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Perspective

Virology—The next fifty years

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SUMMARY

Virology has made enormous advances in the last 50 years but has never faced such scrutiny as it does today. Herein, we outline some of the major advances made in virology during this period, particularly in light of the COVID-19 pandemic, and suggest some areas that may be of research importance in the next 50 years. We focus on several linked themes: cataloging the genomic and phenotypic diversity of the virosphere; understanding disease emergence; future directions in viral disease therapies, vaccines, and interventions; host-virus interactions; the role of viruses in chronic diseases; and viruses as tools for cell biology. We highlight the challenges that virology will face moving forward—not just the scientific and technical but also the social and political. Although there are inherent limitations in trying to outline the virology of the future, we hope this article will help inspire the next generation of virologists.

INTRODUCTION

When *Cell* was launched in January 1974 as a journal of "exciting biology" (Lewin, 1974[1]), the first issue contained two papers on murine leukemia viruses.[2,3] Reflecting the interest of the time in viruses as oncogenic agents, the collection of papers from the second month of publication included a book review on "The Molecular Biology of Tumour Viruses."[4] Over the next 50 years, *Cell* built a home for some of the most impactful research in virology. Many landmark studies have been published, such as those revealing the mechanisms by which retroviruses cause tumorigenesis[5] and the replication of hepatitis B virus via a reverse transcriptase.[6] The happy marriage of *Cell* and virology continued during the coronavirus disease 2019 (COVID-19) pandemic with, for example, the publication of research revealing the roles played by the angiotensin-converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2) receptors of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[7] the identification of the first mutation in the viral genome (D614G in the Spike protein) that dramatically increased virus fitness,[8] and of widespread convergent genomic and phenotypic viral evolution to overcome human immune responses.[9]

Taking inspiration from the 50-year relationship between *Cell* and virology, we outline some of the research questions and themes that we believe may be important in the next 50 years. Of course, it is remarkably hard—perhaps even foolhardy—to discuss the future of an entire discipline during a time of rapid technological change and unrestrained data generation. What follows is therefore by necessity a personal perspective on one possible scientific future.

Even with myriad uncertainties, it is clear that the virology of the future will be characterized by abundant data. One of the challenges facing biomedicine as a whole will be how handle the enormous amounts of data that will continuously flow into our computer terminals. We expect to witness the automation of routine virological tasks using artificial intelligence (AI), hopefully assisted by quantum computers. We also expect the ongoing revolution in protein structure prediction resulting from the development of Alphafold[10] and similar computer technologies to have an ever-greater impact.

Because there are many possible topics to cover, we will focus on what we regard as some of the grand opportunities and challenges facing virology in the coming decades.

CATALOGING THE VIROSPHERE

Metagenomics has ushered virology into a new discovery phase: a massive expansion in descriptions of the scale and diversity of the virosphere, with sequencing performed in diverse and extreme habits, and a move away from only studying viruses associated with overt disease or that directly impact humans.[11–13] Yet we still know less about the biodiversity of viruses than any other group. Although there remain animal taxa to identify, as well as a multitude of bacteria, these numbers pale compared with our miniscule sampling of the virosphere. While

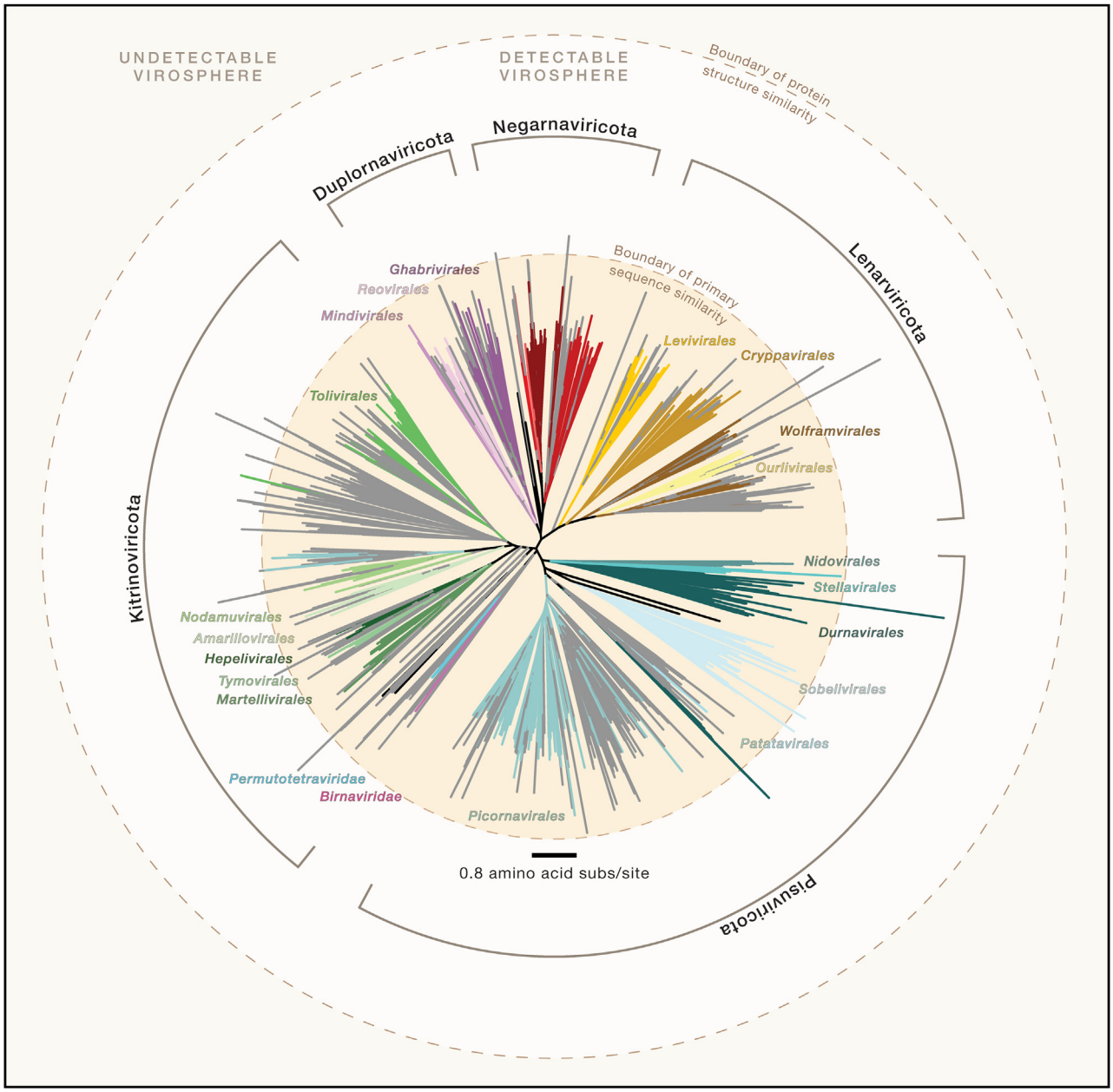

Figure 1. The scale of the RNA virosphere

The inner (unrooted) phylogeny depicts the current large-scale classification of RNA viruses, with the five major phyla marked on the outer rim. Some individual families and orders of RNA viruses are also indicated. Note that because of the enormous genetic distances involved and inherent alignment uncertainties, this phylogeny should not be considered an accurate representation of evolutionary history. The hypothetical outer limit of virus detection using current computational methods based on primary sequence similarity only (e.g., from metagenomics) is marked by the dashed yellow circle. The analysis of patterns of protein structural conservation may extend the detectable virosphere to the outer dashed circle. Beyond that, viruses may be undetectable using any sequence-based approach. Phylogeny adapted from Charon et al.,[14] through Creative Commons, with branch lengths scaled to the number of amino acid substitutions per site (scale bar of 0.8 amino acid substitutions/site).

estimates are little more than educated guesses, we have clearly sequenced less—and likely far less—than 0.1% of all viruses, have formally classified only a subset of these, and biologically characterized an even smaller number.

A core task for the virology of the future will therefore be to understand the size, diversity, and structure of the virosphere (Figure 1). Not only is it important to determine how many viruses there are and what proportion might cause disease, but it is imperative to understand the factors that shape global virus diversity, why some virus groups (such as the *Narnaviridae* and the *Picornavirales*) seem to be more speciose than others, and why some hosts seemingly harbor more viruses than

others. It is also important that many of these viruses are characterized phenotypically. A new synthesis of genotypic and phenotypic approaches should be a goal for the virology of the future.

The study of protein structures, including those predicted by computational approaches, will be central to our future cataloging of the virosphere, leading a trail to the discovery of ever-more diverse and divergent viruses (Figure 1). Obtaining reliable structures for as many viral proteins as possible provides anchor points to explore more of the virosphere. More challenging is estimating phylogenetic trees from structural data, although this will revolutionize attempts to resolve the deep evolutionary history of viruses. Real progress will require models of protein structure evolution that incorporate both the probability of each type of amino acid substitution within a sequence and of how these amino acids interact with each other in a structural context, resulting in a hugely complex evolutionary space.

