diabetes, changes in gut viral community structure were found to precede autoimmunity and changes in gut bacterial communities, suggesting that the gut virome is a sensitive predictor of disease risk (Kostic et al., 2015; Zhao et al., 2018). In fact, phages may be more sensitive than bacteria in predicting at-risk individuals (Moreno-Gallego et al., 2019) and also as biomarkers of disease and pathogenesis (Flores et al., 2013; Gregory et al., 2018). Finally, commensal

phages may have therapeutic potential. In C. *difficile* infection, where fecal microbiota transplant (FMT) has been growing as an alternative to antibiotic therapy, it has been shown that bacteria are not necessary for therapeutic success, raising the possibility that bacteriophages, i.e., the virome that is transferred in FMT, is responsible for recovery from C. *difficile* infection (Ott et al., 2017). Indeed, successful bacteriophage transfer in FMT has been associated with treatment outcome in C. *difficile* (Zuo et al., 2018). In a small open-label study, sustained FMT treatment suggested that viruses and microbes from healthy donors could mitigate autism symptoms in children (Kang et al., 2017). To date, the human virome in SCI remains unexplored, but it is clear that phages are altered in diseases ranging from infectious to metabolic to inflammatory and beyond. One key challenge in interpreting such “human virome” studies is that non-quantitative sample preparations are commonly used, which minimizes the ecological interpretations that can be made from the data (Gregory et al., 2019).

In summary, there are numerous critical interactions between phages and their bacterial hosts: phage-host dynamics alter commensal bacterial communities and determine community assembly trajectory early in life (Lim et al., 2015), provide bacteria with additional survival and virulence genes (De Smet et al., 2016; Torres-Barceló, 2018), and reprogram their bacterial hosts in ways that alter metabolic outputs or confer protection from invasion by pathogenic bacteria at mucosal surfaces (Barr et al., 2019; Mirzaei and Maurice, 2017; Reyes et al., 2012). Like bacteria, gut phage populations change in disease, and sometimes these changes precede disease onset, highlighting the possibility that gut phage profiles could be used as biomarkers to predict disease risk. Like bacteria, phages can also interact with the immune system to promote inflammation or strengthen mucosal defenses and protect the host from disease (Kernbauer et al., 2014). Emerging data suggest that therapeutic phages can work in tandem with the host immune system to resolve bacterial infections in skin (Abedon et al.,2011) and the lung (Roach et al., 2017). Together, these findings suggest that phages likely play an integral role in the microbe-gut-brain axis in disease and should be considered as part of any Eco-Systems biology study.

2. Conclusions

Like so many other human diseases, research in SCI has for decades overlooked the possible impact of microbes and their viruses, largely due to technological limitations that prevented us from seeing these “hidden” movers and shakers in the human body. However, there are now numerous examples where microbes likely play supporting, if not causal, roles in disease pathogenesis. As new tools and approaches become more readily available to the broader research community, the “ecogenomic” toolbox described above will enable a more wholistic, Eco-Systems Biology approach to study SCI that should help unravel the complex interplay along the virus/microbe-gut-brain axis.

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References

Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM, 2011 Phage treatment of human infections. Bacteriophage 1, 66–85. 10.4161/bact.1.2.15845. [PubMed: 22334863]

Abeles SR, Ly M, Santiago-Rodriguez TM, Pride DT, 2015 Effects of long term antibiotic therapy on human oral and fecal viromes. PLoS One 10, e0134941. 10.1371/journal.pone.0134941.

Abu-Shanab A, Quigley EM, 2010 The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 7, 691–701. 10.1038/nrgastro.2010.172. [PubMed: 21045794]

Akhter S, Aziz RK, Edwards RA, n.d. PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity-and composition-based strategies. doi:10.1093/nar/gks406

Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, Andersson AF, Quince C, 2014 Binning metagenomic contigs by coverage and composition. Nat Methods 11, 1144–1146. 10.1038/nmeth.3103. [PubMed: 25218180]

Altschup SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990 Basic local alignment search tool. J Mol Biol 215 (3), 403–410. [PubMed: 2231712]

Amgarten D, Braga LPP, da Silva AM, Setubal JC, 2018 MARVEL, a tool for prediction of bacteriophage sequences in metagenomic bins. Front Genet 9, 1–8. 10.3389/fgene.2018.00304. [PubMed: 29387083]

Ankeny DP, Lucin KM, Sanders VM, McGaughy VM, Popovich PG, 2006 Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. J Neurochem 99, 1073–1087. 10.1111/j.1471-4159.2006.04147.x. [PubMed: 17081140]

Ankeny DP, Guan Z, Popovich PG, 2009 B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. J Clin Invest 119, 2990–2999. 10.1172/JCI39780. [PubMed: 19770513]

Arevalo-Martin A, Grassner L, Garcia-Ovejero D, Paniagua-Torija B, Barroso-Garcia G, Arandilla AG, Mach O, Turrero A, Vargas E, Alcobendas M, Rosell C, Alcaraz MA, Ceruelo S, Casado R, Talavera F, Palazón R, Sanchez-Blanco N, Maier D, Esclarin A, Molina-Holgado E, 2018 Elevated autoantibodies in sub-acute human spinal cord injury are naturally occurring antibodies. Front Immunol 9, 2365 10.3389/fimmu.2018.02365. [PubMed: 30364218]

Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS, 2016PHASTER: a better, faster version of the PHAST phage search tool. Nucl Acids Res 44 10.1093/nar/gkw387.

Arumugam M, Raes J, Pelletier E, Le Paslier D, Batto J, Bertalan M, Borruel N, Casellas F, Costea PI, Hildebrand F, Manimozhiyan A, Bäckhed F, Blaser MJ, Bushman FD, De Vos WM, Ehrlich SD, Fraser CM, Hattori M, Huttenhower C, Jeffery IB, Knights D, Lewis JD, Ley RE, Ochman H, O'Toole PW, Quince C, Relman DA, Shanahan F, Sunagawa S, Wang J, Weinstock GM, Wu GD, Zeller G, Zhao L, Raes J, Knight R, Bork P, Gorvitovskaia A, Holmes SP, Huse SM, 2013 Enterotypes in the landscape of gut microbial community composition. Nature 3, 1–12. 10.1038/nature09944.Enterotypes.

Balmer ML, Slack E, de Gottardi A, Lawson MA, Hapfelmeier S, Miele L, Grieco A, Van Vlierberghe H, Fahrner R, Patuto N, Bernsmeier C, Ronchi F, Wyss M, Stroka D, Dickgreber N, Heim MH, McCoy KD, Macpherson AJ, 2014 The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. Sci Transl Med 6, 237ra66. 10.1126/scitranslmed.3008618.

Banos S, Lentendu G, Kopf A, Wubet T, Glöckner FO, Reich M, 2018 A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. BMC Microbiol 18, 190 10.1186/s12866-018-1331-4. [PubMed: 30458701]

Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M, 2016 The role of Gut Microbiota in the development of obesity and diabetes. Lipids Heal Dis 15, 108 10.1186/s12944-016-0278-4.

Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, Stotland A, Wolkowicz R, Cutting AS, Doran KS, Salamon P, Youle M, Rohwer F, 2019 Bacteriophage adhering to mucus provide a non-host-derived immunity. 10.1073/pnas.1305923110.

BAUER H, HOROWITZ RE, LEVENSON SM, POPPER H, 1963 The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. Am J Pathol 42, 471–483. [PubMed: 13966929]

Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpides NC, Hugenholtz P, 2007 CRISPR Recognition Tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics 8, 209 10.1186/1471-2105-8-209. [PubMed: 17577412]

Boettcher M, Machann J, Stefan N, Thamer C, Häring HU, Claussen CD, Fritsche A, Schick F, 2009 Intermuscular adipose tissue (IMAT): association with other adipose tissue compartments and insulin sensitivity. J Magn Reson Imaging 29, 1340–1345. 10.1002/jmri.21754. [PubMed: 19422021]

Bohórquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F, Liddle RA, 2015 Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. J Clin Invest 125, 782–786. 10.1172/JCI78361. [PubMed: 25555217]

Bolger AM, Lohse M, Usadel B, 2014 Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. 10.1093/bioinformatics/btu170. [PubMed: 24695404]

Borovikova LV, Ivanova S, Nardi D, Zhang M, Yang H, Ombrellino M, Tracey KJ, 2000 Role of vagus nerve signaling in CNI-1493-mediated suppression of acute inflammation. Aut Neurosci 85, 141–147. 10.1016/S1566-0702(00)00233-2.

Boyd JA, 2019 EnrichM [WWW Document]. URL. https://github.com/geronimp/enrichM.

Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Guan NL, Kundu P, Gulyas B, Halldin C, Hultenby K, Nilsson H, Hebert H, Volpe BT, Diamond B, Pettersson S, 2014 The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 6, 263ra158. 10.1126/scitranslmed.3009759.

