Editorial

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# Connecting the dots from viral infection to disease



**Methodological advances have helped identify viruses as causative agents of disease but this is complicated by heterogeneity in patient outcomes and long-term symptoms.**

Viruses that infect animals and plants are now well known as aetiologies of disease. However, unlike bacterial pathogens, viruses were not always easily identified as causative agents owing to their small sizes and their reliance on host cells. About 50 years after bacterial pathogens were identified, the first evidence linking viruses to specific diseases was reported. In the late nineteenth century, Dmitri Ivanovsky reported tobacco mosaic virus infection of plants, while animal infection with foot-and-mouth disease virus was described by Friedrich Loeffler and Paul Frosch. Around the same time, Carlos Finlay reported the first virus shown to cause disease in humans, yellow fever virus, and found that it was transmitted to humans by mosquitoes[1]. Together, these findings laid the foundations for contemporary virology.

But what evidence is required to identify a virus as the cause of disease? Unlike bacteria and fungi, viruses do not fulfil Henle–Koch postulates as aetiologies for disease because they cannot be grown in pure culture[2]. Revised guidelines for viruses include epidemiological, immunological and nucleic acid sequence evidence[2] with an emphasis on the need for multiple and coherent lines of evidence to support viruses as causative agents in disease.

Recent efforts using sequencing approaches to study the viral ecology of emerging viruses suggest greater diversity of viral families with well-known pathogens than previously thought, for example, of *Filoviridae*, *Paramyxoviridae* and *Coronaviridae*. However, often sequences remain the only available information and it is unclear whether these viruses can cause disease. Nonetheless, the potential of Bombali virus, myotis bat morbillivirus, and a bat sarbecovirus to cause disease in humans, all initially detected using sequencing surveys, have been studied in the lab. For example, using in vitro infection assays in human cell lines, in different animal models, or via virus neutralization assays with serum from animals that are immune to related viruses[3–5]. These represent classical methods in virology and can be used to build lines of evidence to support viral aetiologies.

Other methodological advances have provided additional avenues for studying viral infections and thus have advanced our understanding of viral aetiologies. In this issue, Emily Speranza reviews how sequencing techniques and multi-omics approaches enable viral infection to be studied in the context of a microenvironment, shedding light on the roles of immune cells, bystander cells and the extracellular matrix. For example, the ability to distinguish host cells from those that only engulf viral components without being infected per se such as macrophages has been a challenge during efforts to study viral reservoirs and tropism on a molecular level. This can be addressed with methods such as inCITE-seq, which combines detection of viral proteins with intracellular RNA facilitating detection of virus replication on a single-cell level and thus defining cellular tropism. Speranza also highlights advances using spatial proteomics and multiplex immunohistochemistry (for example, IBEX) that allow viral infections of tissues and organs to be studied in situ. These methods can give a more holistic picture of how a virus causes complex disease phenotypes, therefore providing support for viral aetiologies.

The link from virus to disease is not always straightforward. Several viruses can infect multiple organ systems triggering a diverse spectrum of symptoms, some of which can present long after the initial infection and vary across patients. For example, infection with herpes simplex viruses (HSVs) can result in severe encephalitis in some patients, and, despite treatment with the antiviral acyclovir, they can continue to suffer from neurological sequelae. A recent paper by Rybak-Wolf et al. modelled this scenario in brain organoids to better understand the drivers on a molecular level. They applied single-cell sequencing, electrophysiology and imaging methods to show that prolonged inflammation results in neurological sequelae despite antiviral treatment. Their findings suggest that treatment with anti-inflammatory drugs can alleviate post-HSV sequalae[6]. In this case, while HSV infection is the trigger, prolonged inflammation is the cause of symptoms. This highlights the difficulties in unpicking the role of viruses and other factors in disease.

Furthermore, research groups have used multi-omics approaches and large cohorts to study how virus infection can drive long-term symptoms, such as those reported for long COVID. These symptoms are similar to those experienced in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). ME/CFS has been tentatively connected to infection with Epstein–Barr virus but a solid evidence base is lacking. Neurological symptoms such as memory loss, impaired concentration and fatigue have been reported both in patients with ME/CFS and patients with long COVID[7]. Symptoms vary across patients and so determining the role of a virus in these aetiologies is difficult. In a longitudinal study with a cohort of 309 patients, Su et al. used a deep multi-omics approach to reveal an association between SARS-CoV-2 RNA levels, Epstein–Barr virus viraemia, and specific auto-antibodies with risk for developing long COVID[8].

This extensive analysis indicates that several variables are relevant for long COVID development and so disentangling the specific role of a virus in long-term disease is extremely complex, but large cohort studies can be helpful. Comorbidities, infection history and genetic predisposition can also complicate analyses. Therefore, multi-centre collaborations are needed to enable well-documented, large, longitudinal cohort studies. Ideally, these large cohorts would include a diverse set of participants (considering race and/or ethnicity, geographical location, sex, age, pregnancy status, socioeconomic status) in order to unravel the complexities of virus-induced diseases, such as long COVID and ME/CFS.

Pathogenic viruses are a serious public health concern and so our ability to link pathogens and disease is becoming increasingly

important. However, studying the role of viruses in disease can be complex. Research focusing on immunological, metabolic or neurological outcomes of virus infection will help to disentangle these layers, and emerging methods and models will aid our understanding from the single cell to the whole organism. We look forward to highlighting exciting and intriguing research that connects the dots from virus infection to disease development.

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