The grandest challenge will be to determine the core virome of every species on earth, in a similar vein to projects that aim to sequence the genomes of eukaryotic taxa (for example, https://vertebrategenomesproject.org/).[15] No species has had its virome composition determined across its entire geographic range. Cataloging the virosphere will enable us to address many fundamental questions: what is the range of genome sizes in both RNA and DNA viruses? How small can an RNA virus be and still be considered autonomous? Do types of virus exist that have not yet been described? Are there virus-like particles other than viroids? How large and complex can a virus be to still be considered a virus? What is the range of genome structures and replication strategies in viruses, and what factors determine this range? With these data in hand and with improvements in phylogenetic analysis, it may also be possible to reconstruct the evolutionary history of all DNA and RNA viruses, although this raises questions about whether current virus classification schemes are robust to the huge diversity of the virosphere.

REASSESSING VIRUS EVOLUTION AND ECOLOGY

Despite the revolution in virus discovery, important gaps remain in our understanding of virus evolution and ecology. Advances in this area require us to move beyond simple genomic and phylogenetic descriptions of virus diversity. Many of the major questions in virus evolution can only be addressed by virological sampling directed toward a testable hypothesis. These questions include: What are the long-term macroevolutionary trends in viruses (i.e., the processes that lead to the generation of viral species, genera, and families)? Is there a directionality to RNA virus evolution, such as from simple to complex and/or from bigger to smaller? As there have been major transitions in host evolution, such as the evolution of multicellularity, sexual reproduction, and vertebrates, have there been equivalent evolutionary transitions in virus evolution? Do major events in virus evolution reflect the type of host they infect, with differences between the viruses that infect bacteria, plants, invertebrates, and vertebrates? What factors drive genome evolution in viruses?

The science of genomic epidemiology, in which virus genome sequences are used to track the spread of viruses through populations, has blossomed. SARS-CoV-2 genome sequence data has empowered global disease surveillance, enabling the rapid identification of variants of concern/interest and providing a detailed picture of the patterns and dynamics of virus spread through particular localities.[16,17] However, gaps and inequities in global genomic surveillance need to be filled if genomic epidemiology is to become an effective tool on a global scale.[18] The genomic epidemiology of the future will also rely on accurate estimates of key parameters for viruses of interest: (1) evolutionary rates, (2) divergence times, (3) patterns of spatial spread through populations (i.e., phylogeography) and whether there are commonalities to evolutionary and epidemiological patterns for particular types of virus, and (4) population demographics (i.e., rates of population growth and decline). Similarly, at the experimental level, we need precise estimates of other cornerstone parameters[19]: (1) the mutation rate per genome replication (including the rate at which both advantageous and deleterious mutations arise), (2) the fitness effects (i.e., selection coefficients) and potential phenotypic consequences of every mutation in a virus genome (potentially in every host and every cell type), (3) the rate of recombination per replication, (4) the population growth/turnover rate of viruses within individual hosts, and, as a measure of evolutionary interactions, (5) the strength and sign of epistasis, one of the most challenging of all parameters to estimate.[20] Together, when combined with information on immune evasion, these data may give virus evolution some predictability, such that when a new virus emerges, it may be possible to forecast aspects of its spread through populations, in turn informing intervention strategies including optimal vaccine deployment.

Increases in the scale and capacity of single-cell sequencing and multi-omic approaches[21] will enable the in-depth examination of the patterns and dynamics of virus evolution and host interactions during individual infections. A complete characterization of intra-host-virus genetic diversity will be plausible in the near future, including within individual cells and tissues, providing key data on how viruses spatially diffuse within the body and how viruses and other microbes interact. By describing the full range of virus-host evolutionary interactions, we will better understand the evolution of such vexing traits as virulence. Anticipating the trajectory of virulence evolution (i.e., whether it will increase, decrease, or remain the same through time) is of importance when a new virus emerges,[22,23] generating actionable information on how best to undertake population-scale interventions.

A grand challenge in evolutionary virology will be to determine the origin of every human virus (or of any other host species of interest). By revealing evolutionary ancestries, we will be able to determine the most likely pathways by which viruses jump species boundaries and the key mutations involved. Exact evolutionary ancestries are only known for a handful of viruses, with the human immunodeficiency virus 1 (HIV-1) a high-profile example.[24] While strong hypotheses can be drawn in other cases, such as the ultimate origin of SARS-CoV and SARS-CoV-2 in *Rhinolophus* bats,[25] there remains an evolutionary gap of several years that could encompass a variety of mammalian species.[26] In other instances, such as variola virus (the agent of smallpox) and hepatitis C virus (HCV), there is no clear picture of virus origins. Some insights, particularly on the timescale of

virus evolution, may come from the analysis of "ancient" DNA or RNA. Advances in genomic sequencing have made it possible to trace the ancestry of DNA pathogens such as variola virus and hepatitis B virus back over several millennia, revealing their spread through Eurasia.[27,28] Because of their more rapid degradation, the analysis of ancient RNA viruses is more troublesome, with the oldest human viruses sequenced only dating to the early 20th century[29,30] and the genomic signature of some plant viruses spanning 700 years.[31] The ability to sequence ancient RNA viruses would revolutionize the study of virus evolution. Alternatively, extracting antibody preparations from bones or teeth[32] or the sequencing of B cells from well-preserved specimens (e.g., Ötzi the Iceman) could reveal which types of viruses were circulating in historic and prehistoric times.

A similar revolution is needed in virus ecology. As most viruses in most host species do not cause disease, a founding principle should be to consider viruses as players in inter-connected ecosystems rather than simply as disease-causing and emerging pathogens.[33] This re-tuning will place humans as just another host species connected to many others, rather than sitting at the end of a great chain of emergence. A core task will be revealing how viruses move through ecosystems, perhaps by sampling all the viruses within all hosts in a specific ecosystem and/or food web and determining which are shared among hosts and the barriers to cross-species transmission. It will also be important to sample the same ecosystem over multiple time points: this will reveal the changing patterns of virus presence/absence, of virus diversity, and of inter-virus and inter-microbial interactions over time and provide new insights into what drives virus seasonality.[34]

THE ORIGINS OF VIRUSES

Despite the giant strides made in virology over the last 50 years, when it comes to understanding how and when viruses originated, we are still largely in the dark. The challenge, of course, is that viruses leave no fossil record, and their genome sequences are often so divergent that they often cannot be reliably aligned, let alone infer phylogenetic relationships.[14]

There remain two main hypotheses for the origin of viruses: (1) that they are direct evolutionary descendants of ancient replicators with, for example, RNA viruses representing extant members of a lineage that arose in a primordial pre-cellular RNA world, or (2) that viruses are more recently evolved (that is, after the origin of cellular life) "escaped genes" that have their ancestry as mRNA molecules in cellular host organisms that acquired a protective capsid and the ability to replicate autonomously.[35] Determining which of these theories is correct would provide information central to understanding the origin of life itself.

The fragmentary data we have points toward an ancient, pre-cellular virus origin. Homologous protein structures that are more conserved through deep time than primary sequences are found both within and among highly divergent RNA and DNA viruses, suggesting a deep common ancestry.[36] By contrast, there is no strong evidence that any group of viruses have their ancestry as escaped host genes or as "regressed" cellular organisms. Even giant and complex DNA viruses from the order *Megavirales*, such as the *Mimiviridae*, which are often said to blur the distinction between bacteria and viruses, contain genes that clearly link their evolutionary history to that of other DNA viruses.[37] Similarly, although it was once thought that deltaviruses, such as human hepatitis D virus, originated as escaped intron sequences, more recent metagenomic studies have identified related viruses in a range of animal taxa indicative of an ancient viral ancestry.[38,39,40]

Yet there is currently no direct evolutionary link between extant viruses and the hypothetical pre-cellular world, and RNA viruses have not been detected in some assuredly ancient taxa. Of most note is the lack of bona fide RNA viruses in the domain *Archaea*.[41,42] Does this mean that RNA viruses never existed in archaea, as expected if they are more recently involved escaped genes, or are they unable to survive in the hypersaline or hyperthermal environments that are commonly home to archaea? Could archaeal viruses be so divergent in genome sequence that they are effectively invisible to the sequence similarity detection methods that are intrinsic to metagenomics? The analyses of protein structures may help answer these questions.

A better understanding of the earliest events in virus evolution will shed important new light on other intriguing questions. What did the primordial RNA and DNA viruses look like? What are the oldest lineages of RNA and DNA viruses currently circulating? And what role, if any, did viruses play in the origin of variant (i.e., non-canonical) genetic codes?