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF, 2011 Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 108, 16050–16055. 10.1073/pnas.1102999108. [PubMed: 21876150]

Brommer B, Engel O, Kopp MA, Watzlawick R, Müller S, Prüss H, Chen Y, DeVivo MJ, Finkenstaedt FW, Dirnagl U, Liebscher T, Meisel A, Schwab JM,2016 Spinal cord injury-induced immune deficiency syndrome enhances infection susceptibility dependent on lesion level. Brain 139, 692–707. 10.1093/brain/awv375. [PubMed: 26754788]

Brown DG, Soto R, Yandamuri S, Stone C, Dickey L, Gomes-Neto JC, Pastuzyn ED, Bell R, Petersen C, Buhrke K, Fujinami RS, O'Connell RM, Stephens WZ, Shepherd JD, Lane TE, Round JL, 2019 The microbiota protects from viral-induced neurologic damage through microglia-intrinsic TLR signaling. Elife 8 10.7554/elife.47117.

Browning KN, Travagli RA, 2014 Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. Compr Physiol 4, 1339–1368. 10.1002/cphy.c130055. [PubMed: 25428846]

Brum JR, Ignacio-Espinoza JC, Roux S, Doulcier G, Acinas SG, Alberti A,Chaffron S, Cruaud C, de Vargas C, Gasol JM, Gorsky G, Gregory AC, Guidi L, Hingamp P, Iudicone D, Not F, Ogata H, Pesant S, Poulos BT, Schwenck SM, Speich S, Dimier C, Kandels-Lewis S, Picheral M, Searson S, Bork P, Bowler C, Sunagawa S, Wincker P, Karsenti E, Sullivan MB, Coordinators TO, 2015 Ocean plankton. Patterns and ecological drivers of ocean viral communities. Science (80) 348, 1261498. 10.1126/science.1261498.

Bushnell B, 2019 BBTools [WWW Document]. URL. https://sourceforge.net/projects/bbmap/.

Cao Y, Li C, Gregory A, Charlifue S, Krause JS, 2017 Depressive symptomatology after spinal cord injury: A multi-center investigation of multiple racial-ethnic groups. J Spinal Cord Med 40, 85–92. 10.1080/10790268.2016.1244314. [PubMed: 27844565]

Caspi R, Altman T, Dreher K, Fulcher CA, Subhraveti P, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Pujar A, Shearer AG, Travers M, Weerasinghe D, Zhang P, Karp PD, 2012 The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res 40, D742–D753. 10.1093/nar/gkr1014. [PubMed: 22102576]

Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG, 2014 Gut Microbiota: the neglected endocrine organ. Mol Endocrinol. 10.1210/me.2014-1108.me20141108.

Craig A, Guest R, Tran Y, Middleton J, 2017 Cognitive impairment and mood states after spinal cord injury. J Neurotrauma 34, 1156–1163. 10.1089/neu.2016.4632. [PubMed: 27717295]

Davidoff GN, Roth EJ, Richards JS, 1992 Cognitive deficits in spinal cord injury: epidemiology and outcome. Arch Phys Med Rehabil 73, 275–284. [PubMed: 1543433]

Davies AL, Hayes KC, Dekaban GA, 2007 Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. Arch Phys Med Rehabil 88, 1384–1393. 10.1016/j.apmr.2007.08.004. [PubMed: 17964877]

De Smet J, Zimmermann M, Kogadeeva M, Ceyssens P-J, Vermaelen W, Blasdel B, Bin Jang H, Sauer U, Lavigne R, 2016 High coverage metabolomics analysis reveals phage-specific alterations to Pseudomonas aeruginosa physiology during infection. ISME J 10, 1823–1835. 10.1038/ismej.2016.3. [PubMed: 26882266]

de Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, Lara E, Berney C, Le Bescot N, Probert I, Carmichael M, Poulain J, Romac S, Colin S, Aury JM, Bittner L, Chaffron S, Dunthorn M, Engelen S, Flegontova O, Guidi L, Horák A, Jaillon O, Lima-Mendez G, Lukeš J, Malviya S, Morard R, Mulot M, Scalco E, Siano R, Vincent F, Zingone A, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Acinas SG, Bork P, Bowler C, Gorsky G, Grimsley N, Hingamp P, Iudicone D, Not F, Ogata H, Pesant S, Raes J, Sieracki ME, Speich S, Stemmann L, Sunagawa S, Weissenbach J, Wincker P, Karsenti E, Coordinators TO, 2015 Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. Science (80-) 348, 1261605. 10.1126/science.1261605.

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martinez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM, 2006 Community genomics among stratified microbial assemblages in the ocean's interior. Science (80-) 311, 496–503. 10.1126/science.1120250.

DeVivo MJ, Black KJ, Stover SL, 1993 Causes of death during the first 12 years after spinal cord injury. Arch Phys Med Rehabil 74, 248–254. [PubMed: 8439250]

Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S, 2011 Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 108, 3047–3052. 10.1073/pnas.1010529108. [PubMed: 21282636]

Dick GJ, Andersson AF, Baker BJ, Simmons SL, Thomas BC, Pepper Yelton A, Banfield JF, 2009. Community-wide analysis of microbial genome sequence signatures. Genome Biol 10, 85 10.1186/gb-2009-10-8-r85.

Donaldson GP, Lee SM, Mazmanian SK, 2016 Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14, 20–32. 10.1038/nrmicro3552. [PubMed: 26499895]

Doulcier G, Bolduc B, Jang H. Bin, Sullivan MB, Roux S, You Z-Q, 2017 vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria. PeerJ 5, e3243. 10.7717/peerj.3243.

Duerkop BA, Kleiner M, Paez-Espino D, Zhu W, Bushnell B, Hassell B, Winter SE, Kyrpides NC, Hooper LV, 2018 Murine colitis reveals a disease-associated bacteriophage community. Nat Microbiol 3, 1023–1031. 10.1038/s41564-018-0210-y. [PubMed: 30038310]

Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK, 2006 Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci USA 103, 12511–12516. 10.1073/pnas.0601056103. [PubMed: 16895997]

Dutilh BE, Cassman N, McNair K, Sanchez SE, Silva GGZ, Boling L, Barr JJ, Speth DR, Seguritan V, Aziz RK, Felts B, Dinsdale EA, Mokili JL, Edwards RA, 2014 A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nat Commun 5, 4498 10.1038/ncomms5498. [PubMed: 25058116]

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA, 2005 Diversity of the human intestinal microbial flora. Science (80-) 308, 1635–1638. 10.1126/science.1110591.

Elder CP, Apple DF, Bickel CS, Meyer RA, Dudley GA, 2004 Intramuscular fat and glucose tolerance after spinal cord injury - A cross-sectional study. Spinal Cord 42, 711–716. 10.1038/sj.sc.3101652. [PubMed: 15303112]

Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES, 2000 The sympathetic nerve-an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 52, 595–638. [PubMed: 11121511]

Erny D, de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, Schwierzeck V, Utermöhlen O, Chun E, Garrett WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M, 2015 Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 18, 965–977. 10.1038/nn.4030. [PubMed: 26030851]

Evans CT, Rogers TJ, Burns SP, Lopansri B, Weaver FM, 2011 Knowledge and use of antimicrobial stewardship resources by spinal cord injury providers. PM R 3, 619–623. 10.1016/j.pmrj.2011.03.021. [PubMed: 21777860]

Failli V, Kopp MA, Gericke C, Martus P, Klingbeil S, Brommer B, Laginha I, Chen Y, DeVivo MJ, Dirnagl U, Schwab JM, 2012 Functional neurological recovery after spinal cord injury is impaired in patients with infections. Brain 135, 3238–3250. 10.1093/brain/aws267. [PubMed: 23100450]

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M, 2014 Pfam: the protein families database. Nucleic Acids Res 42, D222–D230. 10.1093/nar/gkt1223. [PubMed: 24288371]

Flannigan KL, Denning TL, 2018 Segmented filamentous bacteria-induced immune responses: a balancing act between host protection and autoimmunity. Immunology 10.1111/imm.12950.

Flores SC, Campbell TB, Bassis CM, Beck JM, Weinstock GM, Schmidt TM, Twigg H, Curtis JL, Lynch SV, Ghedin E, Kleerup E, Schloss PD, Jablonski K, Sodergren E, Venkataraman A, Crothers K, Young VB, Morris A, Fontenot AP, Huang L, 2013 Comparison of the respiratory microbiome in healthy non-smokers and smokers. Am J Respir Crit Care Med 187, 1067–1075. 10.1164/rccm.201210-1913oc. [PubMed: 23491408]

Forsythe P, Bienenstock J, Kunze WA, 2014 Vagal pathways for microbiome-brain-gut axis communication. Adv Exp Med Biol 817, 115–133. 10.1007/978-1-4939-0897-4_5. [PubMed: 24997031]

Frank RG, Chaney JM, Clay DL, Shutty MS, Niels CB, Kay DR, Elliott TR, Grambling S, 1992 Dysphoria: A major symptom factor in persons with disability or chronic illness. Psychiatry Res 43, 231–241. 10.1016/0165-1781(92)90056-9. [PubMed: 1438622]

Gabanyi I, Muller PA, Feighery L, Oliveira TY, Costa-Pinto FA, Mucida D, 2016 Neuro-immune interactions drive tissue programming in intestinal macrophages. Cell 164, 378–391. 10.1016/j.cell.2015.12.023. [PubMed: 26777404]

Galiez C, Siebert M, Enault F, Vincent J, Sö Ding J, 2017 WIsH: who is the host? Predicting prokaryotic hosts from metagenomic phage contigs. Bioinformatics 33, 3113–3114. 10.1093/bioinformatics/btx383. [PubMed: 28957499]

Gater DR, 2007 Obesity after spinal cord injury. Phys Med Rehabil Clin N Am 18 (333–51), vii 10.1016/j.pmr.2007.03.004.

