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Virology in the Next Millennium

Minireview

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Viruses were first inferred to exist by the diseases they caused. Although disease manifestations like rabies, paralytic poliomyelitis, smallpox, and measles were noted in antiquity, viruses as infectious agents were defined just over 100 years ago. Their discovery was made initially possible by the revolution in microbiology led by Louis Pasteur and refined in concept by Robert Koch and Joseph Lister at the last half of the nineteenth century. These and other scientists discovered that microscopic organisms produced disease and the presence of the microbe could be defined by several criteria such as their retention on a Pasteur–Chamberlain filter, visualization by light microscopy, and growth on agars and in broths. However, a subset of diseases failed these experimental tests and a new class of organisms were uncovered. Specifically, independent observations by Ivanovsky (1892, 1899) and by Beijerinck (1898) with a disease of tobacco leaves, and by Loeffler and Frosch (1898) with foot-and-mouth disease of cattle, demonstrated an infectious agent that passed through the porcelain filter to the bottom of the flask, was not visible by light microscopy, and failed to grow in agar/broth cultures (Table 1). Indeed it was not until 40 years later, in the 1930s (Kausche et al., 1939), that tobacco mosaic virus was observed by electron microscopy and nearly 50 years before poliovirus could be reproducibly grown in tissue culture cells (Enders et al., 1954). The ability to grow viruses in cultured cells was the technological breakthrough that freed virologists from the need to passage viruses in animals (Gard, 1954) and allowed sufficient recovery of viruses to allow the birth and development of biochemical virology in the 1960s and its expansion to the molecular biology of viruses in the 1970s–1980s.

With sufficient amounts of virus on hand, viral structure, function, and genomic organization followed, leading to insights in replication, transcription, translation, latency, and integration and the beginning of our understanding of how virus transformed cells to a cancerous state or how viruses persisted in animals. Because viruses are small units of packaged genes and *cis*-acting signals ranging in size from a few hundred bases (viroids) to agents with 300 genes, viruses became useful instruments for the study of genetics, cell biology, structural biology, and biochemistry, allowing the study of many basic parameters of life and development. In addition, cell culture permitted the development of large-scale production of vaccines to prevent, for example, paralytic poliomyelitis (the first vaccine produced in cell culture), measles, congenital abnormalities caused by rubella, and most recently the prevention of cancer resulting from hepatitis B virus chronic infections. These achievements led to the eradication of viral diseases that had killed over 400 million people in the twentieth century alone, over 4-fold more than were killed by all the wars of this century (Oldstone, 1998). An estimated 12% to 20% of human cancers are caused by viruses (Zur Hausen, 1991; Gallo et al., 1999), and so the strategy utilized to prevent liver cancer caused by HBV will surely be applied to other cancers in the twenty-first century. Over the past 40 years viruses have played a central role in our present-day understanding of DNA structure and genomes, transcriptional signals (enhancers, promoters), the transcriptional machinery and transcription factors, RNA processing, splicing, poly A–addition, RNA transport, protein localization in cells, and protein processing. The oncogenes and tumor suppressor genes were first uncovered using viruses. Viruses have been excellent probes into the mysteries of the cells and the host animal. Genetic manipulation of both the virus and host are defining the molecular basis of viral diseases (Oldstone, 1996).

But what does the future hold for virology as the millennium ends and the next one begins? First we need to appreciate that the demographics of human populations in the twenty-first century are changing in ways that will impact viruses that prey upon humans. In the next century the rate of population growth will slow, but we will increase our numbers from six billion (in 2000 AD) to eight to ten billion by 2050 AD. In the twenty-first century we will become a predominantly urban species. Today 47 percent of people live in cities; in 2050 AD the great majority of people will live in cities populated with greater than 10 million people (today there are 20 such cities worldwide). We will be more dependent upon our technologies to manage the potential for emerging viral infections.