UNDERSTANDING DISEASE EMERGENCE

The nexus for infectious disease emergence in humans is the human-animal interface, manifest in such activities as animal hunting and farming for food or fur, the wildlife trade, live animal markets, deforestation, and in those populations that live or work near wildlife habitats such as bat roosts. Enhanced surveillance at the human-animal interface is therefore perhaps the single most effective step to prevent future pandemics.[43] Equivalent interfaces exist for animals and plants of social or economic importance. The COVID-19 pandemic has generated much talk about the need for a global "pandemic radar" to help prevent future outbreaks.[43] Although the science behind such next-generation virus surveillance is relatively simple—likely involving a combination of high-throughput metagenomic sequencing and targeted serological screening[44] in settings like the wildlife trade and live animal markets—a greater challenge is overcoming the complex geopolitical issues that have assuredly worsened during the COVID-19 pandemic. Ultimately, it should be possible (if necessary) to sequence a virus genome from every infected patient (or other hosts) in real time. But to achieve this, it will be critical to roll out genomic sequencing technologies in low- and middle-income countries, overcoming political, legal, and regulatory hurdles on the way. This, in turn, requires us to build essential virological, genomics, and computing infrastructure in these localities and provide relevant training, hence requiring considerable financial investment from wealthier nations.[18]

As well as better and more targeted surveillance, major research questions need to be addressed to understand the drivers of disease emergence. Most debated is whether it is possible to predict disease emergence events before they

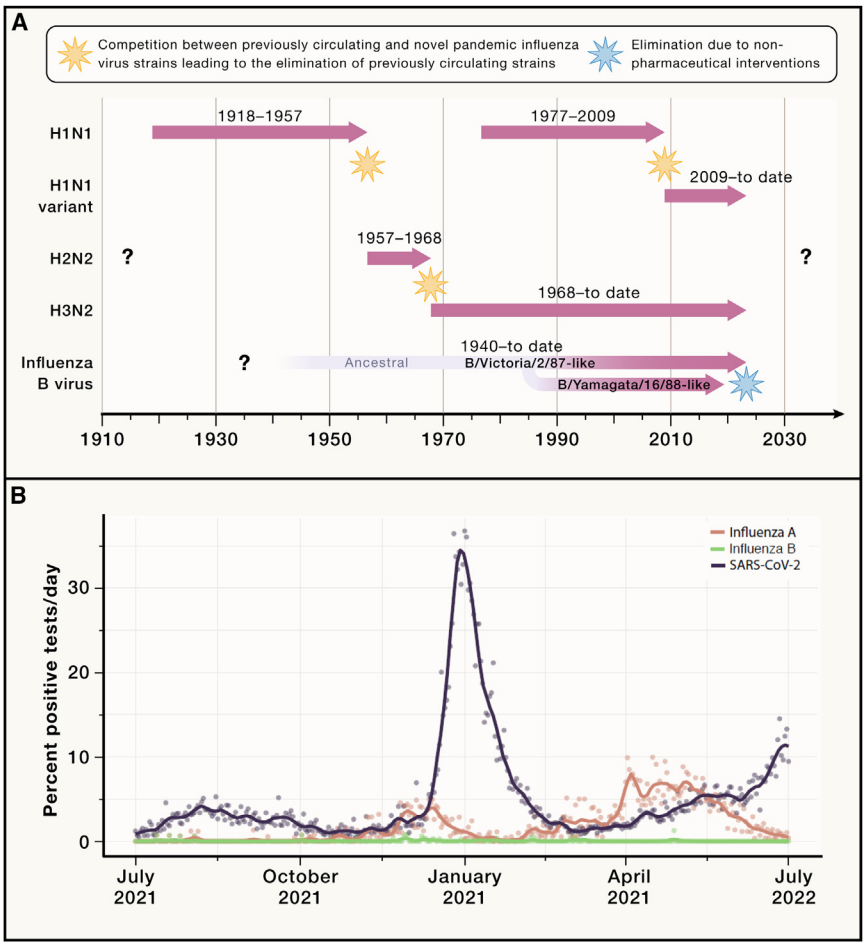

Figure 2. Competition between related and unrelated viruses in humans

(A) Circulation of influenza viruses since 1918. The H1N1 subtype of influenza A virus was present in the human population from 1918 to 1957. In 1957, a different influenza A virus subtype, H2N2, caused a pandemic and outcompeted H1N1 within a short time period, leading to its elimination from the human population. In 1968, another influenza A subtype, H3N2, caused a pandemic leading to the elimination of H2N2. The H1N1 subtype returned in 1977 and circulated until 2009 when a novel swine-origin H1N1 virus caused a pandemic and eliminated seasonal H1N1. The competition between these viruses was likely due to increased serological cross-reactivity leading to elimination of the older, likely less fit virus. Of note, H1N1 and H3N2 are serologically distinct (i.e., the HA and NA come from different phylogenetic groups), which likely allows their co-circulation. Influenza B viruses evolved from an ancestral lineage into the B/Victoria/2/87-like lineage in the mid-1970s and the B/Yamagata/16/88-like lineage in the mid-to-late 1980s. The Yamagata lineage was eliminated in 2020 likely due to non-pharmaceutical interventions imposed during the SARS-CoV-2 pandemic.

(B) Data (% positive tests per day) from a large healthcare system in New York City, USA, suggesting competition between unrelated viruses. In the late autumn of 2021, an increase of H3N2 activity was detected. However, at approximately the same time, the early Omicron variant BA.1 of SARS-CoV-2 started to cause a massive wave of infections in New York City, during which time influenza A virus activity seemed suppressed. With the decline of Omicron BA.1, influenza influenza A virus activity started to rise again. Courtesy of the Mount Sinai Pathogen Surveillance Program.

occur.[45,46] Although there is growing data showing certain host groups, virus types, and perhaps geographical locations are most likely to harbor emerging viruses,[46,47] this does not comprise a predictable, actionable science. In addition, the reasons why some viruses are better to jump species boundaries than others, or why certain host groups such as bats carry and tolerate more viruses than others, are currently opaque and need directed research despite recent progress.[48] At the very least, we require knowledge of the species range of each group of viruses and key aspects of their cellular interactions, such as receptor usage.

THE IMPACT OF CLIMATE CHANGE ON VIRUS (RE-) EMERGENCE

Climate scientists have warned for many years about human-driven increases in global temperatures.[49] As well as causing many general issues, this is a particular concern for viruses transmitted by arthropod vectors. Climate change is allowing vectors species, including mosquitoes, ticks, and their viruses, to extend their range, often from warmer to historically cooler regions.[50,51] This has been observed in Europe in recent years where the Asian tiger mosquito (*Aedes albopictus*)—a vector for many flaviviruses and alphaviruses—has spread north of the Alps, with a similar northward spread observed in *Hyalomma* ticks that transmit the bunyavirus Crimean-Congo hemorrhagic fever virus (CCHFV). This has led to outbreaks of dengue virus infections in Spain, Italy, and France in individuals with no travel history to dengue endemic areas.[52] It has also been suggested that due to possible impacts on virus seasonality, transmission, and human behavior, climate change will increase the burden of human respiratory infections.[53] Understanding the full impact that climate change has on virus (re-)emergence will become a focus of transdisciplinary research through collaborations of experts in virology, vector biology, epidemiology, and climate research.

VIRAL CO-CIRCULATION, INTERFERENCE, AND COMPETITION

We are only beginning to learn about how viruses interact with each other. There have been four influenza pandemics over the past ~100 years (Figure 2). In 1918 a subtype H1N1 virus jumped into humans, resulting in the largest reported virus pandemic. In 1957, a novel H2N2 subtype crossed the species barrier, resulting in another pandemic. Within a short time period, H2N2 outcompeted H1N1, which then disappeared from the population. In

1968, H2N2 was itself displaced by the H3N2 subtype. In 1977, H1N1 returned,[54] likely from a vaccine/challenge experiment,[55] and co-circulated with H3N2 until 2009, when another H1N1 strain jumped into humans from pigs, eradicating seasonal H1N1.[56]

The factors underpinning these subtype replacement events are not well understood, although changing fitness landscapes after adaptation to humans and the induction of cross-reactive antibodies by the new virus are likely part of the explanation.[57] The SARS-CoV-2 pandemic greatly reduced the transmission of other respiratory infections during 2020 and 2021, including influenza (Figure 2). The likely cause was non-pharmaceutical interventions that imposed a much greater toll on viruses like seasonal influenza virus, which has a reproductive number (R_0) of only ~1.5, in contrast to early SARS-CoV-2 variants with R_0 values of 2–3. Like other respiratory viruses, influenza viruses experienced a major population bottleneck in 2020 and 2021, resulting in the disappearance of the B/Yamagata/16/88-like influenza B virus lineage (Figure 2).[58,59] Similarly, the emergence of the rapidly spreading Omicron variant of SARS-CoV-2 in late 2021 was associated with a reduction in H3N2 influenza activity, which returned after the first Omicron wave was over. Because widespread non-pharmaceutical interventions were limited at this time, it is likely that the two viruses directly interfered with each other. Since there is no expected cross-reactivity from adaptive immunity between influenza A virus and SARS-CoV-2, alternative mechanisms may have underpinned this competition. We do not understand the mechanisms involved in these viral competition dynamics: they may include innate immune responses, competition for susceptible individuals/substrate, changed behavior, and cross-reactive adaptive immunity in the case of closely related viruses. This is an important area for future research as competition can be sufficiently powerful to eliminate virus species or subtypes from the global population, such that understanding these phenomena should enable the development of better strategies for virus control, perhaps even eradicating some respiratory viruses completely. If these viruses do not have an extensive animal reservoir, as is the case for influenza B virus, they may no longer pose an epidemic threat.