Gogokhia L, Buhrke K, Bell R, Casjens SR, Longman RS, Round Correspondence JL, 2019 Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. Cell Host Microbe 25, 285–299. 10.1016/j.chom.2019.01.008. [PubMed: 30763538]

Goodus MT, Sauerbeck AD, Popovich PG, Bruno RS, McTigue DM, 2018 Dietary green tea extract prior to spinal cord injury prevents hepatic iron overload but does not improve chronic hepatic and spinal cord pathology in rats. J Neurotrauma 35, 2872–2882. 10.1089/neu.2018.5771. [PubMed: 30084733]

Gorgey AS, Mather KJ, Poarch HJ, Gater DR, 2011 Influence of motor complete spinal cord injury on visceral and subcutaneous adipose tissue measured by multiaxial magnetic resonance imaging. J Spinal Cord Med 34, 99–109. 10.1179/107902610X12911165975106. [PubMed: 21528633]

Gorgey AS, Dolbow DR, Dolbow JD, Khalil RK, Castillo C, Gater DR, 2014 Effects of spinal cord injury on body composition and metabolic profile - part I. J Spinal Cord Med 37, 693–702. 10.1179/2045772314Y.0000000245. [PubMed: 25001559]

Graham ED, Heidelberg JF, Tully BJ, 2017 BinSanity: unsupervised clustering of environmental microbial assemblies using coverage and affinity propagation. PeerJ 5, e3035. 10.7717/peerj.3035.

Gregory AC, Sullivan MB, Segal LN, Keller BC, 2018 Smoking is associated with quantifiable differences in the human lung DNA virome and metabolome. Respir Res 19, 1–13. 10.1186/s12931-018-0878-9. [PubMed: 29295703]

Gregory AC, Zablocki O, Howell A, Bolduc B, Sullivan MB, 2019 The human gut virome database. bioRxiv 655910. 10.1101/655910.

Gregory AC, Zayed AA, Conceição-Neto N, Temperton B, Bolduc B, Alberti A, Ardyna M, Arkhipova K, Carmichael M, Cruaud C, Dimier C, Domínguez-Huerta G, Ferland J, Kandels S, Liu Y, Marec C, Pesant S, Picheral M, Pisarev S, Poulain J, Tremblay J, Vik D, Babin M, Bowler C, Culley AI, de Vargas C, Dutilh BE, Iudicone D, Karp-Boss L, Roux S, Sunagawa S, Wincker P, Sullivan MB, Coordinators TO, 2019b Marine DNA viral macro- and microdiversity from pole to pole. Cell 177, 1109–1123.e14. 10.1016/j.cell.2019.03.040. [PubMed: 31031001]

Gu S, Chen D, Zhang JN, Lv X, Wang K, Duan LP, Nie Y, Wu XL, 2013 Bacterial community mapping of the mouse gastrointestinal tract. PLoS One 8, e74957. 10.1371/journal.pone.0074957.

Guidi L, Chaffron S, Bittner L, Eveillard D, Larhlimi A, Roux S, Darzi Y, Audic S, Berline L, Brum JR, Coelho LP, Espinoza JCI, Malviya S, Sunagawa S, Dimier C, Kandels-Lewis S, Picheral M, Poulain J, Searson S, Coordinators TOC, Stemmann L, Not F, Hingamp P, Speich S, Follows M, Karp-Boss L, Boss E, Ogata H, Pesant S, Weissenbach J, Wincker P, Acinas SG, Bork P, de Vargas C, Ludicone D, Sullivan MB, Raes J, Karsenti E, Bowler C, Gorsky G,2016 Plankton networks driving carbon export in the oligotrophic ocean. Nature 532, 465 10.1038/nature16942.5327600. [PubMed: 26863193]

Gungor B, Adiguzel E, Gursel I, Yilmaz B, Gursel M, 2016 Intestinal microbiota in patients with spinal cord injury. PLoS One 11, 1–10. 10.1371/journal.pone.0145878.

Guo L, Karpac J, Tran SL, Jasper H, 2014 PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156, 109–122. 10.1016/j.cell.2013.12.018. [PubMed: 24439372]

Han M, Yang P, Zhong C, Ning K, 2018 The human gut virome in hypertension. Front Microbiol 9, 1–10. 10.3389/fmicb.2018.03150. [PubMed: 29403456]

Handley SA, Devkota S, 2019 Going Viral: A Novel Role for Bacteriophage in Colorectal Cancer. 10.1128/mBio.02248-18.

Handley SA, Thackray LB, Zhao G, Presti R, Miller AD, Droit L, Abbink P, Maxfield LF, Kambal A, Duan E, Stanley K, Kramer J, Macri SC, Permar SR, Schmitz JE, Mansfield K, Brenchley JM, Veazey RS, Stappenbeck TS, Wang D, Barouch DH, Virgin HW, 2012 Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell 151, 253–266. 10.1016/j.cell.2012.09.024. [PubMed: 23063120]

Hannigan GD, Duhaime MB, Ruffin MT, Koumpouras CC, Schloss PD, 2018 Diagnostic Potential and Interactive Dynamics of the Colorectal Cancer Virome. 10.1128/mBio.

Hawasli AH, Rutlin J, Roland JL, Murphy RKJ, Song SK, Leuthardt EC,Shimony JS, Ray WZ, 2018 Spinal cord injury disrupts resting-state networks in the human brain. J Neurotrauma 35, 864–873. 10.1089/neu.2017.5212. [PubMed: 29179629]

Hayes KC, Hull TCL, Delaney GA, Potter PJ, Sequeira KAJ, Campbell K, Popovich PG, 2002 Elevated serum titers of proinflammatory cytokines and CNS autoantibodies in patients with chronic spinal cord injury. J Neurotrauma 19, 753–761. 10.1089/08977150260139129. [PubMed: 12165135]

Hergenroeder GW, Moore AN, Schmitt KM, Redell JB, Dash PK, 2016 Identification of autoantibodies to glial fibrillary acidic protein in spinal cord injury patients. Neuroreport 27, 90–93. 10.1097/WNR.0000000000000502. [PubMed: 26629661]

Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS, Bushman FD, Artis D, 2012 Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. Nat Med 18, 538–546. 10.1038/nm.2657. [PubMed: 22447074]

Holmes GM, Blanke EN, 2019 Gastrointestinal dysfunction after spinal cord injury. Exp Neurol. 10.1016/j.expneurol.2019.113009.

Hooper LV, Littman DR, Macpherson AJ, 2012 Interactions between the microbiota and the immune system. Science (80-) 336, 1268–1273. 10.1126/science.1223490.

Hotamisligil GS, 2017a Inflammation, metaflammation and immunometabolic disorders. Nature 542, 177–185. 10.1038/nature21363. [PubMed: 28179656]

Hotamisligil GS, 2017b Foundations of immunometabolism and implications for metabolic health and disease. Immunity 47, 406–420. 10.1016/j.immuni.2017.08.009. [PubMed: 28930657]

Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P, 2018 eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res 47, 309–314. 10.1093/nar/gky1085.

Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A,2011 Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A 108, 5354–5359. 10.1073/pnas.1019378108. [PubMed: 21402903]

Imelfort M, Parks D, Woodcroft BJ, Dennis P, Hugenholtz P, Tyson GW, 2014 GroopM: an automated tool for the recovery of population genomes from related metagenomes. PeerJ 2, e603. 10.7717/peerj.603.

Imhann F, Vich Vila A, Bonder MJ, Fu J, Gevers D, Visschedijk MC, Spekhorst LM, Alberts R, Franke L, van Dullemen HM, Ter Steege RWF, Huttenhower C, Dijkstra G, Xavier RJ, Festen EAM, Wijmenga C, Zhernakova A, Weersma RK, 2018 Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. Gut 67, 108–119. 10.1136/gutjnl-2016-312135. [PubMed: 27802154]

Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR, 2009 Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–498. 10.1016/j.cell.2009.09.033. [PubMed: 19836068]

Jašarević E, Morrison KE, Bale TL, 2016 Sex differences in the gut microbiome - Brain axis across the lifespan. Philos Trans R Soc B Biol Sci. 10.1098/rstb.2015.0122.

