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Biological Individuality: The Microbiome as a Dynamic in HCV Liver Disease Progression

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BACKGROUND INFORMATION

The microbiome is "the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space" [1]. Bacterial composition varies in diversity and abundance from person to person [2], and can also vary between different sites of the body [3]. The effects of the microbiome as a regulator of our health can be seen through its impact on endocrinology and immunology. Certain microbes are capable of metabolizing compounds using bacterial enzymes, while other microbes synthesize hormone-like metabolites to influence host metabolism [4]. Deregulation of the microbiome has been correlated with inflammation and autoimmune responses, leading to various disease states and notably, carcinogenesis [2],[5]. As the microbiome impacts immune response, it follows that changes in the microbiome may affect host defense against infectious diseases. Characteristic microbiota shifts from human immunodeficiency virus (HIV) infection have been shown to contribute to variations in viral and bacterial infectivity [6]. Additionally, microbial compositions were found to differ in HIV-positive patients, indicating that viral infectious diseases may reciprocally impact the microbiome [6].

HCV is another type of viral infectious disease; approximately 75% of the time, HCV infection leads to chronic hepatitis C that affects individuals over decades. Metabolism deregulation and inflammation from HCV infection are responsible for the development of severe liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC). Currently, almost all cases of HCV can be cured, but liver disease progression following treatment and recovery from infection hasn't been adequately addressed.

As the liver is in close proximity to the intestinal tract, it is vulnerable to bacterial endotoxin and metabolite exposure from the microbiome. Liver diseases such as non-alcoholic fatty liver disease (NAFLD) have been correlated to changes in the microbiome [7], and NAFLD patients are similarly at risk for eventually developing cirrhosis and hepatocellular carcinoma [8].

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Gut microbiota has been found to be significantly altered when patients develop cirrhosis [9] and when undergoing liver transplantation [10]. Liver transplantation has been found to decrease microbial diversity and impact rates of infection [11]. Patients with chronic hepatitis B cirrhosis have also been found to have distinct microbial compositions [12]. For stage-4 HCV patients, a consistent change in microbial composition has been found in addition to a decreased microbial diversity [13]. However, there has yet to be any information of the microbiome following successful antiviral treatment.

"For stage-4 HCV patients, a consistent change in microbial composition has been found in addition to a decreased microbial diversity"

RESEARCH QUESTIONS

It is notable that the nature of HCV-induced liver disease progression is aligned with conditions associated with microbiome deregulation - specifically metabolism deregulation and increased inflammation. Therefore, it would be of importance to investigate whether certain microbial compositions may affect HCV infection susceptibility as well as drive liver disease progression. Since the microbiome can be quickly and reproducibly altered through dietary lifestyles [14], it also lends itself to be an ideal target for personalized treatment.

Research questions of interest will be presented to consider how the microbiome may impact HCV infection and liver disease progression. Firstly, we will examine whether microbiome individuality may impact variations in disease severity (acute versus chronic) seen in hepatitis C patients. Secondly, we will explore if and how the microbiome may increase susceptibility to HCV infection, specifically re-infection following liver transplantation. And finally, it would be of interest to further elucidate the role of the microbiome in the development of HCC after HCV infection is treated and cleared.

PROJECT NARRATIVE

To answer these important research questions involving the role of the microbiome in HCV liver disease progression, a longitudinal study could be conducted looking at the changes in the microbiome over the course of infection, disease progression and treatment. In particular, the target population would be intravenous drug-users in downtown Vancouver that are at high risk of receiving HCV infection. Stool and/or blood samples could be initially collected from HCV-negative drug users, who are susceptible to infection due to the high frequency of sharing needles. These individuals would then be monitored and additional samples would be collected upon developing chronic infection and after subsequent treatment. More samples from other drug users could also be obtained from the BC Centre for Disease Control.

A typical microbiome analysis workflow includes sample extraction and processing, sequencing, and data analysis [15]. Sequencing methods usually include 16S rRNA amplicon sequencing, and/or shotgun metagenomic sequencing. 16S rRNA is specific to bacteria, effectively eliminating the interference of the human genome. Small base pair differences can provide species level identification and classification [16]. Both types of sequencing can yield microbial composition and relative abundance, but metagenomic analyses following shotgun sequencing can also provide the functional importance (i.e. metabolic and signaling capabilities) of differing taxa [17]; metagenomic analyses can sometimes also identify and characterize unculturable species [18]. The resources and services required for a microbiome analysis are also available in Vancouver, such as the company Microbiome Insights. With this potential study design in mind, we can progress to explore the first research question regarding the divergence in HCV infection disease severity.