DETERMINING VULNERABILITY TO VIRAL INFECTION

There is a large heterogeneity in the human population in the susceptibility to virus infection: some people exposed to a virus become infected while others do not. In many cases, resistance or susceptibility is temporary, and an individual who was not susceptible at a certain time point may be highly susceptible later. Similar observations have been made for disease severity. Approximately 70% of the world's population is affected with herpes simplex virus 1 (HSV-1), and infected individuals may suffer cold sores 3–4 times over a year. However, others may never experience a cold sore, and asymptomatic shedding of HSV-1 and herpes simplex virus 2 (HSV-2) appears to be the rule.[60,61] Many factors play a role in susceptibility, including age, sex, health of the innate and adaptive immune system, the host microbiome, and viral genetics (Figure 3), although the contribution of these individual factors or some assortment of them is often unclear. Socioeconomic factors, health inequities and disparities, stress, and community issues may similarly feed into susceptibility and disease severity. Determining patterns of susceptibility or resistance when exposed to the same virus dose is clearly a major topic for future virology. The lessons learned could lead to better countermeasures including vaccines, as well as therapeutics that act on the innate immune system, or easily implemented behavioral changes. Well-controlled multidisciplinary longitudinal studies in settings that allow all appropriate parameters to be determined are of major importance. This also applies to heterogenic responses to vaccines and heterogeneity in vaccine-induced protection.

VIRAL INFECTION AND CHRONIC DISEASE

While many virus infections are self-limiting and cleared within days following acute infection, some viruses persist for months, years, or even for the lifetime of the host, often in the absence of overt disease. Host health, the magnitude and speed of infection, and the immune response to infection are major determinants of disease outcomes of viral infection (Figure 3). Chronic disease is more typically associated with, but not limited to, viruses that persist. It is estimated that the average human harbors 8–12 persistent infections.[62] Persistent infections establish metastable but dynamic relationships with their host, ranging from continuous low-level replication to latent infection marked by episodic reactivation. Persistent viral infections may result in chronic diseases that are episodic due to changes in health state or stresses or may result in disease or cancers that only afflict a proportion of those infected. Understanding the complex relationship of persistent viruses and their effects on the long-term health of those infected has given way to major strides in controlling or curing these infections. However, our understanding of the relationship between viruses that do not cause disease or that contribute to chronic pathology in only a small proportion of those infected, or only decades after initial infection, and host health remains in its infancy. Understanding the etiological role of viruses—either acute or persistent—in chronic disease represents a major challenge. In addition, metagenomics and AI (see below) will undoubtedly play torch-bearing roles in parsing the human virome, helping to reveal relationships between pathogens within a host and their roles in health and disease.

Viral strategies for long-term persistence result from a mastery of host biology acquired through co-evolution, and particularly their skill in evading host immune functions that would otherwise result in clearance. At the extreme, some viruses, such as herpesviruses, establish life-long latent infections, in which viral genomes are maintained in a reversibly quiescent state. For latency not to be a dead end, the virus must reactivate, re-initiating its replicative program for transmission to a new host. Viruses that establish latency have therefore evolved mechanisms to sense changes in the host environment or physiology, integrate signals, and respond with changes in viral gene expression and commitment to replication. The mechanisms by which viruses interface with and manipulate infected cells for viral persistence or latency remains a field rich in unanswered questions. The frequency and magnitude of asymptomatic reactivation events, their imprint on our biology, the immune responses that underpin control, and their contribution to chronic disease or other infections are central to understanding the relationship of viral persistence to

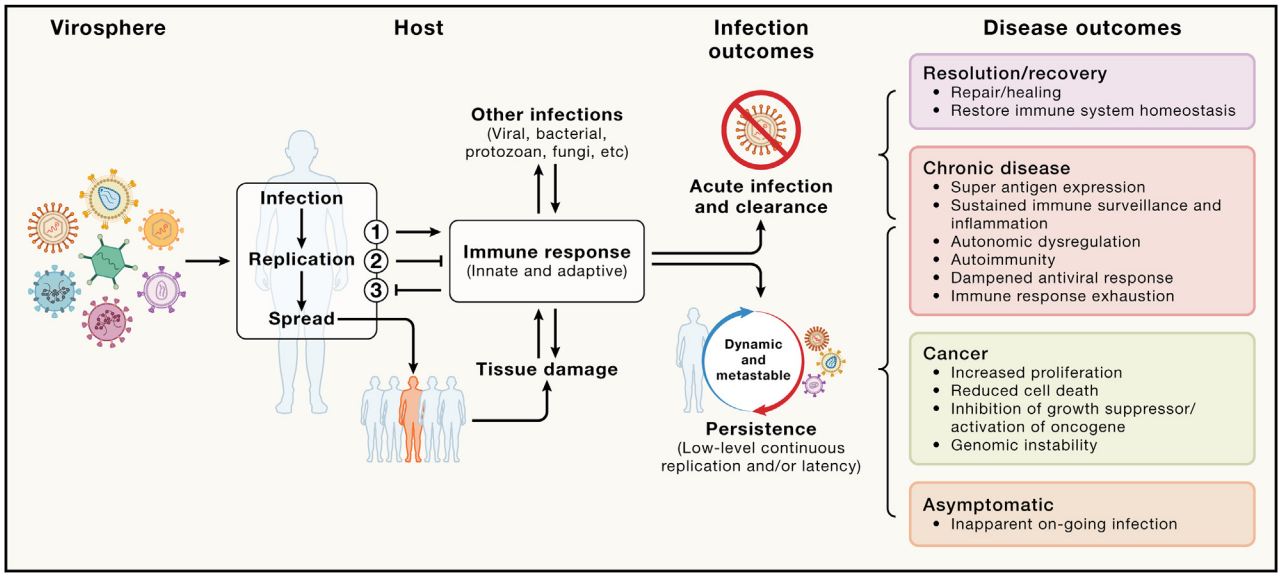

Figure 3. Patterns of virus infection and disease outcomes

Upon infection of a permissive host, virus replication will result in the production of viral progeny for spread to other cells/tissues within the host and to secondary hosts. The outcome of infection (acute infection and clearance versus persistence) and the associated disease outcomes are in large part determined by the balance between the nature and strength of the immune response, the modulation of host response by virus-encoded factors, and, in some cases, the emergence of escape variants. Replication products first stimulate innate and then adaptive immune responses (1, arrow), viral replication products in turn inhibit or manipulate host responses (2, bar) as immune responses attempt to limit infection (3, bar). Other infections (e.g., viral, bacterial, fungal, protozoan, etc.) impact infection and responses to infection in ways that are still poorly understood. The interplay between the infecting virus and the host will often result in clearance of the virus. While clearance will typically result in complete resolution and recovery, in rare cases, immune responses or other host biology altered by the infection may not fully resolve and can result in chronic disease, with chronic inflammation or autoimmunity as an underlying cause. However, some viruses (e.g., herpesviruses, HIV, hepatitis B and C viruses, papillomaviruses, and polyomaviruses) are capable of evading host defenses and are not cleared, establishing persistent or latent infections. In either case, a dynamic and metastable state is reached within the host. Viral persistence may result in chronic or episodic disease, driven by sustained immune response and inflammation, or immune exhaustion and the inability to control replication. Alterations in biology driven by some viral infections may result in transformation and oncogenesis. Alternatively, persistent viruses may result in an inapparent, asymptomatic infection in a healthy immune competent host.

human health. We are just beginning to understand the potential costs or possible benefits of viral persistence to human health in the absence of overt viral disease.

Reflecting their nature as foreign invaders, viruses stimulate inflammatory responses and as such may be co-factors or modulators in cancers and autoimmune disorders (Figure 3). Pinpointing their role in the etiology of chronic disease is challenging as viruses may trigger chronic disease but be inapparent by the time of disease onset (i.e., "hit and run"). Other viruses may be difficult to detect or endemic in the population where only a fraction of carriers develop chronic disease due to other genetic or environmental factors, including co-infections. Further, defining the key changes in the host inflammatory state and those driven by viral infection is a worthy challenge and key to understanding and controlling the etiological role of viruses in chronic diseases.