Jenne CN, Kubes P, 2013 Immune surveillance by the liver. Nat Immunol 14, 996–1006. 10.1038/ni.2691. [PubMed: 24048121]

Jing Y, Yang D, Bai F, Zhang C, Qin C, Li D, Wang L, Yang M, Chen Z, Li J, 2019 Melatonin treatment alleviates spinal cord injury-induced gut dysbiosis in mice. J. Neurotrauma 36, 2646–2664. 10.1089/neu.2018.6012. [PubMed: 30693824]

Jones TB, Basso DM, Sodhi A, Pan JZ, Hart RP, MacCallum RC, Lee S, Whitacre CC, Popovich PG, 2002 Pathological CNS autoimmune disease triggered by traumatic spinal cord injury: implications for autoimmune vaccine therapy. J Neurosci 22, 2690–2700 doi:20026267. [PubMed: 11923434]

Joshi NA, Fass JN, 2011 Sickle: A Sliding-Window, Adaptive, Quality-Based Trimming Tool for FastQ Files (Version 1.33) [Software]. [WWW Document]. URL. https://github.com/najoshi/sickle.

Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M, 2015 KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44, 457–462. 10.1093/nar/gkv1070.

Kang DD, Froula J, Egan R, Wang Z, 2015 MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3, e1165. 10.7717/peerj.1165.

Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, Khoruts A, Geis E, Maldonado J, McDonough-Means S, Pollard EL, Roux S, Sadowsky MJ, Lipson KS, Sullivan MB, Caporaso JG, Krajmalnik-Brown R, 2017 Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: An open-label study. Microbiome 5, 10 10.1186/s40168-016-0225-7. [PubMed: 28122648]

Kernbauer E, Ding Y, Cadwell K, 2014 An enteric virus can replace the beneficial function of commensal bacteria. Nature 516, 94 10.1038/nature13960. [PubMed: 25409145]

Kigerl KA, Hall JCE, Wang L, Mo X, Yu Z, Popovich PG, 2016 Gut dysbiosis impairs recovery after spinal cord injury. J Exp Med 213, 2603–2620. 10.1084/jem.20151345. [PubMed: 27810921]

Kim M-S, Bae J-W, 2016 Spatial disturbances in altered mucosal and luminal gut viromes of diet-induced obese mice. Environ Microbiol 18, 1498–1510. 10.1111/1462-2920.13182. [PubMed: 26690305]

Kopp MA, Watzlawick R, Martus P, Failli V, Finkenstaedt FW, Chen Y, DeVivo MJ, Dirnagl U, Schwab JM, 2017 Long-term functional outcome in patients with acquired infections after acute

spinal cord injury. Neurology 88, 892–900. 10.1212/WNL.0000000000003652. [PubMed: 28130472]

Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen A-M, Peet A, Tillmann V, Pöhö P, Mattila I, Lähdesmäki H, Franzosa EA, Vaarala O, de Goffau M, Harmsen H, Ilonen J, Virtanen SM, Clish CB, Orešič M, Huttenhower C, Knip M, Xavier RJ, 2015 The dynamics of the human infant gut microbiome in development and in progression toward Type 1 diabetes. Cell Host Microbe 17, 260–273. 10.1016/j.chom.2015.01.001. [PubMed: 25662751]

Krause JS, Kemp B, Coker J, 2000 Depression after spinal cord injury: relation to gender, ethnicity, aging, and socioeconomic indicators. Arch Phys Med Rehabil 81, 1099–1109. [PubMed: 10943762]

Kreydin E, Welk B, Chung D, Clemens Q, Yang C, Danforth T, Gousse A, Kielb S, Kraus S, Mangera A, Reid S, Szell N, Cruz F, Chartier-Kastler E, Ginsberg DA, 2018 Surveillance and management of urologic complications after spinal cord injury. World J Urol. 10.1007/s00345-018-2345-0.

Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D, 2011 Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci USA 108, 11548–11553. 10.1073/pnas.1108924108. [PubMed: 21709219]

Krych L, Hansen CH, Hansen AK, van den Berg FW, Nielsen DS, 2013 Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. PLoS One 8, e62578. 10.1371/journal.pone.0062578.

Kulikov AS, Prjibelski AD, Tesler G, Vyahhi N, Sirotkin AV, Pham S, Dvorkin M, Pevzner PA, Bankevich A, Nikolenko SI, Pyshkin AV, Nurk S, Gurevich AA, Antipov D, Alekseyev MA, Lesin VM, 2012 SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19, 455–477. 10.1089/cmb.2012.0021. [PubMed: 22506599]

Kurokawa S, Kishimoto T, Mizuno S, Masaoka T, Naganuma M, Liang KC,Kitazawa M, Nakashima M, Shindo C, Suda W, Hattori M, Kanai T, Mimura M, 2018 The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: An open-label observational study. J Affect Disord 235, 506–512. 10.1016/j.jad.2018.04.038. [PubMed: 29684865]

Lal S, Kirkup AJ, Brunsden AM, Thompson DG, Grundy D, 2001 Vagal afferent responses to fatty acids of different chain length in the rat. Am J Physiol Gastrointest Liver Physiol 281, G907–G915. 10.1152/ajpgi.2001.281.4.G907. [PubMed: 11557510]

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR, 1985 Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A 82, 6955–6959. 10.1073/pnas.82.20.6955. [PubMed: 2413450]

Langmead B, Salzberg SL, 2012 Fast gapped-read alignment with Bowtie 2. Nat Methods 9, 357–359. 10.1038/nmeth.1923. [PubMed: 22388286]

Laslett D, Canback B, 2004 ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32, 11–16. 10.1093/nar/gkh152. [PubMed: 14704338]

Lee YK, Menezes JS, Umesaki Y, Mazmanian SK, 2011 Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 108 (Suppl), 4615–4622. 10.1073/pnas.1000082107. [PubMed: 20660719]

Levatte MA, Mabon PJ, Weaver LC, Dekaban GA, 1998 Simultaneous identification of two populations of sympathetic preganglionic neurons using recombinant herpes simplex virus type 1 expressing different reporter genes. Neuroscience 82, 1253–1267. [PubMed: 9466444]

Level N, 2018 Spinal Cord Injury Facts and Figures at a Glance Re-Hospitalization Cause of Death.

Ley RE, 2010 Obesity and the human microbiome. Curr Opin Gastroenterol 26, 5–11. 10.1097/MOG.0b013e328333d751. [PubMed: 19901833]

Ley RE, Turnbaugh PJ, Klein S, Gordon JI, 2006 Microbial ecology: human gut microbes associated with obesity. Nature 444, 1022–1023. 10.1038/4441022a. [PubMed: 17183309]

Li H, Durbin R, 2009 Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. 25 pp. 1754–1760. 10.1093/bioinformatics/btp324.

Li D, Liu C-M, Luo R, Sadakane K, Lam T-W, 2015 MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676. 10.1093/bioinformatics/btv033. [PubMed: 25609793]

Liddle RA, 2019 Neuropods. Cell Mol Gastroenterol Hepatol 7, 739–747. 10.1016/j.jcmgh.2019.01.006. [PubMed: 30710726]

Lim ES, Zhou Y, Zhao G, Bauer IK, Droit L, Ndao IM, Warner BB, Tarr PI, Wang D, Holtz LR, 2015 Early life dynamics of the human gut virome and bacterial microbiome in infants. Nat Med 21, 1228–1234. 10.1038/nm.3950. [PubMed: 26366711]

Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, Chaffron S, Ignacio-Espinosa JC, Roux S, Vincent F, Bittner L, Darzi Y, Wang J, Audic S, Berline L, Bontempi G, Cabello AM, Coppola L, Cornejo-Castillo FM, d'Ovidio F, De Meester L, Ferrera I, Garet-Delmas MJ, Guidi L, Lara E, Pesant S, Royo- Llonch M, Salazar G, Sánchez P, Sebastian M, Souffreau C, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Gorsky G, Not F, Ogata H, Speich S, Stemmann L, Weissenbach J, Wincker P, Acinas SG, Sunagawa S, Bork P, Sullivan MB, Karsenti E, Bowler C, de Vargas C, Raes J, coordinators TO, 2015 Ocean plankton. Determinants of community structure in the global plankton interactome. Science (80-) 348, 1262073. 10.1126/science.1262073.

Lowe TM, Eddy SR, 1997 tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25 (5), 955–964. [PubMed: 9023104]

Lu YY, Chen T, Fuhrman JA, Sun F, 2016 COCACOLA: binning metagenomic contigs using sequence COmposition, read CoverAge, CO-alignment and paired-end read LinkAge. Bioinformatics 33, btw290. 10.1093/bioinformatics/btw290.

Lucin KM, Sanders VM, Jones TB, Malarkey WB, Popovich PG, 2007 Impaired antibody synthesis after spinal cord injury is level dependent and is due to sympathetic nervous system dysregulation. Exp Neurol 207, 75–84. 10.1016/j.expneurol.2007.05.019. [PubMed: 17597612]

Luedtke K, Bouchard SM, Woller SA, Funk MK, Aceves M, Hook MA, 2014 Assessment of depression in a rodent model of spinal cord injury. J Neurotrauma 31, 1107–1121. 10.1089/neu.2013.3204. [PubMed: 24564232]

Ly M, Jones MB, Abeles SR, Santiago-Rodriguez TM, Gao J, Chan IC, Ghose C, Pride DT, 2016 Transmission of viruses via our microbiomes. Microbiome 4, 64 10.1186/s40168-016-0212-z. [PubMed: 27912785]

Mabon PJ, LeVatte MA, Dekaban GA, Weaver LC, 1997 Identification of sympathetic preganglionic neurons controlling the small intestine in hamsters using a recombinant herpes simplex virus type-1. Brain Res 753, 245–250. [PubMed: 9125409]

Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, Costa FRC, Tiniakou E, Greiling T, Ruff W, Barbieri A, Kriegel C, Mehta SS, Knight JR, Jain D, Goodman AL, Kriegel MA, 2018 Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science (80-) 359, 1156–1161. 10.1126/science.aar7201.

