Chapter 3
The Threat from Viruses

Abstract Infectious disease represent the most significant threat to human health. Significant geologic cataclysmic events have caused the extinction of countless species, but these "Wrath of God" events predate the emergence of Homo sapiens. Pandemic infections have accompanied the rise of human civilization frequently re-occurring leaving a lasting imprint on human history punctuated by profound loss of life. Emerging infections become endemic and are here to stay marking their presence with an annual death toll. Each decade brings a new onslaught of emerging infectious agents. We are surprised again and again but are never prepared. The long-term consequences often remain unrecognized and are always inconvenient including cancer, cardiovascular disease and immune associated diseases that threaten our health. Reliance on clusters of clinical symptoms in the face of diverse and non-descriptive viral infection symptoms is a foolhardy form of crisis management. Viral success is based on rapid replication resulting in large numbers. Single-stranded RNA viruses with their high replication error rate represent a paradigm for resilience.

Keywords Emerging infectious disease · Endemic · Outbreak · Pandemic · Epidemic · Zoonotic · Epidemiologic

Introduction

Contemplating recent career paths, I reviewed a broad range of scientific questions. Most prominent was, why study an area of science that has no significant impact? In fact, why study anything that is not the most highly impactful area? This question demands a definition, what constitutes high impact? I decided to create a definition that impact and threat to life are related. Further, threat to human life is the highest impact and it is likely that things threatening human life may also threaten all life.

What presents the greatest threat to human life today? History should provide critical insights to answer this question. A comprehensive look would seek causes of mass extinctions over the past 3.5 billion years of life on earth. This perspective has been a trending subject in science with focus on five mass extinctions. There are

P. L. Iversen, *Molecular Basis of Resilience*,
https://doi.org/10.1007/978-3-319-98164-2_3

Table 3.1 Mass extinctions

Name	Million years ago	Percent species extinct	Duration of event	Cause of extinctions
Ordovician-Silurian	446	65	4 M years	Unknown
Late Devonian	360	75	25 M years	Unknown
Permian-Triassic	250	85	7 M years	Volcanic eruptions
Triassic-Jurassic	202	62	<1 M years	Climate change
Cretaceous-Paleogene	66	75	0.07 M yrs	Asteroid impact

inherent biases in focusing on mass extinctions such as these events fail to appreciate small living things such as single celled organisms or bacteria. Another bias is in order to appreciate life in the past, the life form must occasionally produce a fossil when it dies. Those concerns aside- geologic events have threatened the survival of living things (Table 3.1).

The actual events causing these extinctions are frequently debated, for example, the asteroid impact 66 million years ago off the coast of Mexico was accompanied by a massive tsunami that was responsible for mass extinctions in Montana, the impact may have also triggered an enormous volcano in India resulting in ash and lava flow responsible for regional species extinctions, and the collective dust resulted in prolonged climate change which was probably responsible for even more species extinctions. The lesson remains the same, volcanic eruptions; climate change and asteroid impact threaten life in a highly significant way and are responsible for extensive selection pressure. Unfortunately, these events are unpredictable and so enormous little can be done about them. A mass extinction may not represent a legitimate threat or selection pressure because large numbers of species are completely eradicated leaving few survivors to drive evolution. Finally, outside of removing dinosaurs so that mammals could thrive, what impact did these events have on human life; humans were not yet on the earth when these events took place.

Refining the search to dramatic changes in human populations moves the period up to the last 100,000 years. Mass migrations took place about every 23,000 years, which is in line with the precession of the earth leading to a possible linkage between earth's precession and human migration. This may have been due to regional climate change like ice ages in the northern hemisphere. However, human population changes on this timescale are not easily documented so estimates of climate and human population dynamics will be limited. Time to refine the search.

Consider the exponential growth, outbreak, of tent caterpillars, *Malacosoma distria*, in Montana reported by David Quammen in his book Spillover (Quammen 2012). The extensive caterpillar population consumed all of the leaves from the local elm and cottonwood trees in the summer of 1993. The caterpillar activity produced a crackle sound, "like a distant brushfire." The city attacked these insects with a broad arsenal of modern countermeasures but to no effect. However, a nuclear polyhedrosis virus (NPV), uses the caterpillar host density like a critical mass in a nuclear weapon to explode destroying the caterpillars in an epic battle. The caterpillar population retreated to undetectable levels in a single season as a result of NPV.

Human populations changed over the past 20,000 years with particular emphasis on the most recent 7000 years. A human population curve over this segment of time shows an exponential growth period interrupted in the middle ages by the plague. Hypothesis: The plague and other pandemic infectious disease events appear to be the greatest threat to human life. Infectious disease kills 15 million people each year, 26 percent of the total 57 million annual deaths in the global population (Fauci and Morens 2012).

A brief review of pandemic infections over the past 3000 years illuminates several events that reduced human populations. The plague of Athens 430 BCE killed 25 percent of the human population (Table 3.2). Both bacterial and viral pandemic

Table 3.2 Brief overview of historical pandemics

Name	When	Infectious agent	Impact
Plague of Athens (Scientific American 2006)	430 BC	Typhus	Killed 25% of population in 4 years
Antonine Plague	165–180 AD 251–266 AD	Variola virus	~5 million killed
Plague of Cyprian			5000 deaths per day in Rome
Plague of Justinian	541–750	*Yersinia pestis*	Killed 50% of Europe's population
Black death	1347–1352	*Yersinia pestis*	75 million deaths
Smallpox	1518–1568	Smallpox virus	80% of children under 5 died
			Mexico's population ↓ 20 to 3 million
Plague of London	1665–1666	*Yersinia pestis*	100,000 killed; 20% of the population
Tuberculosis	1800's	Mycobacterium tuberculosis	25% of the adult population of Europe died
Cholera (Lee 2003)	1816–1826	Virbio cholerae	15 M in India; China, Indonesia
	1829–1851		23 M deaths in Russia 1865–1917
	1863–1875		30,000 of 90,000 Mecca Pilgrims in Africa
	1881–1896		50,000 deaths in America
	1899–1923		120 K in Spain; 90 K in Japan; 60 K in Persia
			800 K in India; 200 K in Phillipines
Influenza pandemic	1889–1890	H3N8/H2N2	Russian flu- 1 million deaths
	1918–1919	H1N1	Spanish flu- 50 M deaths; 2% world's pop
	1957–1958	H3N2	Asian flu- 2 M deaths
	1968–1969		Hong Kong flu- 1 M deaths

infections are significant human killers with bacteria like *Yersina pestis* killing half of the population of Europe and the smallpox virus killing most of the human population of Mexico. Pandemic infections are not restricted to history long before our time, the Spanish flu pandemic of 1918 killed 50 million people. My grandparents would tell stories of watching horse drawn hearses daily carrying the dead through their Indiana small village in 1919. It is safe to conclude pandemic infections are currently relevant and represent one of the most significant threats to human survival.

Yersinia pestis is a bacterium causing plague. Fleas can be infected with *Y pestis* which transmit the bacterium to rodents, the primary hosts. Changes in the environment may lead to the movement of rats into populated areas where humans become infected. Homer points to such an infection in the *Iliad* in his description of the Trojan War in 1190 BCE. Plague has returned several times since the Trojan War imposing enormous loss of human life (Table 3.2). The most recent plague epidemic killed over ten million people in India in the early 20th century. *Y pestis* is still out there ready for favorable conditions to pounce on human populations but outcomes are likely to be less dramatic due to understanding of sanitation practices, quarantine, and availability of antibiotics.