As up to 15% of all cancers may be caused by viruses,[63] advancing the prevention and control of many cancers requires understanding the viruses that initiate or modulate them. Most virus-driven cancers occur decades after the initial infection and result from the virus prolonging survival and proliferation of infected cells that go on to acquire additional mutations or functions that result in evasion of immune responses or aberrant differentiation programs. Epstein-Barr virus (EBV) particles detected in cancer cells of a pediatric Burkitt's lymphoma resulted in the discovery of the first human tumor virus in 1964.[64] EBV persistently infects >90% of adults worldwide and is a major cause of infectious mononucleosis, causing fatigue that may endure for 6 months and establishes a life-long infection, with cancer arising in only a small proportion of those infected and decades following the initial infection. Other cancers associated with EBV include Hodgkin's lymphoma, nasopharyngeal cancer, gastric cancers, and post-transplant lymphoproliferative disease. While EBV encodes multiple genes that drive the development of cancer through dysregulation of cellular proliferation and survival, EBV nuclear antigen-1 (EBNA-1) directly binds palindromic repeat sequences and induces chromosomal breaks that may impact expression of a tumor suppressor and a proto-oncogene.[65] Hence, latent viruses may directly induce genomic instability in addition to altering cellular growth control.

More than 50% of liver cancers are caused by viruses, with hepatitis B virus (HBV) and HCV responsible for 75% of hepatocellular carcinomas worldwide. While there is no cure, the HBV vaccine prevents infection and reduces the incidence of liver cancers. Direct-acting antivirals offer benefits in >90% of those infected with HCV, and a vaccine is currently being sought. Access to HCV antivirals remains a major challenge in reducing HCV disease globally, and long-term chronic complications, such as hepatic scarring, remain unexplored even in those on existing effective therapies. In another example, high-risk human papillomavirus (HPV) types are associated with 99% of cervical

cancers, as well as other anogenital and oropharyngeal cancers. HPV is the most commonly sexually transmitted virus, and the time of infection to clearance or control can be more than 6 months. HPV vaccines have reduced infections with HPV types that cause cancer and genital warts by >80%, and the percentage of HPV-associated cervical precancers has dropped by 40% among vaccinated women. Vaccinating boys for HPV lags that of girls but is important in preventing spread of HPV and the development of HPV-associated cancers.[66–68] Viruses have long been powerful tools in understanding mechanisms of cellular growth control and oncogenesis. Developing targeted, virus-specific treatments and vaccines that prevent virus-driven cancers is an important goal and will also drive the discovery of mechanisms of oncogenesis in general.

In addition to its role in cancers, EBV is a key trigger for multiple sclerosis (MS), increasing the risk by 32-fold and possibly more with more severe infectious mononucleosis.[69,70] EBV latency is unstable in individuals with MS, driving a proinflammatory state, high levels of EBV-specific antibodies, and pathogenic phenotype in memory B cells.[70] MS pathogenesis is linked to poor control of cross-reactive, EBV antigen-specific antibodies, while distinct cytotoxic natural killer (NK) cells driven by cytomegalovirus (CMV) infection are protective in people with high antibody levels.[71] This suggests an intriguing possible benefit of co-infection in preventing MS. The link between EBV and MS is a major advancement and yields multiple viable avenues toward improved treatment. An EBV vaccine is in clinical trials, providing hope for preventing EBV-related cancers as well as MS. Possible associations between EBV and other viruses with infection-associated complex chronic debilitating conditions, such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), long COVID, and others, need increased attention and causal confirmation.

While causative evidence in humans is difficult, many viral infections are associated with neurocognitive decline and disease, including Alzheimer's disease (AD). HSV-1 infection has been postulated to contribute to AD since the 1980s.[72] HSV-1 infection or chronic reactivation has been shown to induce the upregulation of expression, maturation, or activity of proteins associated with AD pathology, such as amyloid precursor protein (APP) cleavage, amyloid-beta (Aβ), and Tau. Dementia has also been associated with HIV-1 infection.[73,74] While anti-retroviral therapy (ART) has reduced severe and progressive neurocognitive disorders associated with HIV-1 infection, mild neurocognitive disorders persist even with ART. It is interesting to speculate that the frequency of reactivation events, asymptomatic or not, may impact neurodegeneration and the likelihood of developing AD. It will be important to define how different outcomes of viral brain infection, asymptomatic to mild versus encephalitis, are associated with the development of AD and the role of host genetic, immune, and metabolic profiles or co-infections in the development of AD following viral infection. The association of viral infection and AD offers the hope that early diagnosis of viral infection followed by antiviral treatment may stave off the development of chronic debilitative diseases, such as AD.

While research often focuses on viruses that cause disease, some virus or virus remnants make up large parts of our virome or genomes, and we have a poor understanding of how they affect health or disease. Anelloviruses, small (2–3.9 kb) single-stranded circular DNA viruses, are the most abundant component of the human virome and are exceptionally diverse human viruses.[62] Anellovirus genome sequences are commonly elevated in immunocompromised hosts,[75,76] yet we have little understanding of their potential impact on health due to the lack of systems to culture or propagate them for study. Similarly, remnants of ancient endogenous retroviruses (ERVs) carried through our evolution over millions of years comprise up to 8% of our genome.[77] Although ERVs typically cannot replicate to produce new infectious viruses, they are transcribed, producing superantigens that stimulate adaptive responses, stimulate interferon (IFN)-γ-inducible innate immune responses impacting biology and health.[62,78] ERVs play an active role in the biogenesis of the mammalian placentas and are controlled to some extent by the developing embryo.[79] While ERV activation is upregulated in many cancers, their role as a contributor to the initiation or progression of oncogenesis is controversial, although increased expression of ERVs may be associated with reduced survival in some cancers.[80] Immature ERVs, such as human ERV-K, likely entered our genome only thousands of years ago. ERV-K is restricted by apolipoprotein B mRNA-editing enzyme, catalytic polypeptide (APOBEC), but retains the ability to replicate and produce progeny and therefore may impact health in certain contexts.[81] Collectively, anelloviruses and ERVs demonstrate the vast unknown territory in the virosphere and the importance it likely holds for understanding human evolution, development, and the contribution of viruses to health and disease.

Finally, acute viral infections or short-term persistence may also result in chronic pathologies. Post-acute sequelae of SARS-CoV-2 infection (PASC; "long COVID") in which one or more COVID-19 symptoms persist, including muscle pain, pain when breathing, fatigue, and neurological effects, affects 1 in 8 people infected. The study of human samples collected from healthy controls or from people who recovered from SARS-CoV-2 infection versus those with persisting symptoms identified decreases in serotonin (gut-derived neurotransmitter), which may explain symptoms associated with reduced hippocampal and vagus nerve activation.[82] Increases in nonconventional monocytes and activated B cells and decreases in type 1 conventional dendritic cells and central memory cells, as well as the stress hormone cortisol, have also been associated with long COVID.[83] SARS-CoV-2 has also been shown to impact mitochondria function in long COVID patients, which has implications for the production of energy in infected cells and can impact multiple organ systems.[84] A causative or interconnected role for changes in immune profiles, cortisol or serotonin levels, and mitochondrial function still needs to be definitively shown, but these are promising early findings for what has been an intractable lingering consequence of the pandemic. Vaccination has been shown to reduce the incidence of PASC, in part by limiting inflammation, providing hope that vaccines and antivirals will limit chronic pathology.[85,86]

Syndromes similar to long COVID have been described after infection with other viruses, including influenza virus (i.e., post-acute sequelae of non-persistent viral infections), coxsackie B virus, and chikungunya virus.[87] Systematic and longitudinal

studies for other viral infections, including those typically classified as ''common cold'' viruses, are needed to determine the extent to which different viruses contribute to chronic disease. It would be unsurprising if many acute virus infections could cause long-term sequelae at different rates and contribute to complex diseases such as diabetes mellitus and systemic lupus erythematosus. Another issue that requires future study is if the many respiratory infections that humans experience over a lifetime, which induce transient local and systemic inflammation, contribute to or cause degenerative diseases at older age, akin to EBV infections increasing the risk of MS. Understanding the spectrum of immune response to viral infections, how other aspects of host biology impact them, and mechanisms of persistence will be paramount to making inroads to defining viral etiologies and approaches to treatment of chronic disease.

