2018

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Type 1 diabetes (T1D) is characterized by the autoimmune destruction of insulin-producing pancreatic beta cells. Although environmental factors interplay with genetic susceptibility to promote immune dysregulation and disease, it remains unclear as to which potential environmental factors are causative and not simply correlative. Despite many hints that the microbiome can have a profound effect on T1D, significant changes in bacterial gut flora and diversity appear to emerge only after the detection of early signs of T1D. Surprisingly, we recently found significant differences in the gut virome preceding the initial signs of T1D, raising the tantalizing possibility that the state of the virome may influence or predict whether susceptible individuals progress on the path to disease. The challenge will be to discern whether there is likely a causative relationship between detected virome differences and T1D.

Keywords: virome, type 1 diabetes, CRESS DNA virus, microbiome, bacteriophages

Viruses in Type 1 Diabetes: Friend or Foe?

Type 1 diabetes (T1D) usually rears its head at a young age, although T1D can develop later on in life as well (Diaz-Valencia et al., 2015). Initially, the appearance of autoantibodies that are specific for the pancreatic islets, or "seroconversion," serves as a non-pathogenic biomarker of preclinical disease development. As autoimmunity progresses, patients develop T cell-mediated autoimmune destruction of the pancreatic beta cells, resulting in loss of insulin production and, thus, dysglycemia (Coppieters et al., 2012b; Katsarou et al., 2017). Many genetic factors confer susceptibility to T1D, particularly certain variants of the highly diverse human leukocyte antigen (HLA) locus important for antigen presentation (Pociot and Lernmark, 2016). However, identical twins often do not share the same T1D outcome, indicating that the environment plays a critical role in triggering disease (Redondo et al., 2008). Many candidate environmental factors, including diet, hygiene, psychological stress, commensal microbiota, and viral infections, have been examined for their potential effects on T1D (Rewers and Ludvigsson, 2016).

In animal models, viruses can either trigger or prevent T1D, each via various potential mechanisms (Ghazarian et al., 2013). Although a number of viruses have been proposed as suspect triggers of T1D in people, most lack convincing confirmation or continued major relevance (Coppieters et al., 2012a). For example, despite reports implicating Mumps infection, clearly the near eradication of Mumps in many countries has not stemmed the continued rise of T1D (Honeyman, 2005). However, a growing body of evidence indicates that enteroviruses (family Picornaviridae, genus Enterovirus) may have a triggering role in T1D (Oikarinen et al., 2011; Yeung et al., 2011; Krogvold et al., 2015). The apparent inverse re-lationship between geographic exposure to enterovirus and T1D incidence would seem to argue against a causative relationship (Viskari et al., 2004, 2005); however, as Viskari et al. and others have speculated, it is possible that a lower prevalence of enterovirus in the population would lead to reduced pre-existing immunity and altered timing of infection, perhaps resulting in a disease course that is more likely to lead to T1D (Tracy et al., 2010). Notably, in the non-obese diabetic (NOD) mouse model of spontaneous T1D, enterovirus infection can prevent diabetes if administered early (Tracy et al., 2002; Filippi et al., 2009), but it accelerates diabetes if administered later in the course of disease development (Serreze et al., 2000). Whether enteroviruses are, indeed, causative agents of T1D is a challenging question to conclusively address, perhaps one that could only be truly settled by seeing the effect of enterovirus eradication on T1D incidence.

Beyond enteroviruses and other specific candidate viruses, it has not been explored whether unknown components of our virome may play a role in T1D development. We have but barely scratched the surface of the diversity and biological import of the virome, which can have profound impacts on host health (Virgin, 2014). Individual viral species or groups of viruses may have unexpected roles in promoting or ameliorating T1D.

The Gut Virome in T1D

Gut microbes and microbe-derived molecules interact with the host to cause wide-ranging effects on systemic immunity (Hooper et al., 2012). Further, the pancreas and the gut have an intimate connection via the immune system, with antigens from the gut being presented to immune cells in the pancreatic lymph nodes (Turley et al., 2005). In contrast to

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the mainly candidate virus-based approach of previous work, we took a broad and unbiased approach, shotgun metagenomics sequencing, to determine whether changes in the virome could be linked to development of T1D.