If infections are the greatest threat to human life, they should be critical drivers of evolution? Clearly infections pose selection pressure on the human populations. Origins of evolutionary thought did not include infection as Darwin established key evolution concepts on the Galapagos Islands. These islands are isolated and an unlikely place for the spread of infections. The concepts speciation point to geographical separation of populations so infections would most likely be restricted to isolated populations. In many cases the survival selection pressure is not identified or ascribed to insufficient sources of food. Unfortunately, common single-stranded RNA viruses are so unstable that there are limited data for a viral fossil record.

Pandemic infections remain a threat to human survival in the presence of the information revolution, daily medical breakthroughs, and global travel. The human retrovirus HIV currently a global infection that infects up to 25% of the population in southern and eastern Africa with a projected death toll of up to 100 million by 2025. Measles killed 200 million people in the last 150 years and the development of an effective vaccine in 1963 reduced concerns for this infection but there were 777,000 deaths in the year 2000. Vaccination programs are frequently disrupted due to complacence resulting from vaccine success, conflicts that shift healthcare focus, and social crisis such as the recent Ebola outbreak in West Africa.

Smallpox is also an ancient infection causing fever, skin lesions, and at times death. King Ramses V of Egypt is thought to have died from smallpox around 1200 BCE. Introduced into Mexico in 1520, smallpox killed 3.5 million Aztec Indians or about half of the population in a period of 2 years and then proceeded to decimate the population of South America. Variola is a highly infectious virus killing 300–500 million people during the 20th century inspiring the eradication campaign in 1967. Variola was eradicated by December of 1979 (De Cock 2001), a rare triumph of public health. The WHO deserves acknowledgment for this unprecedented accomplishment and proof of concept that human suffering is not inevita-

ble. However, variola is a DNA virus with limited rate of mutation and a narrow host range so that animal reservoirs do not exist. The eradication of other viral infections will be more challenging.

The world continues to confront a broad array of microbial threats. Progress and preparedness make our engagement a likely success for those microbes that resurface and infections for which we have experience. Medical and epidemiological uncertainties surround emerging infectious disease, those that challenge us with their novelty.

Emerging Infectious Disease (EID) Becomes Endemic

Pandemics dominate the infectious disease "fear factor" but each pandemic began as a much more frequent occurrence, an epidemic. Most but not all epidemics come from emerging infectious agents, the most significant problem facing life on earth today. The concept of emerging is a human centric term as most of these infections are endemic in an animal host that serves as a viral reservoir. A 2005 report from the University of Edinburgh identified 1407 human pathogens and 177 are emerging or re-emerging of which 75% are zoonotic, that is jump from an animal host to human. Numerous emerging infections caused by viral agents have imposed high impact on human survival (Table 3.3). All the viral agents in Table 3.3 have genomes based on single-strands of RNA except HBV which should focus scientific attention on RNA. There are numerous questions that strike investigators as they ponder a collection of viral agents like those in Table 3.3. The viral polymerase errors in replicating single-stranded RNA genomes are not corrected so the species are constantly changing. The apparent success of these viruses is that as they move from reservoir hosts to humans and as humans become immune to the initial infection, the population of diverse genomes offers multiple chances to adapt by finding a "fit" genome version which can propagate until the next transition requiring adaption.

Acquired Immunodeficiency Syndrome (AIDS) is caused by human immunodeficiency virus 1 (HIV-1), a retrovirus. These viruses have a single-stranded RNA genome that is converted into DNA, a paradigm shift in the flow of genetic information from DNA to RNA. The 36,000,000 human deaths caused by HIV-1 (Table 3.3) is accompanied by a spectrum of clinical signs; eg. fever, diarrhea, peripheral neuropathy, pelvic inflammatory disease, cervical cancer, cytomegalovirus retinitis, Kaposi's sarcoma, lymphoma, *Mycobacterium avium* infection, recurrent pneumonia, and wasting syndrome. HIV-1 genome diversity includes base substitution, insertion, deletion, recombination, and gain or loss of glycosylation sites which all arises from the limited fidelity of the viral reverse transcriptase. These mutations are found in clusters or hypervariable regions indicating the fit virus is selected for from vast numbers of less-fit genome sequences. HIV-1 emphasizes key observations: (1) EID is a significant contemporary concern, (2) we are not prepared for the novel characteristics introduced by EID, (3) clinical signs can be diverse and often mimic symptoms of other diseases, and (4) the replication mechanisms are often error prone resulting in an array of fit viruses.

Table 3.3 Emerging infections that are endemic

Agent	When	Origin	Impact
Dengue	1953	Manila	DHF/DSS 29,803 cases/year
	2010	USA	1986–1990 267,692 cases/year
		Worldwide	1,785,059 cases, 2,398 deaths
Hepatitis B (HBV)	1885	Bremen shipyard	191 cases
	2004	Worldwide	350,000,000 cases, associated with 600,000 deaths/year- WHO
Hepatitis C (HCV)	1987	Global	150,000,000 cases, associated with 350,000 deaths/year
Hepatitis E (HEV)	~1800	Central Asia; Global	20,000,000 infections/year, 3,000,000 cases and 57,000 deaths/year
Human immunodeficiency virus (HIV-1)	1981	Cameroon	35,000,000 people live with HIV, 36,000,000 deaths since 1981, 2,000,000 deaths/year
	As of 2013		
Rabies	2000 BCE	Mesopotamia	54,000 deaths in 1990
	1990	Global	
Influenza	2003	H5N1	13,000,000 cases, 390,000 deaths
	2009–2010	H1N1 pandemic	3 M cases, 250,000 deaths/yr.
	Annual	Global	
Lassa	1950s	Bornea, Nigeria	300,000 cases, 5,000 deaths/yr
Chickungunya	1952	Mozambique	1,250,000 cases
	2006	India	1,118,763 cases, 24,682 deaths
	2013–2014	Americas	
Ebola (Zaire EBOV)	1976	Zaire, DRC	318 cases, 280 deaths
	1994	Minkouka, Gabon	49 cases, 29 deaths
	1995	Kikwit, DRC	315 cases, 242 deaths
	1996	Mayibout, Gabon	31 cases, 21 deaths
(Sudan SUDV)	2007	Kasai DRC	264 cases, 187 deaths
	1976	Maridi, Sudan	284 cases, 151 deaths
	2000	Gulu, Uganda	425 cases, 224 deaths
(Bundibugyo BDBV)	2007	Bundibugyo, Uganda	149 cases, 37 deaths
(Gueckedou)	2013–2015	West Africa	28,616 cases, 11,310 deaths
Measles	500 AD	Persia	200,000,000 deaths
	1855–2005	Global	1,374,083 cases, 630,000 deaths
	1990	Global	
Deaths per year			3.98 Million

Dengue is a flavivirus with a positive sense single-stranded RNA (+ssRNA) genome carried by mosquitos to man. Dengue has been a tropical disease for hundreds of years, typically a disease of young children but in 1953 an emerging severity was recognized in Manila (Table 3.3), hemorrhagic fever (DHF) and dengue shock syndrome (DSS). More than 2 billion people are at risk of dengue infection but only a small fraction of those infected will develop DHF or DHS. Infected people develop antibodies that can lead to antibody-dependent enhancement (ADE) in subsequent infections and more severe DHF or DSS outcomes. It appears ADE events occur in people that produce immunoglobins (IgGs) with enhanced affinity to the activating Fc receptor due to the IgG1 subclass and lack of a fucose glycan modification of the IgG (Wang et al. 2017) *Aedes aegypti* is the primary vector transmitting dengue but a new vector, *Aedes albopictus*, now carries dengue to the southern United States. Emergence of dengue in the southern United States is likely due to used tires imported from Japan which provided a place for the Asian tiger mosquito to live. Outbreaks of dengue fever have become more numerous and more severe over the past three decades.