CO-INFECTIONS: IMPLICATIONS OF VIROME INTERACTIONS

Virome interactions may also be an important disease determinant (Figures 2 and 3). The increasing importance of long COVID has led to active research into the role of co-infections in disease. It remains to be determined if persistent/latent viral infection increases susceptibility to long COVID or if SARS-CoV-2 infection drives reactivation of latent virus that compounds pathology or contributes to COVID-19 outcomes. Increased antibody responses to EBV measured in long COVID patients suggest that concordant reactivation of EBV with SARS-CoV-2 infection could contribute to chronic disease.[83] EBV reactivation was associated with fatigue, while HIV-1 infection was associated with neurocognitive dysfunction in long COVID. By contrast, prior CMV infection correlated with a reduced likelihood of neurocognitive symptoms.[88] However, CMV infection could potentially enhance infection with SARS-CoV-2 as it upregulates the ACE2 receptor, although it may also result in an immune environment less favorable to infection.[89] To understand how these inter-virus relationships might be exploited to better affect outcomes of virus infection, it will be important to disentangle chicken and egg scenarios. It has been recently shown that some variants of CMV elicit potent NK responses that are protective to the development of MS in individuals with high EBV antigen-specific antibody producing cells,[71] offering important mechanistic insight into the relationships between co-infecting viruses and strategies to prevent pathology. These studies also illustrate the complex relationship between new acute infections and existing chronic or latent infections. Understanding pathology and relationships in the context of the entire virome rather than of pathogens in isolation will be a key component of the virology of the future.

The impact of persistent or acute viral infection on other pathogens, including bacterial, fungal, and single or multicellular parasitic pathogens, and how other pathogens impact the outcomes of viral infection, are critical questions in understanding disparate disease outcomes across a population. For example, intestinal helminths increase reactivation of latent gamma herpesviruses and exacerbate pathology of HSV-2 in mice.[90,91] Further, helminths provide better control of respiratory syncytial virus (RSV) yet compromise control of flaviviruses.[92,93] These effects are due to cellular responses and soluble mediators of inflammation, such as IFN and interleukins. Not surprisingly, persistent parasites impact the immune environment, which alters responses to subsequent viral infection in ways that are co-dependent on host factors, such as age and overall health status. Collectively, these studies underscore the significance of diverse co-infections on outcomes of viral infection but are the tip of the iceberg in understanding how colonization of a host organism with multiple persistent pathogens impacts health and the ability to respond to viral infection.

NOVEL ANTIVIRALS, VACCINES, AND OTHER COUNTERMEASURES

In response to COVID-19, novel vaccines and antivirals were developed at record speed, and new technologies were rolled out at large scale for the first time.[94] This will likely catalyze the development of dearly needed countermeasures against both viruses already circulating in the human population as well as emerging pathogens.

On the vaccine side, we witnessed the first licensure of mRNA vaccines, the first large-scale rollout of vectored vaccines, the first licensure of whole inactivated coronavirus vaccines, the first licensure of a DNA vaccine, and an emphasis on protein engineering for stabilizing antigens for both SARS-CoV-2[95] and RSV.[96] We learned that traditional platforms such as inactivated viral vaccines as well as new technologies like those utilizing mRNA can have a massive impact on pandemics and save millions of lives.[97] In addition, several vaccine approaches were developed with technologies that enable vaccines to be produced in low- and middle-income countries.[98,99]

Where do we go from here? The renewed interest in vaccines, as well as investments in vaccine companies during the COVID-19 pandemic, have enabled the development of novel and exciting vaccine candidates (Figure 4). This includes those against viruses circulating in humans, against those for which we have currently available vaccines such as human metapneumovirus, parainfluenzavirus, norovirus, CMV, EBV, and HSV, and also against emerging viruses like Nipah virus, Lassa virus, and Zika virus. In addition, the combination of mRNA technology and engineering of stable pre-fusion viral surface glycoproteins is leading to the development of combined respiratory virus vaccines. New vaccine technologies, especially mRNA, will allow us to rapidly respond to future emerging viruses, with vaccines hopefully available within 3 months of the start of an outbreak.[100] We are also making progress in the development of vaccines that provide protection against antigenically variable viruses or that cover a broad spectrum of related viruses (Figure 4). Examples are advances in the development of ''universal'' influenza virus vaccines[101,102] and approaches to develop variant-proof SARS-CoV-2 vaccines or even pan-coronavirus vaccines.[103,104] While these technological advances should enable better protection from circulating human viruses and significantly increase pandemic preparedness, many issues remain. These include how novel vaccines can be equitably shared globally, difficult immunological hurdles (for example, with HIV-1 and dengue), and addressing large-scale vaccine hesitancy and skepticism that have recently led to significant

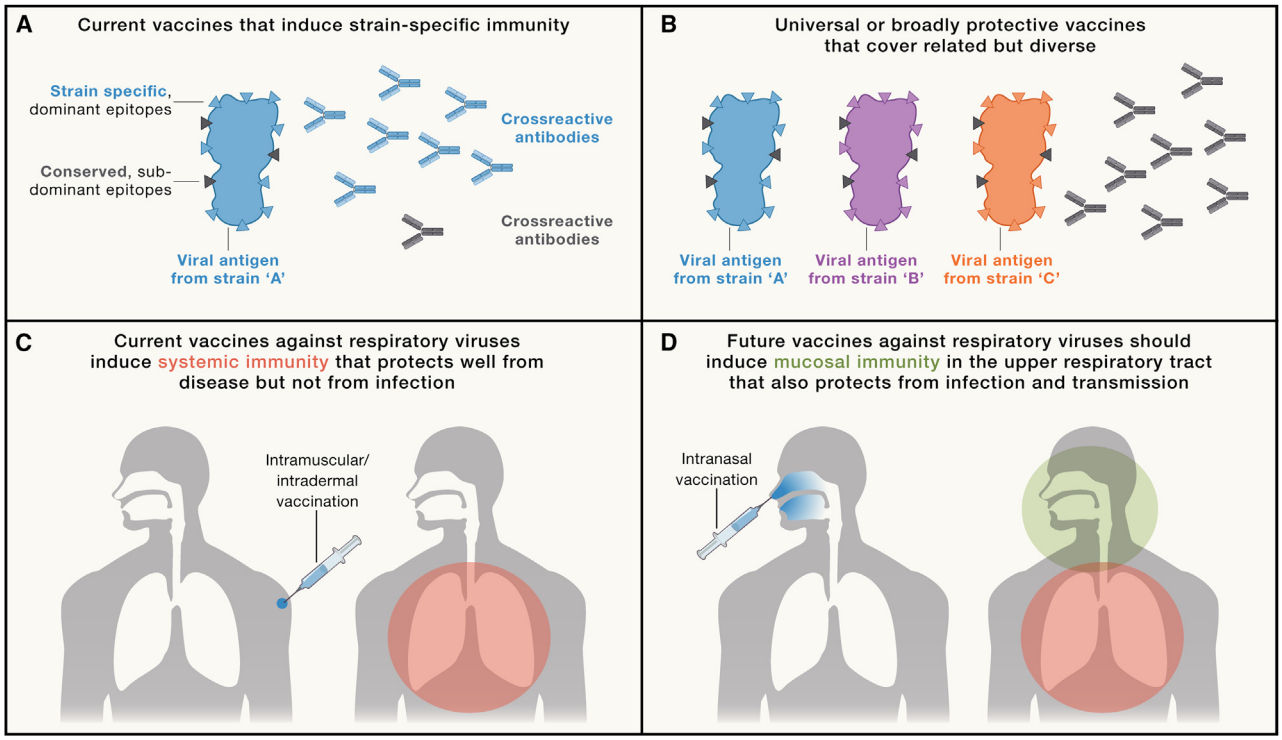

Figure 4. Emerging concepts for viral vaccines

(A) Currently used vaccines often induce strain-specific immunity. However, many RNA viruses like influenza virus and SARS-CoV-2 are antigenically unstable and undergo antigenic evolution (i.e., "drift"). In addition, vaccines that can broadly target related but antigenically distinct viruses are needed for pandemic preparedness.

(B) Broadly protective or universal vaccines that refocus immune responses on conserved epitopes shared by variants or related viruses would enhance both protection from antigenically drifting viruses and pandemic preparedness.

(C) Most vaccines against respiratory viruses are injected and induce systemic immunity that protects against symptomatic and severe disease. However, these vaccines do not effectively induce immunity in the upper respiratory tract.

(D) To avoid (breakthrough) infections and transmission within the population, vaccines that induce strong immune responses in the upper respiratory tract are needed. Vaccination via mucosal routes (intranasal, oral, etc.) may provide this type of protection. In general, mucosal immunity is currently understudied but has become a focus for future exploration.

immunity gaps as exemplified by large-scale measles outbreaks.