Before our examination of the virome, our colleagues Kostic et al. examined the bacterial component of the gut microbiome, because modulation of the intestinal bacterial flora has a profound effect on T1D development in the NOD mouse model (Knip and Siljander, 2016). To determine whether predictive bacterial signatures could be discerned for human T1D, Kostic et al. (2015) performed a longitudinal case-control study in Finland and Estonia, neighboring countries with differing standards of living. Finland has the highest incidence of T1D in the world, whereas the prevalence of autoimmune disease is increasing rapidly in Estonia as its standard of living rises. Monthly stool samples were collected from genetically susceptible infants between the ages of 1 month through 3 years, providing a densely collected view into microbiome changes during the period of life when the microbiome plays an important role in the maturation and education of the immune system (Francino, 2014; Wang et al., 2016). In the end, the study encompassed 11 seroconverters (including 4 who further progressed to diagnosed T1D during the study) along with controls matched for HLA risk allele genotype, age, sex, birth delivery route, and country. For those children who progressed to T1D, Kostic et al. (2015) noted a decrease in bacterial diversity between the detection of seroconversion and the onset of T1D; however, no significant earlier bacterial signatures were found that might predict seroconversion.

Seroconversion is a defining turning point in T1D development that indicates risk of progression to clinical disease (Ziegler et al., 2013). We wondered whether examining the virome in the Finland/Estonia cohort might reveal viral signatures predictive of the early event of seroconversion. All stool samples collected before seroconversion for the 11 seroconverters and corresponding matched controls were processed and analyzed to favor detection of viral sequences (Zhao et al., 2017). A highly diverse set of viruses was detected, with sequence relationships to 178 defined viral genera, including 33 genera of eukaryotic viruses. Of the eukaryotic viruses, the only viral family to show a statistically significant difference between eventual seroconverters and control subjects was the Circoviridae, which contains nonenveloped viruses with single-stranded, circular DNA genomes. These novel Circoviridae-related sequences classify within the broader group of related circular Rep-encoding ssDNA (CRESS DNA) viruses (Delwart and Li, 2012). We were fascinated to find that CRESS DNA viruses were detected only in control subjects (5 of 11) and not at all in the seroconverters. Could it be that these viruses, which have thus far been considered possible commensal viruses with no known link to disease in humans (Virgin et al., 2009), actually play a protective role in T1D?

Viruses Can Be Beneficial to the Host

Each person harbors on average 8–12 chronic infections of characterized viruses (Virgin et al., 2009), not to mention all the viruses only glimpsed in deep sequencing of the virome. Therefore, our bodies not only repel acute viral infections but also coexist, perhaps at times in symbiosis, with persistent

viral infections. The interplay between these viruses and our organismal cells and systems has a pervasive influence on our development and health. Although dissecting this interplay is experimentally challenging, recent work has revealed important insights. Kernbauer et al. (2014) showed that introducing a persistent infection of murine norovirus resulted in a type I IFN-dependent reversal of intestinal abnormalities typically seen in germ-free mice, indicating that a virus can supply signals that are important for host physiology. Other work by Yang et al. (2016) suggests that toll-like receptor (TLR)-mediated host recognition of enteric viruses maintains intestinal homeostasis and resistance to intestinal injury. Along similar lines, Sun et al. (2015) have shown that type I IFN in response to persistent viral infection promotes epithelial cell turnover and wound repair.

Could TLR-mediated type I IFN responses influence the progression of T1D? In the NOD mouse model, TLR-mediated immune signaling can repress or accelerate T1D, depending on the specific TLR and the experimental conditions used (Okada et al., 2010; Tai et al., 2016). In genetically susceptible children, a transient type I IFN transcriptional signature was noted in the peripheral blood preceding seroconversion, suggesting that type I IFN might actually promote disease (Ferreira et al., 2014); arguing otherwise, however, is work showing that type I IFN-deficient NOD mice are not less susceptible to T1D compared with wild-type mice (Quah et al., 2014). Beyond TLR-mediated responses, viruses might ameliorate T1D progression via a number of potential mechanisms: antigenic competition between viral antigens and autoantigens, bystander suppression of autoimmune responses on upregulation of regulatory immune cells in response to viral infection, and perhaps diverse impacts on the immune system milieu that counter specific T1D triggers (Virgin et al., 2009; Okada et al., 2010). For example, if enteroviruses are truly triggers of T1D, could infection with another component of the virome stimulate the immune system in a manner that decreases the risk of enteroviruses reaching and infecting the pancreatic islets? Such a virus-to-virus effect is quite possible, as it has been shown that chronic herpesvirus infection can render the host resistant to other infections (Barton et al., 2007).