Viral "fitness" is constrained by the requirements imposed by the natural host so that it is a low probability event for a virus to move from the natural host to a human. While an insect frequently plays the role of vector carrying a virus from an animal reservoir to humans, several zoonotic viruses are transmitted by placing humans near rodents. Several arenaviruses, minus sense single-stranded RNA viruses, jump from their rodent natural hosts directly to humans through contact with rodent urine or saliva. Notable arenaviruses are named for the hemorrhagic fever (HF) region of their zoonosis; Bolivian HF is caused by Machupo (MACV), Argentine HF is caused by Junin (JUNV) and Venezuelan HF is caused by Guanarito (GOTV). These South American outbreaks are caused by new world arenaviruses in contrast to Lassa HF (LASV), an African or old world arenavirus. LASV is an endemic disease of West Africa (Table 3.3) and can be confused with DHF or Ebola infections.

Viruses that are transmitted by arthropod vectors are called arboviruses. In 1930, only yellow fever of six known arboviruses caused disease in humans. The discovery of arbovirus caused human disease expanded beginning in the late 1930s with western and eastern equine encephalomyelitis (WEEV, EEEV) and St. Louis encephalitis. Both Chickungunya and Zika (Table 3.4) are arboviruses that have emerged to become global infections in the twenty-first century. Zika not only became infamous for causing microcephaly in newborns of infected mothers and Guillain-Barre syndrome but is now sexually transmitted between humans.

I worked on a therapeutic for the treatment of Ebola infections from 2007 to 2013. The genome sequence recovered from each outbreak from 1976 to 2013 has been different. Studies were conducted at USAMRIID in their BSL4 facility by expert Ebola investigators. Rhesus monkeys (*Macaca mulatta*) were injected with 1000 plaque forming units (pfu) of Zaire ebolavirus (ZEBOV) Kikwit into their thigh muscle. All of the untreated control monkeys died between days 8 and 10 following viral injection. We measured viral burden by quantitative polymerase chain reaction (qRT PCR) a measure of genome copies per milliliter (copies/mL) of plasma and plaque formation a measure of infectious viruses per mL (pfu/mL) plasma. We found

Table 3.4 Emerging viral infectious diseases

Agent	When	Origin	Impact
Bovine spongiform encephalopathy (BSE- a prion disease)	1991	Unknown	vCJD 2.95 M exposed, ~60,000 cases (CJD), ~60 cases vCJD/yr
Crimean-Congo hemorrhagic virus (CCHV)	12th Century	Tajikistan	3,128 cases, 156 deaths
	2002–2008	Yozgat, Turkey	
Venezuelan Equine Encephalitis (VEEV)	1995	Venezuela and Columbia	11,390 cases, 16 deaths
HIV-2		Senegal, Cote d'Ivorie	Less pathogenic than HIV-1, tests for HIV-1 detect HIV-2
Human T-cell lymphotropic virus (HTLV-1)	1977	Japan	20–30 million infected
Human parvovirus B19	1975	Global	Epidemic every 4 years
Hendra virus	1995	Australia	Race horses; several deaths
Hantavirus	1950–1953	Korea	Soldiers 3,000 cases, ~300 deaths
(Oran, Laguna Negra)	1996	Andes	25–35% case fatality
(Sin Nombre)	1993	Southwest USA	24 cases, 12 deaths
Human herpesvirus 6 (HHV-6)	1986	Global	Roseola
Human papillomavirus (HPV)		Global	12% of all females infected
SARS CoV	2003	China	8089 infected, 774 Died; >$1B cost
MERS-CoV	2014	Middle East	929 cases, 372 deaths
Monkeypox	2003	Midwest USA	Imported African rodents
Japanese encephalitis		Asia	70,000 cases, >700 deaths/yr
La Crosse (LACV)	1965	Wisconsin	787 cases, 11 deaths
	2004–2013	United States	
Marburg (MARV)	1967	Entebbe, Uganda	31 cases, 7 deaths
	2004	Angola	252 cases, 227 deaths
Norwalk	1968	Norwalk, Ohio	18% of all acute gastroenteritis
Rift Valley Fever Virus (RVFV)	1918	Kenya	1977; 100,000 cases in Egypt
Ross River (RRV)	1928	New South Whales	Infects 5,000 people/year
Rotavirus	1943	USA pre-vaccine	2,700,000 cases, 37 deaths/year

(continued)

Table 3.4 (continued)

Agent	When	Origin	Impact
Yellow Fever Virus (YFV)	2000 BCE	Africa	10% of population died (~4000)
	1648	Yucatan	
	1793	Philadelphia	3400 cases, 452 deaths
	1905	New Orleans	127,000 cases, 45,000 deaths
	2013	Africa	
West Nile Virus (WNV)	1937	Uganda	39,557 cases, 1,668 deaths (neuroinvasive disease emerging)
	1999–2013	United States	
Zika	1947	Zika Forest, Uganda	
	2016	Global	111,333 cases, 10 deaths
	2017	USA	38,303 cases

an average of $1.7x10^8$ genome copies per mL on day 8 post infection and $1.2x10^6$ pfu/mL on day 8. Nearly 100 genomes present for each successful virus in a nonhuvwas not observed, the dominant population of viral genome sequence did not change from one individual to another or from one time to another within the same individual. The existence of defective viral genomes may offer potential for rapid change but in a stable host, change was not rapid. When one considers the magnitude of differences in conditions as a virus jumps from a reservoir host to a human, rapid adaptability is a great advantage. A virus that kills all the hosts is not a successful virus. Perhaps defective viral particles facilitate host immune responses giving them a chance to catch up to the rapidly proliferating virus.

The Ebola outbreak of 2014 illuminated the collateral damage that accompanies EID outbreaks. First, the first responders are local physicians and care givers are killed leaving a population lacking doctors and nurses. Even physicians trained to exercise appropriate caution when interacting with patients such as Dr. Sheik Humarr Khan died of the Ebola infection (Bausch et al. 2014). Second, women are the main caregivers in their families and 75 percent of Ebola deaths in the 2014 outbreak were women. This leaves families lacking traditional structure. Third, the outbreak disrupts production, labor markets and trade resulting in scarcity and inflation of food prices. Food security and nutrition are diminished which preferentially affects poor people as they spend 50 to 70% of their income on food. Finally, public health measures are discontinued. Before the 2014 outbreak, 97 percent of children in West Africa were receiving routine vaccinations but that figure fell below 27 percent. The West Africa loss of measles vaccinations was followed by measles out-

breaks in the United States, suggestive of a global response to a regional loss of public health.