Manrique P, Dills M, Young M, Young MJ, 2017 The human gut phage community and its implications for health and disease. Viruses 9, 141 10.3390/v9060141.

Markle JGM, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, Von Bergen M, McCoy KD, Macpherson AJ, Danska JS, 2013 Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science (80-) 339, 1084–1088. 10.1126/science.1233521.

Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman D, 2011 The human gut virome: Inter-individual variation and dynamic response to diet. Genome Res 21, 1616 10.1101/gr.122705.111. [PubMed: 21880779]

Mirzaei MK, Maurice CF, 2017 Ménage à trois in the human gut: interactions between host, bacteria and phages. Nat Rev Microbiol 15, 397–408. 10.1038/nrmicro.2017.30. [PubMed: 28461690]

Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, Lankowski A, Baldridge MT, Wilen CB, Flagg M, Norman JM, Keller BC, Luévano JM, Wang D, Boum Y, Martin JN, Hunt PW, Bangsberg DR, Siedner MJ, Kwon DS, Virgin HW, 2016 Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe 19, 311–322. 10.1016/j.chom.2016.02.011. [PubMed: 26962942]

Moreno-Gallego JL, Chou SP, Di Rienzi SC, Goodrich JK, Spector TD, Bell JT, Youngblut ND, Hewson I, Reyes A, Ley RE, 2019 Virome diversity correlates with intestinal microbiome

diversity in adult monozygotic twins. Cell Host Microbe 25, 261–272.e5. https://doi.Org/10.1016/j.chom.2019.01.019. [PubMed: 30763537]

Morton SC, Shekelle PG, Adams JL, Bennett C, Dobkin BH, Montgomerie J, Vickrey BG, 2002 Antimicrobial prophylaxis for urinary tract infection in persons with spinal cord dysfunction. Arch Phys Med Rehabil 83, 129–138. 10.1053/apmr.2002.26605. [PubMed: 11782843]

Myers SA, Gobejishvili L, Saraswat Ohri S, Garrett Wilson C, Andres KR, Riegler AS, Donde H, Joshi-Barve S, Barve S, Whittemore SR, 2019 Following spinal cord injury, PDE4B drives an acute, local inflammatory response and a chronic, systemic response exacerbated by gut dysbiosis and endotoxemia. Neurobiol Dis 124, 353–363. 10.1016/j.nbd.2018.12.008. [PubMed: 30557659]

Neufeld KM, Kang N, Bienenstock J, Foster JA, 2011 Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 23, 255–64, e119. 10.1111/j.1365-2982.2010.01620.x. [PubMed: 21054680]

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S,2012 Host-gut microbiota metabolic interactions. Science (80-) 336, 1262–1267. 10.1126/science.1223813.

Nicotra A, Critchley HD, Mathias CJ, Dolan RJ, 2006 Emotional and autonomic consequences of spinal cord injury explored using functional brain imaging. Brain 129, 718–728. 10.1093/brain/awh699. [PubMed: 16330503]

Noble LJ, Wrathall JR, 1989 Distribution and time course of protein extravasation in the rat spinal cord after contusive injury. Brain Res 482, 57–66. 10.1016/0006-8993(89)90542-8. [PubMed: 2706482]

Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, Kambal A, Monaco CL, Zhao G, Fleshner P, Stappenbeck TS, McGovern DPB, Keshavarzian A, Mutlu EA, Sauk J, Gevers D, Xavier RJ, Wang D, Parkes M, Virgin HW, 2015 Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 160, 447–460. 10.1016/j.cell.2015.01.002. [PubMed: 25619688]

O'Connor G, Jeffrey E, Madorma D, Marcillo A, Abreu MT, Deo SK, Dietrich WD, Daunert S, 2018 Investigation of microbiota alterations and intestinal inflammation post-spinal cord injury in rat model. J Neurotrauma. 10.1089/neu.2017.5349.

Ochoa-Repáraz J, Mielcarz DW, Haque-Begum S, Kasper LH, 2010 Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. Gut Microbes 1, 103–108. 10.4161/gmic.1.2.11515. [PubMed: 21326918]

Olsen I, Yamazaki K, 2019 Can oral bacteria affect the microbiome of the gut? J Oral Microbiol 11, 1586422. 10.1080/20002297.2019.1586422.

Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, Cassidy L, Tholey A, Fickenscher H, Seegert D, Rosenstiel P, Schreiber S,2017 Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. Gastroenterology 152, 799–811.e7. 10.1053/j.gastro.2016.11.010. [PubMed: 27866880]

Parks D, 2017 UniteM [WWW Document]. URL. https://github.com/dparks1134/UniteM.

Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil P-A, Hugenholtz P, 2018 A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36, 996 10.1038/nbt.4229. [PubMed: 30148503]

Peng Y, Leung HCM, Yiu SM, Chin FYL, 2012 IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28, 1420–1428. 10.1093/bioinformatics/bts174. [PubMed: 22495754]

Perez-Burgos A, Wang L, McVey Neufeld KA, Mao YK, Ahmadzai M, Janssen LJ, Stanisz AM, Bienenstock J, Kunze WA, 2015 The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. J Physiol 593, 3943–3957. 10.1113/JP270229. [PubMed: 26084409]

Popovich PG, Horner PJ, Mullin BB, Stokes BT, 1996 A quantitative spatial analysis of the blood-spinal cord barrier I. Permeability changes after experimental spinal contusion injury. Exp Neurol 142, 258–275. 10.1006/exnr.1996.0196. [PubMed: 8934558]

Prüss H, Tedeschi A, Thiriot A, Lynch L, Loughhead SM, Stutte S, Mazo IB, Kopp MA, Brommer B, Blex C, Geurtz LC, Liebscher T, Niedeggen A, Dirnagl U, Bradke F, Volz MS, DeVivo MJ, Chen

Y, von Andrian UH, Schwab JM,2017 Spinal cord injury-induced immunodeficiency is mediated by a sympathetic-neuroendocrine adrenal reflex. Nat Neurosci 20, 1549–1559. 10.1038/nn.4643. [PubMed: 28920935]

Quince C, Walker AW, Simpson JT, Loman NJ, Segata N, 2017 Shotgun meta-genomics, from sampling to analysis. Nat Biotechnol 35, 833–844. 10.1038/nbt.3935. [PubMed: 28898207]

Rabosky DL, 2014 Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. Cit Rabosky DL 9, 89543 10.1371/journal.pone.0089543.

Rankin KC, O'Brien LC, Segal L, Khan MR, Gorgey AS, 2017 Liver adiposity and metabolic profile in individuals with chronic spinal cord injury. Biomed Res Int 2017, 1364818. 10.1155/2017/1364818.

Rappé MS, Giovannoni SJ, 2003 The uncultured microbial majority. Annu Rev Microbiol 57, 369–394. 10.1146/annurev.micro.57.030502.090759. [PubMed: 14527284]

Ren J, Song K, Deng C, Ahlgren NA, Fuhrman JA, Li Y, Xie X, Sun F, 2018 Identifying Viruses from Metagenomic Data by Deep Learning.

Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, Gordon JI, 2010 Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466, 334–338. 10.1038/nature09199. [PubMed: 20631792]

Reyes A, Semenkovich NP, Whiteson K, Rohwer F, Gordon JI, 2012 Going viral: next-generation sequencing applied to phage populations in the human gut. Nat Rev Microbiol 10, 607–617. 10.1038/nrmicro2853. [PubMed: 22864264]

Roach DR, Leung CY, Henry M, Morello E, Singh D, Di Santo JP, Weitz JS, Debarbieux L, 2017 Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. Cell Host Microbe 22, 38–47.e4. https://doi.Org/10.1016/j.chom.2017.06.018. [PubMed: 28704651]

Roth E, Davidoff G, Thomas P, Doljanac R, Dijkers M, Berent S, Morris J,Yarkony G, 1989 A controlled study of neuropsychological deficits in acute spinal cord injury patients. Paraplegia 27, 480–489. 10.1038/sc.1989.75. [PubMed: 2608301]

Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, Chao CC, Patel B, Yan R, Blain M, Alvarez JI, Kébir H, Anandasabapathy N, Izquierdo G, Jung S, Obholzer N, Pochet N, Clish CB, Prinz M, Prat A, Antel J, Quintana FJ, 2016 Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. Nat Med 22, 586–597. 10.1038/nm.4106. [PubMed: 27158906]

Rothhammer V, Borucki DM, Tjon EC, Takenaka MC, Chao CC, Ardura-Fabregat A, De Lima KA, Gutiérrez-Vazquez C, Hewson P, Staszewski O, Blain M, Healy L, Neziraj T, Borio M, Wheeler M, Dragin LL, Laplaud DA, Antel J, Alvarez JI, Prinz M, Quintana FJ, 2018 Microglial control of astrocytes in response to microbial metabolites. Nature 557, 724–728. 10.1038/s41586-018-0119-x. [PubMed: 29769726]

Round JL, Mazmanian SK, 2009 The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9, 313–323. 10.1038/nri2515. [PubMed: 19343057]

Roux S, Enault F, Hurwitz BL, Sullivan MB, 2015 VirSorter: mining viral signal from microbial genomic data. PeerJ 3, e985. 10.7717/peerj.985.