In the twenty-first century, many countries will have for the first time in history three times more people over the age of 60 years than under the age of 4 years. Because of this, influenza epidemics could be the most important emerging and evolving virus. Few people in the year 2050 AD will have been immunized for smallpox, a disease eradicated in the twentieth century. Is there any possibility it or a relative could return? The human immunodeficiency virus and hepatitis C virus will surely require novel approaches in the next century if we are to control these infections. The human immunodeficiency virus has caused a downturn in the population with a lessening of the mean expectation of life (in Africa) for the first time since the 1918/1919 influenza pandemic. While HIV and hepatitis C viruses have already spread throughout the world, the hemorrhagic fever viruses (ebola, dengue) and hantaan viruses appear to wait in the wings before widespread diseases appear. Animal viruses such as African swine fever virus are on the move; from Africa to Portugal in 1957, to Spain in 1960 and the Caribbean and South America by the late 1960s and early 1970s. New viruses are continuously being uncovered although for several their role in disease is still undetermined.

Table 1. Selected Milestones in Virology

Date(s)	Virologist(s)[References]	Discovery
1892,1898	D.I. Ivanofsky, M. Beijerinck[1–3]	First demonstrations of a filterable plant virus: tobacco mosaic virus.
1898	F. Loeffler & P. Frosch[4]	First demonstration of a filterable animal virus: foot-and-mouth disease virus.
1901	W. Reed et al. (U.S. Army Yellow Fever Commission)[5,6]	First human virus: yellow fever virus. First use of consent form for human clinical investigation.
1904–1908	V. Ellermann & O. Bang, H. Vallee & H. Carre[7,8]	First demonstration of a leukemia-causing virus, retrovirus.
1908	C. von Pirquet[9]	First report of virus causing immunosuppression: measles virus.
1909	K. Landsteiner & E. Popper[10,11]	Isolation of poliomyelitis virus.
1911	P. Rous[12]	First demonstration of a solid tumor virus: Rous sarcoma virus.
1915	F. Twort[13]	Discovery of bacteriophages.
1917	F. d'Herelle[14]	Bacteriophages, plaque assay.
1923–1928	A. Carrel, H. Maitland & M. Maitland[15,16]	Tissue culture of embryo explants and first tissue culture cultivation of virus: Rous virus, vaccinia virus.
1931	J. Furth[17]	Use of mice as a host for viruses.
1931	A. Woodruff & E. Goodpasteur[18]	Use of embryonated hen's eggs as a host for viruses.
1933	W. Smith, C.H. Andrews & P.P. Laidlow[19]	Isolation of human influenza virus.
1933	R. Shope[20]	Rabbit papilloma virus: first DNA tumor virus.
1936	P. Rous, J. Beard[21]	Rabbit papilloma virus induces carcinomas in a different species.
1939	G. Kausche, P. Ankuch, H. Ruska[22]	First electron micrograph of a virus: tobacco mosaic virus.
1946	W. Stanley, J. Summer, J. Northrop[23,24]	Preparation of a viral protein in a pure form: tobacco mosaic virus.
1948–1955	J. Enders, F. Robbins, T. Weller[25–27] H. Eagle[28]	Routine use of tissue culture to grow and study viruses. Development of optimal media for growing cells.
1937–1951	M. Theiler, H. Smith[29,30]	Development of 17D strain human yellow fever vaccine: made in animal and embryonic cultures.
1954–1961	J. Salk, A. Sabin, H. Koprowski, J. Enders, S. Katz, S. Krugman[31–34]	Development of poliomyelitis virus vaccine. Development of measles virus vaccine: made in tissue culture.
1957	A. Isaacs, J. Lindenmann[35,36]	Discovery of interferon.
1950–1970s	S. Luria, M. Delbrück, J. Monod, E. Wollman, A. Hershey, S. Benzer, S. Cohen, D. Nathans, R. Dulbecco, D. Baltimore, H. Smith, W. Arber, H. Temin, P. Sharp[37–48]	Quantitative plaque assay, origins of molecular biology and molecular virology.
1950, 1960, 1980s	B. Sigurdsson, B. Blumberg, C. Gajdusek, S. Prusiner, J. Stevens et al.[49–54]	Persistent, latent, and slow virus infections. Prions.
1954	W. Rowe[55]	Role of thymus in immune responses to virus.
1970s–1980s	R. Zinkernagel & P. Doherty, M. Oldstone, B. Fields, B. Moss et al.[56]	Major histocompatibility restriction and cytotoxic T lymphocytes, immune mediated viral diseases, molecular pathogenesis.
1969–1976	R. Heubner, P. Vogt, M. Bishop, H. Varmus et al.[57,58]	Oncogenes.
1977	World Health Organization—Many health workers and virologists[59]	Eradication of smallpox as a disease that killed over 300 million people in the twentieth century.
1978–1985	S. Harrison, A. Olson, J. Hogle, M. Rossman, R. Rueckert[60–62]	First atomic structure of a plant (tomato bush stunt) and animal (poliomyelitis, rhinovirus) virus.
1979	D. Lane, L. Crawford, D. Linzer, A. Levine[63,64]	SV40T-antigen-p53, virus host cell interaction-tumor suppressors.
1981	J. Skehel, D. Wiley, I. Wilson et al.[65]	Structure/function of influenza virus hemagglutinin. First atomic structure of a glycoprotein.
1981–1984	R. Gallo, F. Barré-Sinoussi, L. Montagnier, Y. Hinuma[66–71]	First human retrovirus.
1984–2000+	M. Hilleman et al.[72]	First molecular recombinant virus vaccine: hepatitis B virus. First vaccine to successfully treat cancer: hepatitis B virus-induced liver cancer.
2000–2015	World Health Organization—Many health workers and virologists	Planned eradication/elimination of poliomyelitis virus (by 2002 to 2005) and measles virus (by 2015) as a disease.