Can microbiome individuality explain the variations in disease severity (acute versus chronic) seen in hepatitis C patients?

The difference between patients with acute and chronic infection is likely due to how well individuals can minimize and clear infection. The differing microbial composition can influence disease susceptibility [19], such as the level of defense one has against pathogens. Certain species may be able to produce antivirals against the infection or stimulate the host to produce anti-viral peptides [20]. Alternatively, species may inhibit infection and replication by directly modifying the virion [20]. In the case of HIV, lipopolysaccharides

(LPS) from Gram-negative bacteria activate innate immune responses [21]. It follows that such findings can also be expanded to other viruses, particularly an RNA virus like HCV.

From the study design described above, the focus point for this research question would be to look at the differences in microbial composition between patients that develop acute versus chronic infection. Samples could be taken upon initial diagnosis of infection and after a six-month mark, when patients are deemed to have developed chronic infection. There may a lower diversity of species in individuals that eventually develop a chronic infection. Perhaps individuals that are able to clear the infection have a greater abundance of protective species or conversely, individuals that develop chronic infection may have a greater abundance of species that weaken defenses or increase susceptibility to viral infection. Upon identifying such species, additional infectivity assays can be performed to find the direct correlation between those particular bacteria and HCV. By understanding how microbial composition impacts ability to clear the infection, we can administer treatments to target these potentially detrimental species or promote the growth of beneficial species as a "preventative" measure for chronic infection.

However, in the circumstance that the disease progresses in the case of chronic infection, a patient may develop cirrhosis and liver cancer. Liver transplants are required for individuals with these end-stage liver diseases. Unfortunately, there are financial and supply limitations to liver replacements, and regrettably, an individual that has a received a transplant may be susceptible to a re-infection. Approximately 50-90% of patients develop reoccurrence of HCV within a year of receiving a liver transplant and some as soon as 3 months [22]. Additionally, approximately 15% develop cirrhosis within 5-7 years [22]. This, therefore, leads to the next research question, which investigates the impact of the microbiome in HCV re-infection.

Can the microbiome increase susceptibility to HCV re-infection following liver transplant?

Although the transplanted liver should have a "healthy" microbiome from the organ donor, the organ recipient gut microbiome may play a role in re-infection through bacterial translocation. Microbiome changes can impact the gut lining, allowing bacteria and bacterial products to cross the gut barrier to the blood [23]. Bacterial endotoxins, specifically LPS, can induce expression of adipocyte enhancer-binding protein (AEBP1), which prevents macrophages from clearing cholesterol from the body and thereby resulting in fat accumulation in the liver [24]. Fat accumulation in the liver can also occur from modifications in gut microbes responsible for bile acid metabolism [25]. This then generates an environment ideal for HCV infection, which requires up-regulated lipid production in the liver for viral replication. The "diseased state" microbiome is therefore hypothesized to increased susceptibility to a more severe infection or re-infection of HCV following liver transplantation.

By performing a microbiome analysis on individuals prior to infection, we can tentatively establish the microbiome associated with a "healthy state" and identify any changes in diversity, composition or abundance. Using the experimental design and technologies described above, we can monitor the microbiome changes of HCV-infected patients after they receive a liver transplant. Additionally, we can compare the microbiome of non-HCV infected patients undergoing liver transplantation to those that are HCV-positive. This can establish if changes in the microbiome specifically due to HCV infection are likely to affect susceptibility to re-infection.

Moreover, recurrence of HCV infection after transplantation is affected by the length of pre-transplantation antiviral treatment [26], but individuals on the list for transplantation often have severe liver conditions and are limited for time. Many patients also respond poorly to anti-viral treatment post transplantation [26]. By conducting such a study, we can identify targets for treatment, such as re-establishing microbial diversity or by minimizing overgrowth of detrimental bacteria. The aim would be to decrease the susceptibility of re-infection by targeting the microbiome and therefore improve patient outcomes.