Contemporary Emerging Infectious Disease

Coronaviruses are unique in their large single-stranded RNA genome (20,000 bases) and are often associated with mild disease, bronchitis and gastroenteritis. A 2003 outbreak of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in China rapidly spread to 8098 cases in 37 countries (Table 3.4). Retrospective analysis of this outbreak linked zoonosis to a culinary trend in which people sought out exotic animals to eat. These nontraditional food animals appeared in street markets in urban areas bringing people close to the reservoir host triggering the outbreak. The civet cat was identified as the source of the first human cases but this is not the natural host. The natural host is the fruit bat, which infects a variety of small mammals including the civet cat. A point of intervention has been removal of the civet cat from markets but the natural bat host remains in the environment maintaining the virus for the next outbreak.

A second coronavirus outbreak in 2014 of Middle East Respiratory Syndrome coronavirus (MERS CoV) rapidly involved 23 countries including Africa, Western Europe, and Southeast Asia. The number of cases is relatively low but the case fatality ratio is of great concern (Table 3.4). The camel is reservoir host in this case but it may not be the natural host since camels often get sick as well. Transition from animal-to-human transmission to efficient human-to-human transmission was observed quickly in the case of both SARS-CoV and MERS-CoV. This rapid adaptability of a highly infectious virus group should raise concern over endemic coronaviruses in companion animals since these viruses can clearly become pathogenic, can jump from animal to human and then become transmitted from human-to-human.

An ongoing outbreak of Yellow Fever in Africa (Table 3.4) is a striking reminder that EID can re-emerge into virtually naïve populations. Some people recover from the acute symptoms but liver damage causes yellow skin, the reason for the name for the disease. The liver damage leads to bleeding and kidney damage and occasionally death. Yellow Fever is a flavivirus that originated in Africa but was distributed to the world on barges and sailing ships to tropical ports. The virus arrived in the Americas on slave ships. This virus interrupted the building of the Panama Canal which attracted the attention of Walter Reed, the military physician responsible for demonstrating transmission by mosquitos. An effective vaccine is used worldwide with immunity lasting over 10 years. The limitation of this vaccine like most is that populations need to be vaccinated regularly which in a world constantly changing due to political and economic drivers leads to vaccination gaps.

Tumor Viruses

Cancer is one of the leading causes of human death but estimates are that 20 percent of all human cancers are directly caused by viruses (Table 3.5) (Morales-Sanchez and Fuentes-Panana 2014). The first tumor virus was identified by Peyton Rous in 1911. He took cell lysates from a chicken sarcoma which he passed through a filter known to hold back bacteria and then injected the filtered lysate into chickens. A tumor developed at the injection site. The virus is now known as Rous Sarcoma Virus (RSV), a single-stranded RNA virus. Rous won a Nobel Prize in Physiology or Medicine for the discovery in 1966. RSV is a retrovirus that captured a tyrosine kinase, src gene, that triggers uncontrolled growth in infected host cells.

The Avian Leukemia Virus (ALV) was discovered in Denmark early in the twentieth century capable of causing disease in blood forming tissues of chickens. A strain of ALV, avian myeloblastosis virus (AMV) was described in 1952 that provided a convenient source of tissue for biochemical studies. During the 1930s a strain of inbred mice was found to develop leukemia between 6 and 18 months of

Table 3.5 Viruses associated with cancer

Virus	Cancer	Impact
Epstein Bar Virus- 1964	Burkitt Lymphoma	7800 Africa Endemic
[Human Herpesvirus 4-HHV4]	Gastric Carcinoma	933,900 (9% linked to EBV)
	Hodgkin Lymphoma (US)	62,400 (46% linked to EBV)
	Nasopharyngeal Carcinoma (China)	80,000 (98% linked to EBV)
	TOTAL	197,450
Human T-cell lymphotropic virus [HTLV-1] -1980	T-cell leukemia/lymphoma (ATLL)	1 of 1000 infected are symptomatic
	1980	1 in 1500 HTLV-1 carriers
Hepatitis B Virus (HBV)- 1965	Hepatocellular Carcinoma (HCC) 0.5% of HBV infections	60–80% of the worlds HCCs, HCC is the 6th most frequent cancer,
	3.5 M * 0.005 = 1.75 M HCCs	5% of all cancers worldwide
Human Papillomavirus (HPV)-1985	Cervical Carcinoma (HPV-16,18)	528,000 cases, 266,000 deaths
	[75-90% caused by HPV]	12,900 cases, 4,100 deaths USA
Hepatitis C Virus (HCV)- 1989	Hepatocellular Carcinoma (HCC)	HBV and HCV lead to:
		720,000 deaths cirrhosis/year
		420,000 deaths from HCC/year
Kaposi's Sarcoma Virus (KSHV)	Kaposi Sarcoma	rare
[Human Herpesvirus 8-HHV8]		
Merkel Cell Virus (MCV)-2008	Merkel Cell Carcinoma	1700 cases/yr USA
Polyomaviruses: SV40, BK, JC	Solid Tumors	

age were cultivated at Cold Spring Harbor. A murine leukemia virus (MLV)was isolated in 1951 from these mice extending tumor viruses into mammals. Soon a feline leukemia virus (FeLV) was isolated in 1967 followed shortly by isolation of a feline sarcoma virus (FeSV). These viruses established the cancer virus concept.

Epstein Bar Virus (EBV) Denis Burkitt identified an unusual tumor in Uganda in 1957 at the Mulago Hospital in a 5-year-old boy with swelling in his jaws (Burkitt's Lymphoma 2014). After that he saw a second case at the Jinja district hospital on the shores of Lake Victoria this became more than a curiosity. A review of hospital case notes confirmed the prevalence of these tumors of the jaw and they were accompanied by swellings in kidneys, ovaries, adrenal glands and liver. He assembled data from 38 patients and histological examination led to description of "lymphoma syndrome" or the "African Lymphoma."

Burkitt received a grant in 1961 for £250 to visit 57 hospitals in eight African countries; Uganda, Kenya, Tanzania, Malawi, Mozambique, Zimbabwe, Zambia, and Republic of South Africa to investigate African lymphoma. The tumor was found everywhere at the equator in areas where year-round temperature was above 60 °C but not in areas over 5000 feet in elevation. Burkitt further determined the tumors only occurred where the annual rainfall was above 20 inches and not seen in the dry savannah of Nigeria. Burkitt contacted doctors in Papua New Guinea to discover these tumors were the most common childhood tumor in that country but only in wet coastal regions and not in the dry highland areas.

Burkitt's working hypothesis was that an insect transmitted virus infection was responsible for the lymphoma syndrome. He then observed adults with the lymphoma but only in individuals that moved into the "African lymphoma belt." These tumors were then observed in the United States and Europe at a rate of 1–3 per million or about 100X less frequently than in Africa. Biopsy samples did produce viruses but none that came from insects so the working hypothesis was abandoned.

A new hypothesis emerged between 1963 and 1966 in which *P falciparum* (a parasite causing malaria) was the causative agent for the lymphoma. It is believed that severe malaria does play a role in the development of Burkitt's lymphoma. At the advice of Peter Clifford, Burkitt administered methotrexate to treat patients which produced encouraging results. In 1961 Burkitt gave a lecture at Middlesex Hospital, "The Commonest Children's Cancer in Tropical Africa: A Hitherto Unrecognized Syndrome." Anthony Epstein attended the lecture which changed his life and provides a punctuation mark in the history of science.

Epstein introduced himself after the lecture and the two sat down for tea. Burkitt agreed to send tumor specimens to Epstein and Epstein received a grant from the US National Institutes of Health in 1963 for $45,000 which allowed him to employ two research assistants. Yvonne Barr was one of the research assistants that was able to propagate a virus after 26 tries with tumor specimens. The other research assistant was Bert Achong, an electron microscopist, who was first to identify the virus as a member of the Herpes Virus family. The virus bears the name Epstein Barr Virus or EBV and is a causative agent of Burkitt's Lymphoma.