How are we going to respond? The World Health Organization predicts the worldwide eradication of poliovirus by the end of the first decade of the next century. Measles is anticipated to be eliminated by 2015 AD. Shortly thereafter, the hepatitis B vaccinations of the twentieth century will prevent expected liver cancers in the Far East. New disciplines will be developed to respond to these challenges. Viral diagnoses in the field (PCR, large volume DNA sequencing) will develop a new field of molecular epidemiology, which could be rapidly

translated to new modes of vaccine development and novel methods of immunization using dendritic cells or cytokines to enhance responses to antigens. Renewed efforts in viral drug development will produce novel drugs that selectively control or eliminate some viruses. Technology of DNA assays and informatics will provide a molecular understanding of virus–host cell responses both in vitro and in vivo.

The field of gene therapy, which has promised a great deal, but only produced limited successes in the twentieth century, will depend upon the development of virus vectors in the twenty-first century. Recombinant and mutant viruses will be used in vaccines, in gene replacement therapy, and for the selective killing of tumor cells in people. Viral agents that cause old diseases will be uncovered, perhaps for type I diabetes, multiple sclerosis, or other neurological syndromes. Viruses that have evolved a way to enhance or inhibit inflammatory reactions, immunosuppress the host, or evade an immune response will be characterized and understood. These very viral genes and approaches will then be applied to relieve autoimmune diseases in humans.

But surely viruses will continue to lead the way in the basic sciences, as well as in clinical sciences. Great questions remain in virology and its research laboratories. How does herpevirus latency in neurons or lymphoid cells occur and what are the events in reactivation of these viruses? How do viruses persist or maintain chronic active infections in their host? What new diseases do they cause? How do the cancer viruses cause a tumor to arise? We need to understand changes in viral evolution, altered host range specificity, the causes of virulence and attenuation via mutations, and the control of mutation rate in viruses. The RNA viruses are unique and live on the edge of their "error threshold", which means that their mutation rates and their rate of evolution will be a big problem in our future. Viruses of our evolutionary past (retroviruses) may comprise 1%–10% of our genome. Did they play a role in our evolution and have we overlooked their role in disease? What is their impact upon our diversity, immune response, or response to other viruses?

Finally, viruses will continue to be the tools in our ever-expanding revolution in the biological sciences. Basic science laboratories will continue to utilize viral genes and enzymes, viral promoters, viral proteins, and viral capsids to explore the fundamental questions of life processes. The twenty-first century will provide new challenges both to the viruses and to the virologists.

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