Roux S, Brum JR, Dutilh BE, Sunagawa S, Duhaime MB, Loy A, Poulos BT, Solonenko N, Lara E, Poulain J, Pesant S, Kandels-Lewis S, Dimier C, Picheral M, Searson S, Cruaud C, Alberti A, Duarte CM, Gasol JM, Vaqué D, Bork P, Acinas SG, Wincker P, Sullivan MB, Coordinators TO, 2016 Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. Nature 537, 689–693. 10.1038/nature19366. [PubMed: 27654921]

Sauerbeck AD, Laws JL, Bandaru VV, Popovich PG, Haughey NJ, McTigue DM, 2015 Spinal cord injury causes chronic liver pathology in rats. J Neurotrauma 32, 159–169. 10.1089/neu.2014.3497. [PubMed: 25036371]

Scarpellini E, Ianiro G, Attili F, Bassanelli C, De Santis A, Gasbarrini A, 2015 The human gut microbiota and virome: potential therapeutic implications. Dig Liver Dis 47, 1007–1012. 10.1016/j.dld.2015.07.008. [PubMed: 26257129]

Schaedler RW, Dubos R, Costello R, 1965 The development of the bacterial flora in the gastrointestinal tract of mice. J Exp Med 122, 59–66. 10.1084/jem.122.1.59. [PubMed: 14325473]

Schwab JM, Zhang Y, Kopp MA, Brommer B, Popovich PG, 2014 The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury. Exp Neurol 258, 121–129. 10.1016/j.expneurol.2014.04.023. [PubMed: 25017893]

Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D, Huttenhower C, Izard J, 2012 Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol 13, R42. 10.1186/gb-2012-13-6-r42.

Shavelle RM, DeVivo MJ, Brooks JC, Strauss DJ, Paculdo DR, 2015 Improvements in long-term survival after spinal cord injury? Arch Phys Med Rehabil 96, 645–651. 10.1016/j.apmr.2014.11.003. [PubMed: 25616394]

Sheng L, Jena PK, Liu HX, Kalanetra KM, Gonzalez FJ, French SW, Krishnan VV, Mills DA, Wan YJY, 2017 Gender differences in bile acids and microbiota in relationship with gender dissimilarity in steatosis induced by diet and FXR in-activation. Sci Rep 7, 1–12. 10.1038/s41598-017-01576-9. [PubMed: 28127051]

Shi C, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, Leiner I, Li MO, Frenette PS, Pamer EG, 2011 Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. Immunity 34, 590–601. 10.1016/j.immuni.2011.02.016. [PubMed: 21458307]

Shin JC, Goo HR, Yu SJ, Kim DH, Yoon SY, 2012 Depression and quality of life in patients within the first 6 months after the spinal cord injury. Ann Rehabil Med 36, 119–125. 10.5535/arm.2012.36.1.119. [PubMed: 22506244]

Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, Banfield JF, 2018 Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nat Microbiol 3, 836–843. 10.1038/s41564-018-0171-1. [PubMed: 29807988]

Skelton F, Martin LA, Evans CT, Kramer J, Grigoryan L, Richardson P, Kunik ME, Poon IO, Holmes SA, Trautner BW, 2019 Determining best practices for management of bacteriuria in spinal cord injury: Protocol for a mixed-methods study. J Med Internet Res 21, e12272. 10.2196/12272.

Stilling RM, Ryan FJ, Hoban AE, Shanahan F, Clarke G, Claesson MJ, Dinan TG, Cryan JF, 2015 Microbes & neurodevelopment - Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. Brain Behav Immunol 50, 209–220. 10.1016/j.bbi.2015.07.009.

Straub RH, Wiest R, Strauch UG, Härle P, Schölmerich J, 2006 The role of the sympathetic nervous system in intestinal inflammation. Gut 55, 1640–1649. 10.1136/gut.2006.091322. [PubMed: 17047110]

Sullivan MB, 2015 Viromes, not gene markers, for studying double-stranded DNA virus communities. J Virol 89, 2459–2461. 10.1128/JVI.03289-14. [PubMed: 25540374]

Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G,Djahanschiri B, Zeller G, Mende DR, Alberti A, Cornejo-Castillo FM, Costea PI, Cruaud C, d'Ovidio F, Engelen S, Ferrera I, Gasol JM, Guidi L, Hildebrand F, Kokoszka F, Lepoivre C, Lima-Mendez G, Poulain J, Poulos BT, Royo-Llonch M, Sarmento H, Vieira-Silva S, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Bowler C, de Vargas C, Gorsky G, Grimsley N, Hingamp P, Iudicone D, Jaillon O, Not F, Ogata H, Pesant S, Speich S, Stemmann L, Sullivan MB, Weissenbach J, Wincker P, Karsenti E, Raes J, Acinas SG, Bork P, coordinators TO, 2015 Ocean plankton. Structure and function of the global ocean microbiome. Science (80-) 348, 1261359. 10.1126/science.1261359.

Tate DG, Forchheimer M, Rodriguez G, Chiodo A, Cameron AP, Meade M,Krassioukov A, 2016 Risk factors associated with neurogenic bowel complications and dysfunction in spinal cord injury. Arch Phys Med Rehabil 97, 1679–1686. 10.1016/j.apmr.2016.03.019. [PubMed: 27109330]

Tatusov RL, Galperin MY, Natale DA, Koonin EV, 2000 The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28, 33–36. [PubMed: 10592175]

Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, Verschoor CP, Loukov D, Schenck LP, Jury J, Foley KP, Schertzer JD, Larché MJ, Davidson DJ, Verdu EF, Surette MG, Bowdish DME, 2017 Age-associated microbial dysbiosis promotes intestinal permeability, systemic

inflammation, and macrophage dysfunction. Cell Host Microbe 21, 455–466.e4. 10.1016/j.chom.2017.03.002. [PubMed: 28407483]

Tilg H, Kaser A, 2011 Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest 121, 2126–2132. 10.1172/JCI58109. [PubMed: 21633181]

Tillisch K, 2014 The effects of gut microbiota on CNS function in humans. Gut Microbes 5.

Torres-Barceló C, 2018 The disparate effects of bacteriophages on antibiotic-resistant bacteria. Emerg Microbes Infect 7, 1–12. 10.1038/s41426-018-0169-z. [PubMed: 29323102]

Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen K, Chang HW, Podar M, Short JM, Mathur EJ, Detter JC, Bork P, Hugenholtz P, Rubin EM, 2005 Comparative metagenomics of microbial communities. Science (80-) 308, 554–557. 10.1126/science.1107851.

Turnbaugh PJ, Gordon JI, 2009. The core gut microbiome, energy balance and obesity. J Physiol 587, 4153–4158. 10.1113/jphysiol.2009.174136. [PubMed: 19491241]

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI, 2006 An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027–1031. 10.1038/nature05414. [PubMed: 17183312]

Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI, 2008 Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3, 213–223. 10.1016/j.chom.2008.02.015. [PubMed: 18407065]

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF, 2004 Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428, 37–43. 10.1038/nature02340. [PubMed: 14961025]

Ueno M, Ueno-Nakamura Y, Niehaus J, Popovich PG, Yoshida Y, 2016 Silencing spinal interneurons inhibits immune suppressive autonomic reflexes caused by spinal cord injury. Nat Neurosci 19, 784–787. 10.1038/nn.4289. [PubMed: 27089020]

Uritskiy GV, DiRuggiero J, Taylor J, 2018 MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome 6, 158 10.1186/s40168-018-0541-1. [PubMed: 30219103]

van Westerloo DJ, 2010 The vagal immune reflex: a blessing from above. Wien Med Wochenschr 160, 112–117. 10.1007/s10354-010-0761-x. [PubMed: 20364413]

Vik DR, Roux S, Brum JR, Bolduc B, Emerson JB, Padilla CC, Stewart FJ, Sullivan MB, 2017 Putative archaeal viruses from the mesopelagic ocean. PeerJ 5, e3428 10.7717/peerj.3428. [PubMed: 28630803]

Villarroel J, Kleinheinz K, Jurtz V, Zschach H, Lund O, Nielsen M, Larsen M, Villarroel J, Kleinheinz KA, Jurtz VI, Zschach H, Lund O, Nielsen M, Larsen MV, 2016 HostPhinder: a phage host prediction tool. Viruses 8, 116 10.3390/v8050116.