An interesting historical note involves Werner and Gertrude Henle at the Children's Hospital of Philadelphia. They created antibodies to EBV infected cells and their survey of blood samples from American adults revealed over 90% to be EBV positive in 1966. They also showed that normal human lymphocytes could be made immortal by infecting them with EBV in 1967. We now know EBV silently infects children in the Western World but in those infected a bit later in life become susceptible to infectious mononucleosis (the kissing disease) as adolescents.

In 1972, a collaboration between George Klein and the Manolovs led to a discovery of a chromosome change that was specific to Burkitt's lymphoma. This chromosome change was a translocation of 8:14 and rarely 8:2 or 8:22 (Zech et al. 1976). The break points in chromosomes 2, 14, and 22 contained immunoglobin genes that become active in B lymphocytes as they produce antibodies during infection. A Nobel Prize in 1989 to Michael Bishop and Harold Varmus provided the significance to the break point in chromosome 8, bringing active immunoglobin genes near the oncogene *c-myc*.

In 1970, a second cancer common in North and East Africa as well as Southern China was found to be EBV positive, carcinoma of the post-nasal space (Nasal Pharyngeal Carcinoma or NPC). The tumor is observed in epithelial cells not B-cells and is not geographically linked to endemic malaria regions. In this case, it appears that aerosol exposure to environmental carcinogens (possibly in preserved fish) may introduce mutations in nasal epithelial cells. The NPC may be the result of silent EBV infection, carcinogen induced cellular mutations, and people carrying HLA A*0207 and B*46 antigens.

In 1975, several reports describing "fatal infectious mononucleosis" appeared. David Purtilo described X-linked proliferative syndrome (XLP) based on an international registry of boys with fatal EBV infections (Purtilo et al. 1977). Using genetic marker analysis from the international registry they narrowed XLP to a three million base pair segment on the X-chromosome. In 1998, a 384-base pair segment encoding the XLP protein of 128 amino acids was found to be lost or damaged in al XLP patients (Nichols et al. 1998). Curiously, the XLP protein loss leads to a defect in NK and T lymphocytes which are necessary for recognition of EBV infected B lymphocytes. These individuals cannot make antibodies to EBV because their B cells require help from T lymphocytes.

The development of potent immune suppressing drugs like cyclosporine A was linked to lymphoma after organ transplantation. Two girls with acute lymphoblastic leukemia received bone marrow transplants from their HLA-match brothers. The grafts were successful but they developed lymphoblastic leukemia from the transplant (male cells). These lymphomas do not present chromosomal translocation like Burkitt lymphomas revealing a new path from EBV to lymphoma. The immune suppression upsets the EBV host homeostasis crippling T-cells that are required for keeping EBV under control. Another situation where the virus can replicate without control is in HIV/AIDS patients and AIDS-lymphoma is yet another EBV induced casualty.

EBV provides key insights into tumors associated with viral infections. First, the same virus is linked to multiple tumors in different populations including Burkitt's

lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma. Second, tumorigenesis involves more than one mechanistic pathway but interest has focused on the latent membrane protein-1 (LMP1), EBV nuclear antigen 1 (EBNA1), and BamH1-A reading frame-1 (BARF1) genes. Third, dissecting the association between infection with EBV and cancer has unfolded over a period of 30 years and led to a greater understanding of cancer, the immune system, and methods for immortalizing cells to study specific cell clonal populations. EBV has been a boon to scientific research while imposing a challenge to human survival.

Hepatitis B Virus (HBV) Approximately 2 billion people have been infected with HBV and 360 million suffer from chronic infection. Acute HBV infections are usually self-limiting associated with 5.2 million cases a year. Chronic HBV infections lead to complications in 15–40 percent of cases resulting in 0.5 to 1.2 million deaths each year. HB V causes 60–80 percent of the world HCC cases which represents 5 percent of all cancers in the world. The HBx gene is considered key to oncogenesis. Vaccination programs are effective in reducing mortality in infants and have reduced emerging prevalence of the disease. Dietary exposure to aflatoxin enhances HBV-related HCC (Sun et al. 1999) as well as co-infection with HCV and excessive alcohol consumption.

Hepatitis B Virus (HBV) was named due to differences in transmission: hepatitis A type virus follows fecal-oral transmission while B type virus is parenteral. The genome is circular DNA that is not fully double-stranded with the end of the full-length strand linked to DNA polymerase. The genome is 3020–3320 nucleotides long (full length strand) and 1700–2800 nucleotides for the short strand. Four genes are encoded including: (1) C the core protein (HBcAg) synthesized by uORF AUG to make pre-core protein, (2) P is the DNA polymerase, (3) S is the surface antigen (HBsAg) which has three AUG start sites that divide the gene into pre-S1, pre-S2, and S, and (4) X is a gene that is not fully understood but may be a transcriptional transactivator. Non-coding RNA include HBV PREalpha, HBV PREbeta, and HBV RNA encapsidation signal epsilon. There are 10 known genotypes which differ by 8 percent in sequence with distinct geographical distributions and are labeled A to J and at least 24 subtypes. Type F is the most divergent form and found in Central and South America, predominantly Brazil.

Human HBV has a narrow host range infecting humans and higher primates, eg. Chimpanzees. In the 1970s a Woodchuck hepatitis virus (WHV) was discovered which will not infect rodents. A ground squirrel form was identified (GSHV) that is a distant relative of the marmot and woodchuck. Indeed, DHBV infects ducks but not all species of duck, grey herons are infected with HHBV, the Ross' goose with RGHBV, the snow goose with SGHBV, white storks with STHBV, and cranes with CHBV. There are no hepadnaviruses in arthropods or insects.

Human T-cell Lymphotropic Virus (HTLV-1) HTLV-1 is a single-stranded RNA retrovirus, defined by their use of reverse transcriptase, a polymerase, that makes a DNA copy of the RNA 7 kb viral genome. The DNA viral genome is integrated into the host genome where it is referred to as a provirus and is replicated along with the

host genome during cell division. Only 1 percent of infected individuals will develop leukemia and this is observed 20 to 30 years after asymptomatic infection. The HTLV-1 tax protein is likely to initiate cell transformation through interactions with transcription activators and cell cycle regulators.

Hepatitis C Virus (HCV) HCV has infected approximately 3 percent of the world's population (~210 million) but screening of the blood supply has reduced prevalence. HCV is a flavivirus composed of a 9.4 kb single-stranded, positive-sense RNA. HCV is characterized by a single serotype but at least 6 major genotypes. Genotype 1b is the most common genotype seen in the United States and Taiwan. HCV becomes a chronic infection by evading host immune defenses through a combination of: (1) high replication rate (10^{12} virions/day) and (2) lack of error proofreading by the viral polymerase leading to mutations in response to immune pressure. The genetic variability of HCV has limited efforts to design an effective vaccine.

Human Polyomaviruses The polyomaviruses are small (3.4 kbp), double-stranded DNA viruses. Early studies with simian virus 40 (SV40) led to identification of the large tumor antigen (large T; LT). LT is also found in BK and JC viruses which are more suspect human tumor viruses and Merkel cell polyomavirus (MCV) which is now well established as a human tumor virus (Feng et al. 2008). The N-terminus of LT contains an LXCXE motif that interacts with the retinoblastoma protein RB while the C-terminus contains an ATPase/DNA helicase domain that can inactivate p53. The LS-p53 complex activates insulin-like growth factor I (IGF-I) which alone is capable of cell transformation.