The idea that components of the virome could provide protection against T1D is consistent with the hygiene hypothesis, which postulates that decreasing infections and environmental exposures in a given area is a driver of increased autoimmunity (Okada et al., 2010). It is interesting that CRESS DNA viruses (other than porcine circovirus, derived from consuming pork products) were found in human stool samples from Pakistan, Nigeria, and Tunisia but not the United States (Li et al., 2010), indicating that the prevalence of these viruses could be affected by standards of hygiene.

Bacteriophages May Point the Way for Bacterial Changes in T1D

Bacteriophages represent the dominant viral component of the gut virome (Ogilvie and Jones, 2015). Our analysis of bacteriophage sequences in the Finland/Estonia cohort identified bacteriophage contigs that were predictive of age at the time of sample collection, indicating that these bacteriophage signatures were remarkably consistent across individuals and might define the normal development of the virome. However, the bacteriophage component of the virome, nevertheless,

differed in important ways between eventual seroconverters and control children. The richness and Shannon diversity of these families was reduced in children who later seroconverted (Zhao et al., 2017). We further identified a subset of bacteriophage sequences, directly or inversely associated with seroconversion, that was highly predictive for discriminating between seroconverters and controls (Zhao et al., 2017).

It is interesting that these differences in bacteriophage populations before seroconversion were observed despite the lack of distinguishing bacterial signatures from the same samples (Kostic et al., 2015). Is it possible that changes in bacteriophages before seroconversion led to the changes in bacterial populations and decrease in bacterial diversity seen by Kostic et al. after seroconversion? Bacteriophages persisting as prophages integrated in bacterial genomes can be reactivated by various stimuli (Mills et al., 2013). Perturbations to lysogenic-lytic cycles of bacteriophages could lead to changes in bacteriophage abundance with only a delayed effect on bacterial populations.

Beyond their influence on bacterial populations, bacteriophages may interact with the host directly. Phage Ig-like protein domains on viral capsids can bind to mucins, thus concentrating bacteriophages in mucus and providing protection from bacterial infection (Barr et al., 2013). Bacteriophages can gain systemic access from the gut (Duerkop and Hooper, 2013) and have the potential to exert immunomodulatory effects on various immune cells (Mori et al., 1996; Eriksson et al., 2009; Gorski et al., 2012). Discerning whether specific bacteriophages might have more direct effects on host physiology and disease is made more challenging by phage hypervariability and rapid shifts that can lead to change in tropism even from a single nucleotide change (Liu et al., 2002; Minot et al., 2012). The central role of bacteriophages in the gut and host physiology, however, cannot be ignored.

A Long Way from Disease Association to Causal Relationship

Despite tantalizing hints that CRESS DNA viruses may play a role in protecting against seroconversion, it remains to be shown whether their significance can be seen in a separate and larger cohort, whether the viruses we detected replicate in humans or reflect an environmental source, and whether they can show an effect on disease in a T1D model such as the NOD mouse. Caution is warranted when identifying specific signatures in sequencing data as potentially linked to the phenotype in question, and a high burden of proof and confirmation must be satisfied before a particular microbiome signature can be said to prevent or accelerate disease. There is no doubt, however, that a specific virus can have profound effects on host physiology and disease. How such a virus fits into the overall landscape of contributing factors, such as host genetics, host behavior and interactions with the environment, and the milieu of other microbiome components, will need to be understood before we contemplate introducing commensal viruses to at-risk individuals in hopes of preventing disease. Our hope is that the continued advances in technology that have allowed us to glimpse this possibility will carry us forward to determining whether this possibility could become reality.

Disclosure Statement

No competing financial interests exist.

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