Whetstone WD, Hsu JYC, Eisenberg M, Werb Z, Noble-Haeusslein LJ, 2003 Blood-spinal cord barrier after spinal cord injury: relation to revascularization and wound healing. J Neurosci Res 74, 227–239. 10.1002/jnr.10759. [PubMed: 14515352]

Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G, 2009 Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci USA 106, 3698–3703. 10.1073/pnas.0812874106. [PubMed: 19234110]

Woese CR, Fox GE, 1977 Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci U S A 74, 5088–5090. 10.1073/pnas.74.11.5088. [PubMed: 270744]

Woodcroft B, 2019 CoverM [WWW Document]. URL. https://github.com/wwood/CoverM/.

Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, Mathis D, 2010 Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 32, 815–827. 10.1016/j.immuni.2010.06.001. [PubMed: 20620945]

Wu J, Zhao Z, Sabirzhanov B, Stoica BA, Kumar A, Luo T, Skovira J, Faden AI, 2014 Spinal cord injury causes brain inflammation associated with cognitive and affective changes: role of cell cycle pathways. J Neurosci 34, 10989–11006. 10.1523/JNEUR0SCI.5110-13.2014. [PubMed: 25122899]

Wu Y-W, Simmons BA, Singer SW, 2016 MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics 32, 605–607. 10.1093/bioinformatics/btv638. [PubMed: 26515820]

Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY, 2015 Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161, 264–276. 10.1016/j.cell.2015.02.047. [PubMed: 25860609]

Yutin N, Makarova KS, Gussow AB, Krupovic M, Segall A, Edwards RA, Koonin V, 2018 Discovery of an expansive bacteriophage family that includes the most abundant viruses from the human gut. Nat Microbiol 3, 38–46. 10.1038/s41564-017-0053-y. [PubMed: 29133882]

Zhang Y, Guan Z, Reader B, Shawler T, Mandrekar-Colucci S, Huang K, Weil Z, Bratasz A, Wells J, Powell ND, Sheridan JF, Whitacre CC, Rabchevsky AG, Nash MS, Popovich PG, 2013 Autonomic dysreflexia causes chronic immune suppression after spinal cord injury. J Neurosci 33, 12970–12981. 10.1523/JNEUR0SCI.1974-13.2013. [PubMed: 23926252]

Zhang C, Zhang W, Zhang J, Jing Y, Yang M, Du L, Gao F, Gong H, Chen L, Li J, Liu H, Qin C, Jia Y, Qiao J, Wei B, Yu Y, Zhou H, Liu Z, Yang D, 2018 Gut microbiota dysbiosis in male patients with chronic traumatic complete spinal cord injury. J Transl Med 16, 353 10.1186/s12967-018-1735-9. [PubMed: 30545398]

Zhao G, Vatanen T, Droit L, Park A, Kostic AD, Poon TW, Vlamakis H, Siljander H, Härkönen T, Hämäläinen A-M, Peet A, Tillmann V, Ilonen J, Wang D, Knip M, 2018 Intestinal virome changes precede autoimmunity in type I diabetes-susceptible children. Proc Natl Acad Sci 115 10.1073/pnas.1706359114.

Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, Zeng L, Chen J, Fan S, Du X, Zhang X, Yang D, Yang Y, Meng H, Li W, Melgiri ND, Licinio J, Wei H, Xie P, 2016 Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry 21, 786–796. 10.1038/mp.2016.44. [PubMed: 27067014]

Zheng T, Li J, Ni Y, Kang K, Misiakou M-A, Imamovic L, Chow BKC, Rode AA, Bytzer P, Sommer M, Panagiotou G, 2019 Mining, analyzing, and integrating viral signals from metagenomic data. Microbiome 7, 42 10.1186/s40168-019-0657-y. [PubMed: 30890181]

Zuo T, Wong SH, Lam K, Lui R, Cheung K, Tang W, Ching JYL, Chan PKS, Chan MCW, Wu JCY, Chan FKL, Yu J, Sung JJY, Ng SC, 2018 Bacteriophage transfer during faecal microbiota transplantation in Clostridium difficile infection is associated with treatment outcome. Gut 67, 634–643. 10.1136/gutjnl-2017-313952. [PubMed: 28539351]

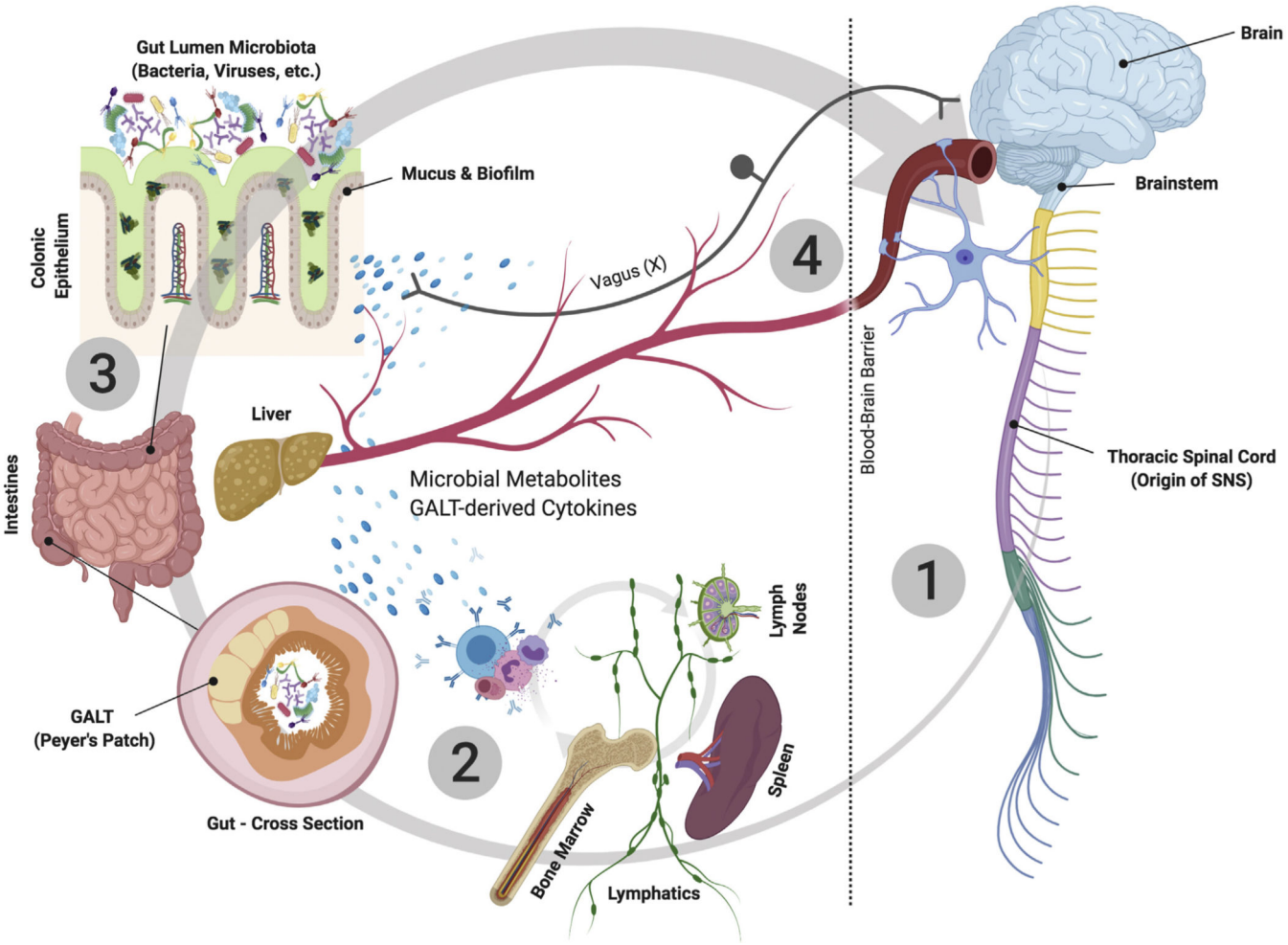

Fig. 1.

Spinal cord injury (SCI) sets in a motion a systematic breakdown of communication between the nervous system, the immune system and the gastrointestinal system (1). As a result of SCI, normal sympathetic control over all body systems is lost, creating an imbalance in autonomic tone in most organs. Consequently, physiological control of hematopoiesis (bone marrow and spleen) and immune surveillance (controlled by bone marrow, spleen, lymph nodes, gastrointestinal- associated lymphoid tissue/GALT) (2) as well as gastrointestinal function (e.g., motility, transit, mucin production) and liver function are lost. This break in homeostasis causes chronic immune dysfunction and gut dysbiosis, i.e., a lasting change in the ecological balance of microorganisms found in the biofilm and gut lumen (3). The metabolites and cytokines produced by microbes and cells in the dysbiotic gut enter the circulation or activate the vagus nerve, creating a feedback loop by which functional changes in the gut-immune axis affect structure and function within the CNS (brain, brainstem and spinal cord) (4).