Cardiovascular Viruses

A recent report of morbidity and mortality reveals heart disease and cardiovascular events are the number one killer with neoplasia and infections following close behind. However, infections frequently cause neoplasia and cardiovascular disease leading to death. If we combine cardiovascular events and neoplasia caused by infection, then infectious disease is the most significant threat to human life and qualifies as the area of greatest impact.

Enterovirus The picornavirus family are small (pico-), single-stranded, positive sense RNA genome (7.4 kb) viruses that synthesize a single polyprotein that is cut into a small collection of functional proteins by virally encoded (2A and 3C) and cellular proteases. Poliovirus is a picornavirus that has served as the prototype for the viral family. The enteroviruses are a group of picornaviruses that have been associated with cardiac disease. Coxsackievirus B (CVB), Coxackievirus A (CVA), and echovirus infections lead to cardiac signs 3.2% of the time. About one-third of patients with acute cardiac disease (inflammation of the heart) are antibody positive

for enteroviruses. While acute myocarditis is often self-limiting, chronic cardiac disease often leads to dilated cardiomyopathy (DCM) which is present with no heart inflammation. DCM is associated with heart failure which can be lethal (Table 3.6). These chronic infections lead to 50 percent of all cardiac transplants worldwide. The enteroviral 2A protease can also degrade dystrophin in the heart leading to cardiac necrosis, reduced ejection fraction, and then to dilated cardiomyopathy. Transmission of these viruses is from contaminated food and water.

Cytomegalovirus (CMV) The human herpesviruses are large double-stranded DNA genome viruses. The human herpesvirus-5 (HHV5) or cytomegalovirus (CMV) has a 235 kbp genome encoding over two hundred genes. One problem in finding associations with CMV infections is that it is not an emerging disease, 50 to 99% of the population has been infected making comparisons to a control group challenging. Most infections are observed in children and in newborns serious clinical findings can be observed. Hence, most adults carry latent infections that are reactivated when individuals become immune suppressed following solid organ transplantation, malignant hematological disease, and AIDS. Reactivation in immune suppressed individuals is associated with increased mortality. The association with cardiovascular disease has been demonstrated in two recent studies. In one, CMV infections were detected in 14.5 percent of coronary artery samples from bypass operations compared to 4 percent in of patients who needed cardiac surgery for reasons other than atherosclerosis Hebar et al. 2015). In the other, CMV reactivation (viremia) was detected in 16.5% of immunocompetent patients admitted for major heart surgery (Roa et al. 2015). The incidence of coronary artery disease is the major contributor to death from heart disease and if 14 to 16.5% of this disease is associated with CMV infections, this infection is a significant human health hazard.

Finding an Emerging Infectious Disease

Recognizing an emerging infectious disease involves well established strategies of surveillance; (1) identify unusual clusters of disease, (2) evaluate the spread of an outbreak, (3) estimate the magnitude of the problem, and (4) if possible identify the

Table 3.6 Viruses associated with cardiovascular disease

Virus	Cardiovascular disease	Impact
Enterovirus Infections	Myocarditis	CVB3-34.6/1000; Mortality in newborns is 75%
Coxsackievirus		Chronic infections-dilated cardiomyopathy (DCM) cause 25% of 750,000 cases of heart failure, 250,000 deaths; Responsible for 50% of cardiac transplants worldwide.
Cytomegallovirus	Heart Disease	Atheroscleotic plaque

infectious agent. The strategy has proven valuable for known infectious and noninfectious diseases but has limited capacity to detect emerging infectious diseases. However, deviation from the traditional approach to surveillance is not likely to gain support.

Al Smith, a veterinary virologist, approached me after a seminar I presented in 1997 in the College of Veterinary Medicine at Oregon State University. I had just joined AVI BioPharma as the head of their research and development program. Al was a veterinarian and professor and had devoted his career to the *Caliciviridae* family of viruses dating back to his time in the naval research station in California. He had isolated the nonhuman vesivirus group of caliciviruses not only from suffering sea lions in the Channel Islands, but from reptiles in the San Diego zoo and whales held in captivity. Al and his capable technician, Doug Skilling successfully propagated the virus isolates in cell culture, an accomplishment not shared by other laboratories at the time. Al developed nucleic acid probes and induced antibodies to these viral isolates. Over decades of research he assembled an extensive collection of vesiviruses which were held in redundant −70° freezers. His singular vesivirus focus gave the appearance of a zealot but his ability to cultivate viral isolates, his extensive collection of isolates, and his one of a kind detection reagents made him a one of a kind virologist. Al was well beyond retirement age and was an "old school" virologist ready at a moment's notice to take his bag into the real world and collect swabs from ailing animals. He maintained careful records of the condition of his patients, their location, and the setting of the animal in the community. Every field trip led to work in the lab propagating virus from his swabs.

Al wanted to know if I would be interested in finding an antiviral agent for these viruses. My prime directive at AVI BioPharma was to explore the capabilities of our proprietary antisense technology and I had yet to investigate targeting a virus. The caliciviruses have positive sense single-stranded RNA genomes which express three genes from a single polyprotein. We identified an active agent following an investigation with small collection of candidates (Stein et al. 2001).

The vesivirus group of caliciviruses are considered animal only and are not believed to infect humans but Al began exploring human samples with his collection of detection reagents. After several years making incremental progress, we found an association between human blood samples seropositive for vesivirus and markers of liver disease (Smith et al. 2006). We felt this evidence of an emerging infectious disease in humans and its potential to cause liver disease would be welcomed by the medical community.

Al and I made a trip to Washington DC at our own expense to relate the findings in person. We meet with virologists at the National Institutes of Health but were met with judicious skepticism. Harvey Alter had been instrumental in the discovery of Hepatitis C Virus (HCV) and felt all viral liver disease is already accounted for by hepatitis viruses. Hence, no interest in our findings. We met with the American Red Cross blood banking group in Shady Grove Maryland and were met with concern. Blood supplies are scrutinized by the nucleic acid test (NAT) to eliminate viral contamination and any further elimination of blood samples would threaten an already limited inventory. Our findings simply add complication to the blood supply

business. They asked for more compelling and more extensive data to add robustness to our claims. The only problem is that they were not interested in providing financial support for the recommended studies. The conundrum is all too common, you need more data to convince granting agencies to support the work but there is no support to add to the limited data.

Life on the cutting edge is frequently discouraging. Our strategy took two paths: one to seek commercial support and the other to create proof of concept data for an antiviral solution. The most logical commercial solution was to meet with Michael Houghton at Chiron. He was the driving scientific force in finding HCV and Chiron might like adding to their reputation by finding another previously unrecognized hepatitis virus. Indeed, Dr. Houghton invited us to bay area to discuss the project. He reviewed our data and the quality of Al's detection reagents. Chiron provided modest support and their in-house laboratory help to confirm our observations, a glimmer of hope. Al created a company, Calicitech, so that he could accept support and license his reagent patents from the university.