There have similarly been major advances in antiviral therapies. This includes classical small molecule antivirals that have been game changers for HCV (i.e., an actual cure) and HIV-2 (antiretroviral therapies including pre-exposure prophylaxis) and that were developed very rapidly for not only SARS-CoV-2, but also monoclonal antibody-based therapeutics. Before COVID-19, only one monoclonal antibody (mAb)-based prophylactic against RSV for use in very young infants was on the market. Even before COVID-19, we saw the development of mAb therapeutics against Ebola virus, with two licensed in 2020[105] and new RSV prophylactic mAbs, one of which was recently licensed in the US.[106] One groundbreaking aspect of this new RSV prophylactic is that the mAb Fc is modified to provide a months-long half-life: this enables pre-season treatment and hence protection for the whole winter season with only one administration. Many mAbs were developed as therapeutics or prophylactics against SARS-CoV-2, although they were outpaced by the rapid evolution of resistance.[107] Fortunately, new mAbs can be identified quickly and developed into effective antivirals against new variants, although testing these new mAbs in clinical trials on timescales that track virus evolution is unsustainable. For the future, we need a regulatory system that allows changes in mAbs akin to strain changes for influenza and COVID-19 vaccines, with very limited clinical testing so that mAb therapies can keep up with variant evolution. Of course, mAb therapeutics could be useful for many viral infections, including emerging viruses, and it is likely that these antivirals will have a huge impact on how we manage seasonal infections and future responses to emerging viruses. Long-lasting mAb-based prophylactics are especially suitable to protect individuals who are immune suppressed. A remaining issue with mAb therapeutics is their cost, making them inaccessible in many countries. Novel developments like mRNA-delivered mAbs could help reduce costs and make this type of therapeutic accessible to the global population.[108,109]

Both classical antivirals and mAb therapeutics act directly on the virus and either neutralize it or block the activity of essential proteins. By contrast, host-directed antivirals target host factors needed by viruses to successfully replicate. These antivirals are in development and have potential to make a significant impact in the future. The core idea is that due to their generally limited number of proteins, viruses need to highjack parts of the cellular machinery and that the cellular components the virus needs can

be targeted to inhibit viral replication instead of the virus itself. These "indirect" antivirals have multiple advantages. First, many viruses interact with similar/the same cellular pathways to enter cells, to replicate, and then to exit cells. Many of the indirect antivirals therefore have very broad antiviral activity and will likely cover whole virus families (or even broader), such that they do not need to be specifically developed for a newly emerged virus. In addition, viruses can sometimes develop resistance with no fitness cost, rendering the antiviral ineffective (e.g., the ion channel or neuraminidase inhibitors for influenza virus).[110] This is unlikely to happen with host-targeting antivirals. However, these indirect antivirals need to be tested extensively for safety and can likely only be used transiently since they directly inhibit cellular proteins and functions. Nevertheless, it is likely that they will have a significant impact, and future therapeutics and prophylactics will be an active area of research.

The COVID-19 pandemic highlighted our poor understanding of the interactions between viruses and the immune system on mucosal surfaces of the upper respiratory tract.[111] For the future, it is important that these interactions are understood to design vaccines and antivirals that not only protect from disease but also block infection and/or prevent transmission. This area of research and the development of mucosal vaccines and antivirals (e.g., mucosally administered mAbs) could provide future solutions to effectively curb seasonal respiratory virus waves within the population.[112] This approach could also be used to limit the spread of respiratory emerging viruses before they cause a pandemic.

The COVID-19 pandemic also told us how non-pharmaceutical interventions can help prevent respiratory virus infections. The use of masks,[113] social distancing, and appropriate ventilation systems in buildings and public transport[114] can significantly reduce the risk of getting infected. Smart planning of new buildings and public transport systems accounting for virus transmission may make the global community much more resilient to respiratory virus seasonal epidemics and pandemics. Essentially, improvements in ventilations could bring the same improvements for respiratory viruses that clean water and hygiene have brought for gastrointestinal tract infections. These structural considerations, together with a better understanding of viral transmission routes, behavior of viruses on upper respiratory mucosal surfaces, and vaccines and antivirals that act efficiently, may make it possible to curb virus spread and ideally eliminate some respiratory viruses from the population.

Finally, a key goal will be the development of additional technologies to reduce the risk of zoonotic infections. One issue that manifests with influenza viruses is that livestock may become infected and then transmit the virus to humans. This occurred in the 2009 H1N1 pandemic that involved transmission from swine. It is now possible to develop genetically engineered livestock that are resistant to certain viruses like influenza.[115] Using CRISPR and other technologies, animals resistant against whole virus families could be produced, representing an exciting research avenue.

CLINICAL METAGENOMICS

There is increasing use of metagenomics as a diagnostic tool (and occasionally an accredited test) in clinical medicine, not only for the initial identification of novel pathogens like SARS-CoV-2 but also for detecting known disease agents.[116] Metagenomics may eventually become a one-stop shop for infectious disease diagnosis. Although in most cases routine diagnostic techniques such as PCR, antigen-based tests, and cell culture provide sufficient information rapidly and at cost, metagenomics provides major advantages. As well as its presence or absence, the genome sequence of the pathogen is obtained (which can be used in subsequent clinical and epidemiological studies), with sequence read count acting as a measure of abundance. Rather than only finding a single pathogen of interest, metagenomics potentially provides data on all the microbes in an individual host and highlights their interactions, and the host genomic data obtained may provide information on host genetic susceptibilities.

At present, clinical metagenomics remains in the development, assessment, and validation phase. Although laboratory validation has been performed in some cases,[117] work is ongoing to optimize the best strategies for sequencing and downstream bioinformatics analysis. Topics for consideration include whether it is best to deplete host sequence reads or enrich for viral ones, the optimal sequence read counts and read lengths per sample, sequencing platform, the best protocols for RNA extraction and library preparation, cost, turnaround time, whether each potential pathogen "hit" should be subject to PCR confirmation, and the most specific and sensitive bioinformatics tools. Although all metagenomic approaches have pros and cons depending on context (for example, whether the aim is to identify known or unknown pathogens), an obvious advantage of shotgun sequencing without dedicated virus capture is that it enables the study of the possible interactions between viruses and co-infecting bacteria and/or parasites. Overall, it is reasonable to expect that metagenomic diagnostic approaches will be validated and standardized over the next decade.

One of the highest hurdles for clinical metagenomics is the enormous amount of sequence data now generated. Ten years into the metagenomics revolution and genome sequencing data is accumulating more rapidly than the computational tools to analyze it. The challenge is to accurately identify disease-causing pathogens from the myriad of commensal microbes and possible contaminants within this huge abundance of data and do so on a timescale that enables a clinical intervention. Again, AI offers an obvious path forward. However, it is also the case that clinicians, public health officials, and technicians must be trained to reliably interpret metagenomic data, and effectively analyzing and storing the huge amount of data generated by metagenomic sequencing may pose problems for public health laboratories. Finally, the increasing use of metagenomics inevitably raises ethical issues, including whether it can and should be routinely linked to host genomic data.

VIRUSES AS TOOL OF CELL BIOLOGY

Viruses have served as powerful pioneering tools in molecular and cellular biology (Figure 5). They have evolved to target pivotal control points in the biology of the host cell and have

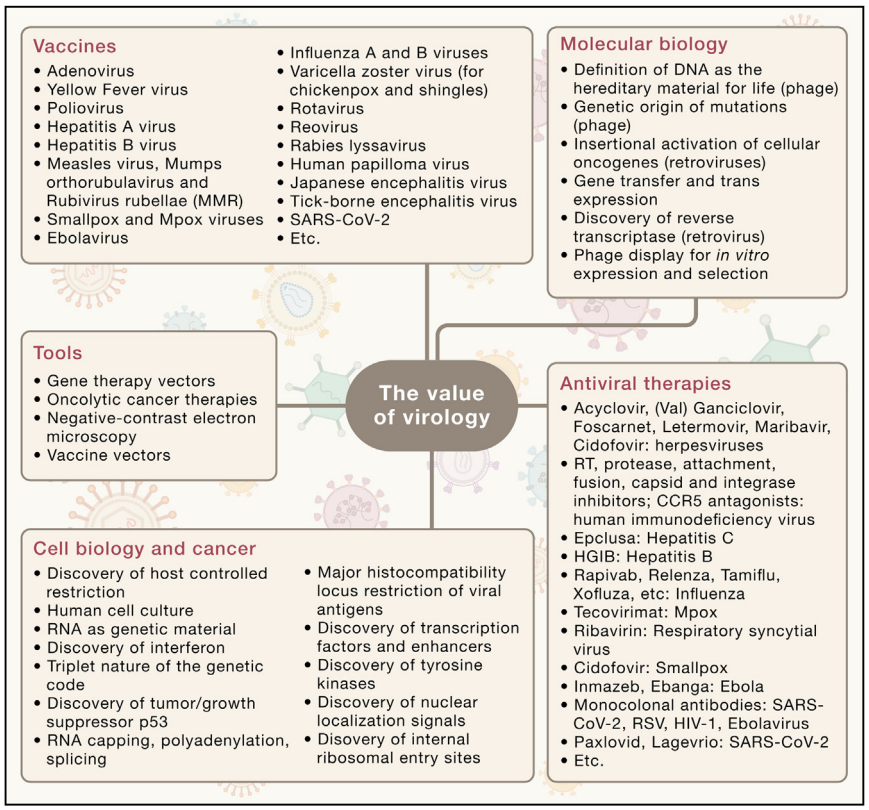

Figure 5. Products of virology

In addition to understanding the basic biology of viral infection and disease, the science of virology has been the driver for the discoveries that underpinned the broad-scale development of vaccines and antiviral therapies, as well tools for the treatment of cancer and genetic disorders. Of equal importance, viruses have been major tools for foundational discovery in molecular and cell biology, defining the basic mechanisms of DNA replication and transcription to oncogenesis. While this figure is not comprehensive, it conveys the scope of how the study of viruses has impacted science and health. RT, reverse transcriptase.