Patients that are going to receive or have received liver transplantation are usually put through a regime of antivirals, and the success rates of HCV clearance are convincing. However, it is still possible for individuals that have cleared HCV infection to eventually develop HCC [27]. The microbiome may be an overlooked factor in contributing to hepatocyte damage. This brings us to the next research question, which investigates possibly causal factors induced by the microbiome that leads to hepatocyte damage and eventual development of HCC.

Does the microbiome stimulate the development of hepatocellular carcinoma after HCV infection is cleared?

In the situation where the microbiome is unable to improve to a "healthy state" even after HCV clearance,

it is likely that it may influence the development of HCC. Microbiome imbalances are both a driving factor and a result of bacterial translocation induced inflammation and mucosal permeability [24]. Bacterial translocation involves the movement of endotoxins through the bloodstream and has been suggested to have clinical relevance with chronic liver diseases. The bacterial products are carried through the hepatic portal vein, inducing hypertension and increased immune response. The endotoxins in the blood also drain into the liver, triggering inflammation and subsequent hepatocyte damage [24]. Increased inflammation and immune response induced by endotoxins also make it difficult for commensal bacteria to re-establish a "healthy state" [28]. Consequently, metabolism deregulation that occurs from the "diseased state" microbiome may accumulate liver cell damage. The gut microbiota can generate a number of hepatotoxic compounds that need to be metabolized by the liver [25]. Metabolism deregulation and increased inflammatory and immune responses induced by the microbiome, therefore, amplify the amount of hepatocyte damage that will likely result in the development of HCC.

It is necessary to first examine the microbiome of patients that have been treated for and have cleared HCV infection using the microbiome analysis workflow described previously, and compare it to the "healthy state" microbiome. From this research, we can identify whether there is an overgrowth of species that are responsible for many harmful bacterial products. For example, LPS from Gram-negative bacteria have been found to be an important player in increased immune response. It is also likely that species adapted to grow in an inflammatory environment will be predominant, and these species may prevent the microbiome from recovering to the "healthy state". By investigating the change in microbial composition and change in the functional profile of these species, we can better understand if and how the microbiome impacts the development of HCC. With the development of successful treatments targeted toward the microbiome in combination with antivirals, we would be able to successfully clear HCV infection and prevent progression of liver disease.

From these three research questions, we have revealed many different ways that the microbiome may impact the susceptibility of different individuals to HCV infection as well as how it may impact liver disease progression. The microbiome may represent a new frontier in precision medicine in the field of HCV infection due to its individuality and flexibility. As we progress, there are many challenges in microbiome research that need to be overcome, but also many opportunities to apply such research to benefit HCV patients.

SUMMARY AND CONCLUSION

By understanding the link between HCV infection and the microbiome, we can move forward to its potential use in precision medicine to alleviate liver disease progression (summarized in Fig. 1). The microbiome can be used as a diagnostic by profiling individuals based on the relative abundance and diversity of different taxa [29]. It can also be used as a therapeutic target, as the microbiome could be advantageously shifted towards a "healthy state", such as through dietary changes, use of pre-biotics and pro-biotics, use of antibiotics and/or possibly fecal transplantation [2]. Certain prebiotic and probiotic mixtures have been shown to improve hepatic steatosis, inflammation and endotoxemia conditions [24]-[25],[30]. The flexibility of the microbiome makes it an enticing option in the frontier of precision medicine.

However, some difficulties that may arise include issues with sample collection - a stool or blood sample is preferable and much less invasive than a liver biopsy. Additionally, there may be issues with getting samples from the same patients, as drug-users may not return frequently to the hospital or clinic. HCV patients are also commonly found to be co-infected with HIV, introducing another variable in addition to the other regulators of the microbiome, such as an individuals' diet, environment, age, and sex [31]-[32]. As a large amount of data is generated from metagenomic analyses, intensive bioinformatics expertise will also be required to accurately process all the data. Bioethical issues will also be a concern, as some drug users are considered of a low-socioeconomic status – these individuals could be potentially contributing to the research without receiving the benefits of the research. Sequencing data is also considered extremely personal and demands a rigorous level of data protection for patient privacy. Despite these challenges, such research can deepen our understanding of the microbiome and potentially improve HCV patient outcomes.