Testing an antiviral agent in humans would be expensive but in the absence of natural history of the infection would make human proof of concept impossible. However, Al had been contacted by a cat rescue facility in Atlanta, Mommy Cat, describing an outbreak of feline calicivirus (FeCV) in their cattery. These cats had all been vaccinated with an approved FeCV vaccine which failed to protect these cats. A veterinarian can decide to use alternative medicines if there are no alternatives and the owner oc the cats can sign the equivalent of informed consent. We provided our FeCV specific antiviral to Mommy Cat along with a detailed protocol for treatment. The infected kittens we treated survived while those not treated died providing encouraging data. We then discovered a similar outbreak in the humane society facility in Eugene Oregon, our neighbors. Again, we reached an agreement to treat infected kittens and were successful (Smith et al. 2008). This was the first time we treated an infection based on symptoms in an "outbread" population of cats. With over 100 kitten patients and remarkable survival differences between treated and untreated, we had our proof of concept.

There is no disease cluster to link to human vesivirus. Norovirus is a calicivirus and is known to infect humans, in fact it is well known on cruise ships, day care centers, and extended care retirement facilities. Our efforts to have human vesivirus recognized as an emerging infectious disease have failed. Chiron was not impressed with our antiviral and no longer were interested in evaluating the detection reagents. If we are lucky, human vesivirus infections will remain mild clinical oddities. Al Smith has an interesting way of responding, "That virus is out there infecting humans. It won't go away."

Non–Pathogenic Viral Infections

The existence of non-pathogenic viral infections led to the emergence of the study of the immune system, vaccination, gene therapy, and concern for future pandemics. We carry evidence of ancient retroviral infections in our genome from integration events that became vertically transmitted making up as much as 8 percent of the human genome (Meyer et al. 2017). These genomic fossils are called endogenous retroviral genomes (ERV). Most ERV sequences accumulate sufficient mutations over evolutionary time that horizontal transmission is unlikely. These viral genome segments are trapped but can still be transcribed and encode some viral proteins. The significance of these integrated genomes is a current topic of investigation.

Adeno-associated Virus (AAV) is a single stranded DNA virus that infects humans but are not known to cause disease. The lack of pathology has led to their use as viral vectors for human gene therapy. AAV can infect dividing and quiescent cells and will persist extra chromosomally without integrating into the genome of the host cell. AAV is a member of the *Parvoviridae* family in the genus *Dependoparvovirus*.

Mopeia Virus (MV) is an Arenavirus closely related to Lassa Virus and shares reservoir hosts. MV is naturally attenuated and nonpathogenic in humans. Infection of humans with MV protects against lethal challenge with LV (Wulff et al. 1977).

GB Virus C (GBV-C)/Hepatitis G Virus (HGV) is a flavivirus (Pegivirus Flaviviridae) named after G. Barker, a surgeon, first identified in 1995. HGV infects one sixth of the world's population but does not cause human disease. A meta-analysis of 15 publications investigating GBV-C infections in HIV-positive individuals indicate coinfection with GBV-C slows the progression of HIV disease in individuals that have been seropositive for 2 years or more (Zhang et al. 2006a). Two of the studies investigated GBV-C 5 years after documented HIV seroconversion estimate the hazard ratio of 0.36 (95% CI).

Human spumaretrovirus (HFV; Spumavirus) is a retrovirus first identified in 1971 from a lymphoblastoid cell culture from a Kenyan patient with nasopharyngeal carcinoma. HFV is homologous to primate foamy viruses and is most closely related to the chimpanzee foamy virus (SFVcpz). Early studies raised alarm for association with autoimmune diseases but more extensive studies with more precise diagnostic reagents fail to find a disease associated with HFV. HFV is a rare human infection and concerns parallel SFV infections in humans.

Simian Foamy Virus (SFV; Spumavirus-retroviridae) is a retrovirus infecting most primates born in captivity and people making contact with infected primates can become infected. Human infections frequently occur in males probably requiring a bite from infected nonhuman primates but are harmless. The infected cells often fuse to form syncytia of giant foamy cells, which gives the virus its name. The

error rate in SFV genomes is exceptionally low, 1.7 x 10^{-8} substitutions per site per year, compared to HIV 10^{-3} substitutions per site per year. Since so-called cross-species, infections have only been observed for a little over a decade the long term consequences are not known. These infections are watch and wait for everything from a zoonotic epidemic to identified disease clusters. Perhaps this is exactly the sort of infection that will emerge as a significant human concern in the future.

Torque Teno Virus (TTV; Alphatorquevirus) is a single-stranded, positive sense DNA genome virus about 3.8 kb in size in the *Anelloviridae* family. Nearly 100 percent of even healthy individuals are infected in some countries. The virus was discovered in 1997 as the "transfusion transmitted virus (TTV)" in a Japanese patient. It is often found in patients with liver disease but does not cause hepatitis on its own. Closely related Torque Teno mini virus (TTMV) were isolated in 2007 and found to have smaller genomes of 2.8–2.9 kb. TTMV infections are also common but do not cause any described human disease.

Human Adenovirus Type 5 (rAd5) is used to create an Ebolavirus (EBOV) vaccine encoding Zaire ebolavirus glycoprotein failed to protect animals immune to Ad5. A replication defective chimpanzee-derived adenovirus (ChAd3-EBO-Z) provided protection against lethal EBOV challenge in macaques but protection wane over several months. They boosted with a modified vaccinia Ankara (MVA-BN-Filo) that led to durable protection (Stanley et al. 2014). This vaccine progressed through phase I, single-blind, randomized human trials in Mali between 2014 and 2015. A single dose of the ChAd3-EVOV-Z is efficient as a prime vaccine strategy followed by MVA-BN-Filo as a boost was well tolerated in humans Tapia et al. 2016).

WU polyomavirus (WUPyV; Polyomavirus) is a 5229 base double-stranded DNA virus infecting less than 5 percent of the human population. Wu, named after Washington University, is found as a co-infection in various respiratory infections but WU does not cause disease on its own. WU is closely related to KI virus that also is not known to cause clinical disease. However, related polyomaviruses that are clinically relevant include BK virus associated with nephropathy, JC virus associated with progressive multifocal leukoencephalopathy, SV40 virus associated with mesothelioma, and Merkel cell polyomavirus associated with cancer.

Vaccinia virus (Orthopoxvirus) is a large double stranded DNA virus closely related to Smallpox. Edward Jenner, the father of immunology, found the milkmaids exposed to cowpox (vaccinia) were immune to smallpox in 1798. This was the first vaccine (named after vaccinia) leading to the modern vaccine that has allowed for the eradication of smallpox.

Viral sequences are constantly mutating with no purpose other than seeking a survival/infectivity benefit. This means viruses with no current pathology represent a pre-mutation reservoir for the next catastrophic human pandemic. The popularity

of RNAseq is likely to expand our catalog of nonpathogenic viral infections. However, the management of such information is in question.

Antiviral Drugs

Technology used to counter viral infections has resulted in over 90 approved drugs for the treatment of nine different human viral infections in just 50 years (De Clercq and Li 2016). Several different antiviral drug groupings have been reported, but the following arise from review of the mechanisms of action of antiviral drugs assembled in Table 3.7: (1) Inhibition of viral attachment and entry, (2) Inhibition of viral uncoating, (3) Viral Polymerase Inhibitors, Nucleotide analogues (NTRTI) and Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTI) and DNA Polymerase Inhibitors, Nucleic Acid Synthesis inhibitors and Nucleotide Pool Size Agents, (4) Latency Reversal Agents, (5) Integrase Inhibitors, (6) Protease Inhibitors for both HIV and HCV, (7) Neuraminidase Inhibitors, (8) Immune Response Modifiers, and (9) Antisense Inhibitors.