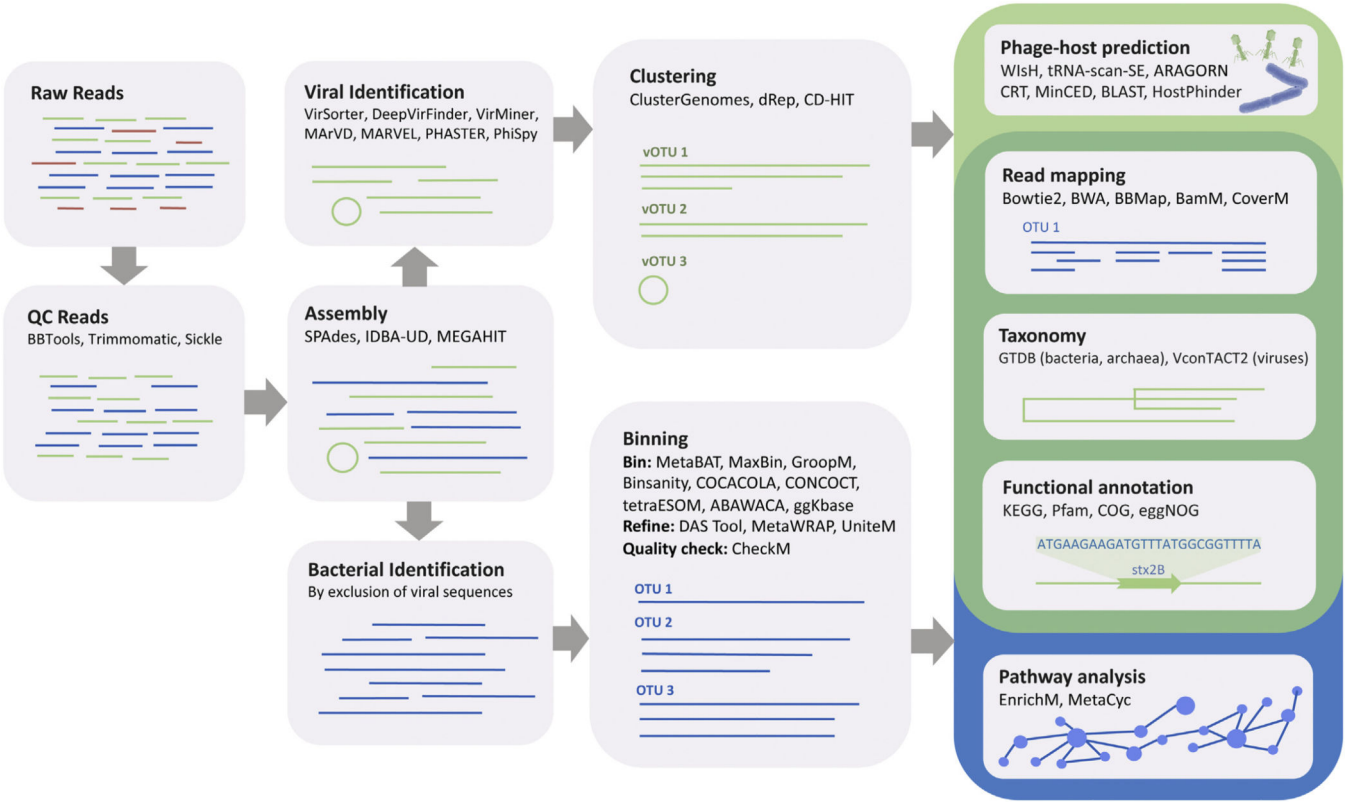

Fig. 2.

The metagenomic pipeline. Raw sequencing reads are provided by the sequencing center. The first step is to quality control (QC) the reads (Bolger et al., 2014; Bushnell, 2019; Joshi and Fass, 2011), which involves removal of reads that map to the model organism from which samples were collected, removal of adaptors and common sequencing center contaminants, and removal of low-quality reads. The final result of this step is a set of clean reads. Next, the clean reads are assembled to form long contigs (Kulikov et al., 2012; Li et al., 2015; Peng et al., 2012). Ideally, these contigs correspond to all or part of the genomes of the microbial taxa (bacteria and viruses) within the sample. In the microbial identification step, bacteria can be grouped (called binning) by operational taxonomic unit (OTU) with a variety of tools (Alneberg et al., 2014; Dick et al., 2009; Graham et al., 2017; Imelfort et al., 2014; Kang et al., 2015; Lu et al., 2016; Parks, 2017; Sieber et al., 2018; Uritskiy et al., 2018; Wu et al., 2016)(ggkbase.berkeley.edu). Viral OTUs (vOTUs) are similarly identified with a variety of tools (.Akhter et al., n.d; Amgarten et al., 2018; Arndt et al., 2016; Ren et al., 2018; Roux et al., 2015; Vik et al., 2017; Zheng et al., 2019). At this point, a user possesses two sets of contigs corresponding to complete or near-complete genomes of the viruses and bacteria present in the original sample. Here, analysis can diverge. Common analyses include read mapping (Bushnell, 2019; Langmead and Salzberg, 2012; Li and Durbin, 2009; Rabosky, 2014; Woodcroft, 2019) to calculate abundances, taxonomy classification (Doulcier et al., 2017; Parks et al., 2018), and functional annotation (identification of genes present within contigs) (Finn et al., 2014; Huerta-Cepas et al., 2018; Kanehisa et al., 2015; Tatusov et al., 2000). For phages (viruses that infect bacteria), host prediction (Altschup et al., 1990; Bland et al., 2007; Galiez et al., 2017; Laslett and

Canback, 2004; Lowe and Eddy, 1997; Villarroel et al., 2016) to identify the bacterial host is a common downstream analysis. For bacteria, pathway analysis (Boyd, 2019; Caspi et al., 2012) may be of interest.

Table 1

Tools for Metagenomic Analysis. Lists the tool on the left, with the title of associated paper on the right. If no published paper is available, a short description is provided with corresponding code repository as requested by the tool's creator.

ABAWACA	A tool for binning metagenomic contigs. Available at: https://github.com/CK7/abawaca
ARAGORN	ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences
BamM	Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence on Phylogenetic Trees
BBTools	BBTools is a suite of fast, multithreaded bioinformatics tools designed for analysis of DNA and RNA sequence data, including bbduk, which filters and trims reads for adapters and contaminants using k-mers and bbmap, a short-read aligner for DNA and RNA-seq data. Available at: https://sourceforge.net/projects/bbmap/
BinSanity	BinSanity: unsupervised clustering of environmental microbial assemblies using coverage and affinity propagation.
BLAST/prophage blast	Basic Local Alignment Search Tool
Bowtie2	Fast gapped-read alignment with Bowtie 2
BWA	Fast and accurate short read alignment with Burrows-Wheeler transform
CD-HIT	Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences
CheckM	CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes
ClusterGenomes	A tool to remove redundancies from contigs or genomes by clustering them, using the longest contig as the representative for the population. Available at: https://bitbucket.org/MAVERICLab/docker-clustergenomes
COCACOLA	COCACOLA: binning metagenomic contigs using sequence COmposition, read CoverAge, CO-alignment and paired-end read LinkAge
COG	The COG database: a tool for genome-scale analysis of protein functions and evolution
CONCOCT	Binning metagenomic contigs by coverage and composition
CoverM	A configurable, easy to use and fast DNA read coverage and relative abundance calculator focused on metagenomics applications. Available at: https://github.com/wwood/CoverM/
CRT/MinCED	CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats.
DAS Tool	Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy
DeepVirFinder	Identifying viruses from metagenomic data by deep learning
dRep	dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication
eggNOG	eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses
EnrichM	A tool for MAG annotation and de novo MAG identification, as well as metabolic pathway analysis and functional analysis. Available at: https://github.com/geronimp/enrichM
ggKbase	A suite of tools, including a popular binning tool which uses phylogeny, coverage, and GC content to separate a set of contigs into bins. Available at: https://ggkbase.berkeley.edu/
GroopM	GroopM: an automated tool for the recovery of population genomes from related metagenomes
GTDB	A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life.
HostPhinder	HostPhinder: A Phage Host Prediction Tool
IDBA-UD	IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth.

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KEGG	KEGG as a reference resource for gene and protein annotation
MArVD	Putative archaeal viruses from the mesopelagic ocean
MARVEL	MARVEL, a Tool for Prediction of Bacteriophage Sequences in Metagenomic Bins
MaxBin	MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets.
Megahit	MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph
MetaBAT	MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities
MetaCyc	The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases
MetaWRAP	MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis
Pfam	Pfam: the protein families database
PHASTER	PHASTER: a better, faster version of the PHAST phage search tool
PhiSpy	PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity- and composition-based strategies
Sickle	A sliding-window, adaptive, quality-based trimming tool for FastQ files. Available at: github.com/najoshi/sickle
SPAdes	SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing
tetraESOM	Community-wide analysis of microbial genome sequence signatures
Trimmomatic	Trimmomatic: a flexible trimmer for Illumina sequence data
tRNA-scan-SE	tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence
UniteM	UniteM implements different ensemble binning strategies to produce a non-redundant set ofbins from the output of multiple binning methods. Available at: https://github.com/dparks1134/UniteM
VconTACT2	vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria
VirMiner	Mining, analyzing, and integrating viral signals from metagenomic data
VirSorter	VirSorter: mining viral signal from microbial genomic data
WIsH	WIsH: who is the host? Predicting prokaryotic hosts from metagenomic phage contigs