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Editorial overview: Viral pathogenesis: New technologies to advance research in human viral pathogenesis

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Viruses have and will continue to play a major role in the health and well-being of virtually all animal species including humans. Progress in the treatment and prevention of virus-associated disease has been based on critical basic and translational scientific discoveries from the advent of Jenner's seminal discovery of the benefits of smallpox vaccination to recent advances in antiviral treatments for human immunodeficiency virus (HIV) and hepatitis C virus (HCV), more effective vaccination to prevent varicella zoster virus reactivation and shingles, and highly promising approaches to novel vaccines for dengue, influenza, Ebola, Zika and respiratory syncytial viruses. In all cases, one of the most important forces propelling these discoveries has been the continued development of new technologies and tools that enhance our ability to interrogate viral pathogenesis and immunity at increasingly more precise levels of accuracy and detail. Some examples of the early enabling research technologies that informed past discoveries include the use of animal models to study viral disease mechanisms, the advent of cell culture and plaque assays to quantify and purify viruses, the development of an ever-increasing number of immune assays including hemagglutination inhibition, complement fixation, gel diffusion, neutralization, solid phase immunoassays and, in the last 30–40 years, a wide variety of increasingly sophisticated virus-specific T cell-based assays. In addition, the molecular biology revolution provided investigators with an ever increasingly large toolbox to examine, at the mechanistic level, the basis for the pathogenic nature of various viral families and the targets on those viruses for prevention and attack by the immune system.

In recent years a variety of powerful new technological advances and approaches have appeared that serve to accelerate our ability to investigate in even greater detail the basis of and the means by which we can modify viral disease progression and host immunity. In this brief collection of review articles be have focused on several of these exciting new technologies, realizing of course, that we are not covering the 'waterfront'. Several important areas of technological advance are not covered here such as new approaches to enhance single cell identification and functional analysis including single cell mass cytometry. With this technology, antibodies are labelled with specific rare elements rather than with fluorophores. The labelled cells are then detected using a time-of-flight mass spectrometer rather than by laser-detected light emission. This technology has substantially increased the number of molecules or other antigenic structures that can be detected on a single cell because of the elimination of spectral overlap that is generally encountered when using multiple fluorophores. This technology has been applied broadly in the last five years in a variety of ways to study

viral pathogenesis and immunity [1]. Another recently introduced technology that we have not covered in this set of reviews focuses on novel high throughput ways to isolate individual human B cells (most often antibody secreting B cells) specific for a particular pathogen. Multiple approaches have been designed to isolate such B cells, and used perhaps most productively in the search for broadly neutralizing human antibodies to HIV and antibodies that neutralize multiple drift strains of influenza virus or even both H1 and H3 viruses [2,3].

There are several other highly valuable new technologies about which the reader should be aware and that are not covered in this set of reviews. One is the use of a variety of new deep sequencing techniques to discover viruses that had not been previously detected using more limited specific technologies such as cell culture, virus-specific PCR, immune electron microscopy and serology. Deep sequencing has the advantage of being unbiased in its ability to detect any nucleic acid-containing organism, known or unknown. These technologies played a critical role in the detection of the SARS coronavirus and other viral pathogens [4,5].

Reviews in this issue cover new technologies for understanding interactions between viruses and their hosts ranging from single cell transcriptomic analysis and genetic screens to organoid culture, development of humanized mice and longitudinal analysis of human subjects. Each has its own challenges and advantages for answering fundamental questions regarding the pathogenesis of viral infections that will enable not only a better understanding of viral disease mechanisms, but also development of effective interventions.

Cristinelli and Ciuffi review approaches to obtaining reliable and interpretable data from single cell RNA sequencing (scRNA-Seq). Techniques for cell enrichment and isolation, amplification and tagging of small amounts of cellular RNA, as well as the bioinformatics necessary for data analysis are being rapidly developed and improved. Used in conjunction with phenotypic analysis of the cells scRNA-Seq has demonstrated heterogeneity of infected cells and allowed detailed characterization of the innate and adaptive immune responses to an increasing number of infections.

Another approach to understanding virus infection at a cellular level is identification of host factors that affect virus replication either because they are necessary for the virus life cycle or because they inhibit virus replication. A variety of approaches to screening for these factors, such as mutagenesis and RNA interference, have been used for genetic screening with success, but the recent development of CRISPR/Cas9 technology has facilitated this analysis with improved versatility and fidelity. McDougall *et al.* review the application of libraries of guide RNAs (gRNAs) that can target the genes of any host cell by creating double strand DNA breaks that lead to mutations and a permanent null phenotype. Host factors required for replication of lytic viruses have been identified through enrichment or dropout of gRNAs in surviving cells. Several programs for analysis are available and have been applied to identify the role of ER proteins in flavivirus replication, receptors for murine norovirus and effectors of transformation for Epstein-Barr virus.

The study of the pathogenesis of viruses that infect humans and are without good animal model systems has been difficult. Three approaches to this problem are represented in this volume: human organoid cultures, humanized mice and patient cohorts. Ramani *et al.* review the rapidly developing field of organoid cultures. These multicellular, physiologically active, self-organizing cultures representing multiple organs can be derived from embryonic and pluripotent stem cells and stem cells isolated from biopsies and surgical specimens. The technology has been particularly useful for investigation of human viruses that infect the gastrointestinal tract and has resulted in expanded identification of target cells, virus strain and host variation and the role(s) of host factors such as interferon. Likewise, brain and lung organoids have provided new insights into cellular susceptibility and responses to Zika virus and RSV. The technology continues to improve with addition of new cell populations and better understanding of the effects of maturation.

An additional approach to developing an animal model for human virus infections has been the long history of progress toward a humanized mouse that would be susceptible to infection and develop an appropriate immune response to infection. Douam and Ploss review the current status of this effort that has been driven by the need for animal models to test treatments and vaccines. Approaches have included transgenic expression of human virus receptors and transplantation of human immune system (HIS) components into immune compromised mice expressing factors that promote human hematopoiesis. Investigations using such mice have identified new aspects of virus-host interactions after infection with HIV, flaviviruses and hepatitis B, C, D, and E viruses. Challenges include generation of physiological relationships between cell populations and overcoming the host-specific effects of many soluble factors.

Lastly, Katzelnick and Harris review the advantages of establishing investigations of longitudinal cohorts of people at risk for acquiring particular virus infections of interest. As illustrated for their studies of dengue virus infections, ongoing evaluation of carefully constructed and well-characterized cohorts enable identification of risk factors, outcome variables, efficacy of interventions and accuracy of diagnostics.

References

1. Sen N, Mukherjee G, Arvin AM: **Single cell mass cytometry reveals remodeling of human T cell phenotypes by varicella zoster virus**. *Methods* 2015, **90**:85-94.
2. McCoy LE, Burton DR: **Identification and specificity of broadly neutralizing antibodies against HIV**. *Immunol Rev* 2017, **275**: 11-20.
3. Neu KE, Henry Dunand CJ, Wilson PC: **Heads, stalks and everything else: how can antibodies eradicate influenza as a human disease?** *Curr Opin Immunol* 2016, **42**:48-55.
4. Skewes-Cox P, Sharpton TJ, Pollard KS, DeRisi JL: **Profile hidden Markov models for the detection of viruses within metagenomic sequence data**. *PLoS ONE* 2014, **9**:e105067.
5. Kennedy PGE, Quan PL, Lipkin WI: **Viral encephalitis of unknown cause: current perspective and recent advances**. *Viruses* 2017, **9**:e138.