been instrumental in discovery of cellular control points and mechanisms. In 1952, Alfred Hershey and Martha Chase, early pioneers in molecular biology, used bacteriophage T2 to demonstrate that nucleic acid, not protein, was the genetic material.[118] In 1972, virology led to the first engineered recombinant DNA using lambda bacteriophage and Simian Virus 40 (SV40),[119] ushering in the revolution of recombinant DNA technology. The basic cellular processes of mRNA capping,[120–122] splicing,[123,124] and polyadenylation[125,126] were all discovered over the next decade using viruses, laying the foundation for understanding how gene expression and protein synthesis are controlled. In 1977, Levine and Crawford discovered the p53 tumor suppressor, a major "guardian of the genome," using adenovirus and SV40.[127,128] Mammalian transcription factors were first identified in 1981, utilizing mouse mammary tumor virus (MMTV) and SV40.[129–131] Further, antiviral defenses, including restriction enzymes, RNAi, and CRISPR, have driven revolutionary advancements in molecular biology. Viruses led the way of discovery in the dawn of the molecular age and will continue to be beacons for discovery of cell biology.

Given their ability to deliver nucleic acids to infected cells for transgene expression, induce immune responses, or induce cell death, viruses have also become tools for human health as vectors for gene therapy (e.g., adeno-associated virus, lentivirus, and herpes simplex), oncolytic therapies (e.g., adenoviruses, herpes simplex, adeno-associated virus, measles, and vaccinia), and as vaccine platforms (e.g., measles, vesicular stomatitis, CMV, and avian paramyxoviruses), which will have enormous impacts in combatting congenital disease and cancers that have been intractable and will be a major driver of personalized medicine. Currently, 70% of gene therapy trials have used viral vectors. Basic virological research is critical for the safe use of viral vectors as we discover how to modulate immune responses to vectors, improve and target *trans* gene expression, and prevent unintended gene activation or inactivation in viruses that integrate into the host genomes.[132] Combining viral vectors with CRISPR-Cas9 gene editing technologies will open limitless possibility for treating human disease.

AI IN VIROLOGY

We are witnessing a revolution in AI. This will similarly impact virology, particularly the computational analysis of virus genome sequences that often involve a variety of repetitive tasks that can readily be learned by machines.

It is inevitable that programs like Alphafold will increase in both accuracy and speed.[10] Alphafold has transformed protein structural predictions, with a myriad of downstream applications and will have huge practical benefits to virology. These include documenting the full range of virus protein structures in nature (particularly as many viral homologs to cellular proteins have almost no primary sequence homology), helping to better dissect protein-protein, virus-cell, and virus-host interactions, resolving the evolutionary relationships among particular structures, such as virus capsids and polymerases, and the rational design of therapeutics.

Although it will not replace experimental systems, AI might eventually enable us to predict all the biological and phenotypic characteristics of a virus, as well as its interaction with host cells, host immune systems, and therapeutic agents, as well as its pathogenicity and pandemic potential, directly from its genome sequence. To succeed in this space, AI algorithms need to be trained to understand the phenotypic consequences of every single and every combination of mutations in a virus genome;

Box 1. Some pressing questions in virology

What is the diversity, size, and structure of the virosphere, and how does it impact ecosystem health?

What were the primordial viruses, and did they play role in the evolution of cellular life?

What direct and indirect factors determine competition among viruses, and can they be harnessed as strategies for virus control?

How do chronic viral infections mechanistically contribute to chronic human disease, such as neurocognitive decline, long COVID, MS, and cancer?

How do persistent infections impact host immune responses, and what is the impact of co-infections on viral persistence and vice versa?

To what extent can vaccination or early detection and treatment of infection, where antivirals exist, prevent chronic disease?

How do we understand the interface of host and viral biology and its complexity with respect to outcomes of infection?

How do we build public trust in the ability of virology to safely forge new directions for human health and ensure preparedness and response for future pandemics, as well as other infectious disease threats?

the structure of every protein and how they interact; how viruses function in a wide range of cell types, receptors, and hosts; and virus interactions with components of the immune system, including their ability to generate antigenic variation, their possible response to antiviral agents, and their epidemic potential. While this may seem an impossible goal, so would have sequencing 16 million genomes of a single virus species (as is now the case for SARS-CoV-2) in 1974, and recent progress has been made in predicting immune escape mutations.[133]

CONCLUSIONS

We have outlined topics that we believe may be of importance for virology in the coming decades, with some key questions outlined in Box 1. Of course, it is inevitable that the future of this discipline will also be shaped by unanticipated events and scientific advances, and that we will almost certainly experience another major pandemic, perhaps dwarfing COVID-19.

Our perspective necessarily falls under the shadow of COVID-19. It would therefore be negligent to discuss the future of virology without reference to the current political environment, which will have far-reaching and often unpleasant consequences for our discipline. We stand at an unprecedented time in the history of virology. The worth of much of the work we and our colleagues have done every day since the birth of *Cell* is being challenged. Virologists are under intense scrutiny, and our work and intentions are subject to abundant misinformation, misrepresentation, and misunderstanding. New restrictions on virological research (e.g., gain-of-function, animal sampling, international collaborations) are being imposed. Despite the lack of any direct evidence, the allegation that SARS-CoV-2 had a laboratory origin and the role played by virologists in this will likely be a contentious issue for years to come. The specter of virologists questioned by members of the US Congress for writing a scientific paper should cause widespread disquiet for those who value free, open, and just societies.

Virology needs to stand firm in the face of these assaults but also to learn important lessons. Virology, like any science, cannot function without public support. We must better communicate our work and acknowledge where failings occur. Most research in virology goes on in the background without overt publicity. In the face of misinformation, virologists must show the worth of their science for everyday life and the greater society, highlighting the success stories. Virology has played a huge role in reducing the burden of infectious disease and was integral to controlling the COVID-19 pandemic. Strong support, yet robust governance, of virology is critical if this is to be true of future pandemics. Rapid communication and data sharing are arguably the most effective ways to mitigate a pandemic, and without effective global dialogue and collaboration, our response to the next pandemic will be increasingly toothless. Better science education and outreach, science communication, and even participatory/community science are needed to involve the population in virology and increase the understanding of what we do in the broad public domain. Decades of prior virology research empowered the rapid development of safe and effective COVID-19 vaccines within a year of identifying SARS-CoV-2. If virology is not nurtured, we cannot hope to detect or respond to threats effectively without the arsenal of knowledge built through steady research and scientific discovery: we are effectively disarming our best defense. The current political environment and the anti-science sentiment it has nourished means that we are arguably less well prepared for a pandemic than we were in January 2020. To compound this issue, the assault on virology will deter the next generation of scientists from entering fields driving the science critical to pandemic response.

The last 100 years of virology have brought remarkable discoveries that broadly impacted science and human health (Figure 5). In 1980, the WHO declared smallpox eradicated due to an unprecedented vaccination campaign. Reverse transcriptase was discovered, and the first recombinant DNA molecules were created. Virology led the discovery of oncogenes and tumor suppressors, defining basic mechanisms of oncogenesis and the role of viruses in cancer. Viruses have taught us how antigens are processed and the role of major histocompatibility complexes in educating immune responses. Through virology, we have gained insight into basic cellular processes that are foundational to life. This has led to the development of treatments and vaccines that have dramatically curbed pandemics from HIV-1 to SARS-CoV-2. Virology has informed areas of cell biology so broadly that the impacts on human health are hard to fully grasp, and it has the potential to transform human health in the future. Restricting virology will come at a heavy price.

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DECLARATION OF INTERESTS

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to influenza virus vaccines and therapeutics, SARS-CoV-2 serological assays, and NDV-based SARS-CoV-2 vaccines, which list F.K. as co-inventor. Several of these inventions are licensed to commercial entities. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. F.K. has consulted for Merck, Seqirus, Curevac, and Pfizer; is currently consulting for GSK, Gritstone, 3rd Rock Ventures, and Avimex; and is a co-founder and scientific advisory board member of CastleVax. The F.K. laboratory has also been collaborating with Pfizer on animal models of SARS-CoV-2 and is collaborating with Dynavax on influenza virus vaccines. E.C.H. has consulted for Moderna and Pfizer on aspects of COVID-19.

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