In summary, we already know that HCV infection induces microbiome changes, and we have good reason to believe that these changes may influence the host immune response and metabolism. Microbiome individuality, as well as HCV-induced microbiome changes, may create an environment that is suitable for HCV infection while inducing hepatocyte damage that progresses liver diseases. With further understanding,

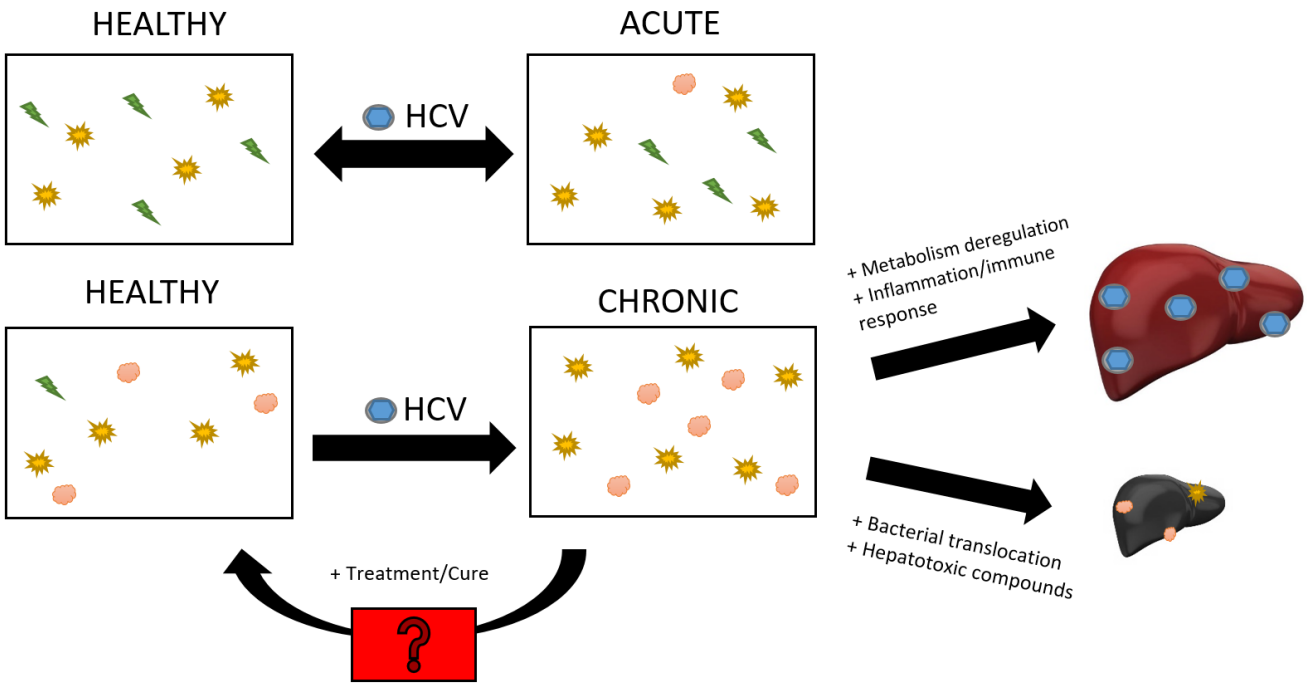

FIG. 1 Individuality in one's microbial composition may influence one's defense against HCV infection and subsequent development into chronic infection. Changes in the microbiome can induce metabolism deregulation and increase inflammation, leading to an ideal environment for HCV infection and replication. Additionally, bacterial endotoxins and hepatotoxic compounds from the microbiome can cause accumulated hepatocyte damage, resulting in hepatocellular carcinoma. The microbiome may be unable to shift to a healthy state after treatment and recovery from HCV infection, leading to re-infection and/or liver disease progression.

we can use HCV as a model system to continue studying the relationship between the microbiome and other viral infections, especially those that result in diseases associated with metabolism deregulation and changes in immune response. The microbiome can then be used as a potential diagnostic or therapeutic target for these disease manifestations. The complexity and individuality of the microbiome firmly establish its importance to human health and offers boundless potential as a precision medicine initiative.

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