Table 3.7 Comprehensive list of antiviral mechanisms of action

Name	Trade name	MOA	Use	Approval date	Number Agents
Amantadine		Inhibit Viral Uncoating	Influenza A	1966	2
Podofilox	Condylox	Antimitotic Agent	HPV	1990	1
IFN-alpha-2b	Intron A	Immune Resp. Modifier	HBV	1986	5
Saquinavir	Fortavase	HIV Protease Inhibitors	HIV-1	1995	12
Idoxuridine	Dendrid	ntRTI/DNA pol Inhib	HSV	1963	34
Oseltamivir	Tamiflu	Neuraminidase Inhibitor	Influenza A	1999	4
VZIG	VZIG	Entry Inhibitor	VZV	1981	7
Fomivirsen	Vitravene	Antisense	CMV retinitis	2006	1
Boceprevir	Victrelis	HCV Protease Inhib	HCV	2011	5
Raltegravir	Isentress	Integrase Inhibitor	HIV-1	2007	5
Panobinostat[a]	Farydak	Latency Reversal Agents	HIV-1	2015	4
Nevirapine	Viramune	NNRTI	HIV-1	1996	5
Simeprevir	Olysio	NS5A RdRp	HCV	2013	10
Foscarnet	Foscavir	Nucleic Acid Synth Inhib	HSV, CMV	1991	1
Ribavirin	Copegus	Nucleotide Pool Size	HCV, Viral HF	1985	1
Imiquimod	Aldara	TLR7 Against	HPV	1997	1

[a]approved for Multiple Myeloma use as antiviral is experimental and off-label

Antibodies

Administration of hyperimmune sera from immunized animals or human donors was the first effective treatment for infectious diseases. The practice has limitations but is still used to treat bacterial toxins and viral infections caused by CMV, RSV, HAV, HBV, RABV, VZV and MEV (Keller and Stiehm 2000). The development of human or humanized monoclonal antibodies (HumAbs) has created a feasible way to rapidly generate novel antiviral therapeutics. HumAbs have advantages over serum therapy in that they are chemically defined reagents with minimal variability, greater activity per mass of protein, and they have no immunological consequences related to serum sickness. Several mAbs have been approved for treatment of infectious diseases including viral and bacterial pathogens (Table 3.8).

The antiviral mAb discovery field is exploding with activity particularly for HIV and HCV infections. A humanized mAb targeting lymphocyte CCR5 receptors called PRO140 has demonstrated potent and prolonged anti-HIV-1 activity and a large margin of safety (Jacobson et al. 2010a). Administration of PRO 140 by the subcutaneous route offers patients a way to self-administer the mAb but importantly the mAb is transported in the lymphatics providing enhanced access to binding to the cellular target (Jacobson et al. 2010b). The next generation of antiviral therapeutics are likely to be dominated by mAbs.

Vaccines

Perhaps the only way to clear a viral infection involves a host immune response (Table 3.9). The innate immune response is particularly effective centered on a type 1-interferon pathway. Unfortunately, many viruses carry mechanisms to evade host innate responses and innate immune effectors do not have the capacity for memory. The adaptive immune response can mitigate infection with antibodies, generally to surface antigens, which prevent the spread of the virus and T-lymphocytes, which can clear virus-infected cells.

Table 3.8 Approved monoclonal antibodies for antiviral passive immunotherapy

Agent	Trade name	Virus	Target	Year
Palivizumab	Synagis	RSV	F protein	1998
Raxibacumab	Raxibacumab	Bacillis anthracis	protective antigen	2012
Siltuximab[a]	Sylvant	HHV-8	IL-6	2014
Obiltoxaximab	Anthem	Bacillis anthracis	protective antigen	2016
Bezlotoxumab	Zinplava	Clostridium difficile	Toxin B	2016
Avelumabb	Bavenco	Polyomavirus	PD-1	2017

[a]Approved for treatment of Castlemen's disease
[b]Approved for treatment of Merkel Cell Carcinoma

Viruses Not Targeted by Antiviral Drugs, Monoclonal Antibodies nor Vaccines

The vast majority of viral infections have no treatment options, neither drug, antibody, nor vaccine. Attention is focused on emerging viruses such as Epstein-Barr virus (EBV), human parvovirus B19, Human norovirus, human rhinovirus, human herpesvirus 6, human coronaviruses (SARS and MERS CoV), human astrovirus, human sapovirus, chickungunya virus, dengue virus, West Nile virus (WNV), Hendra virus, Nipah virus, Venezuelan Equine Encephalitis (VEEV), Eastern Equine Encehalitis (EEEV), Western Equine Encephalitis (WEEV), Ebola virus (EBOV), Marburg virus (MARV), Bundibugyo, Lassa Virus, Junin Virus, Machupo virus and Zika virus.

The first strategy will be to use an existing drug designed for another virus off-label. This seems likely for antiviral drugs like Cidofovir, Foscarnet, and Ganciclovir

Table 3.9 Comprehensive list of viral vaccines

Vaccines	Virus	Trade names	Year	Efficacy	Recommendation
Adenovirus	ADV-4,7				Military recruits
Hepatitis A	HAV	Havrix, Vaqta, Epaxal	1995	95	2 doses IM by age 1
Hepatitis B	HBV	Sci-B-vac, Engenix-B,	1981	85–90	babies of mothers with HBV
Hepatitis E vaccine	HEV	Hecolin			China approved in 2012
Human Papillomavirus	HPV	Gardasil, Cervarix	2006	70	Women 9–25, Men 9–26
Influenza vaccine	IFV A	FluMist, Flozone, Influvac	1930	40–60	Yearly >6 mo, >65 y/o
Japanese Encephalitis	JEV	Ixiaro	1930	90	In areas with endemic JEV
MMR vaccine	MEV	Prionix, MMR II, ProQuad	1963	>75	Children age 1+4
MMR vaccine	Mumps	Prionix, MMR II, ProQuad	1967	>75	Children age 1+4
Polio Vaccine	PV	Kinrix, Pediarix, Ipol	1955	99	All children, 3 doses
Rabies Vaccine[a]	RABV	Imovax, RabAvert	1885		High risk areas
Rotavirus Vaccine	RotV	Rotarix, Rotateq	2006	45	Routine Vaccinations
MMR vaccine	RUBV	Prionix, MMR II, ProQuad	1969	>75	Children age 1+4
MMRV	VZV	Tetra	2005		All Children 1–2
Shingles Vaccine	VZV	Zostavax	2006	51	All adults >60
Smallpox Vaccine[b]	VARV	Dryvax, Imvanex	1796		Virus erradicated
Yellow Fever Vaccine	YFV	YF-VAX	1938	99	Routine where endemic

[a]Developed by Pasteur and Roux

[b]First recognized vaccine attributed to Edward Jenner

particularly for double-stranded DNA viruses like EBV, HPV, and HHV6. Ribaviran has been used for a number of single-stranded RNA viruses including polio, Junin, and Lassa Fever. Secondary strategies will require investment of time and effort beginning with vaccine development and creation of monoclonal antibodies.

Platform Technology RNA-based therapeutics offer rational design for an expansive number of new antiviral strategies. This advantage is superimposed on the theoretical advantages of selectivity, specificity and affinity provided by Watson-Crick base pairing. RNA-based therapeutics are expected to provide a substantially more narrow range of pharmacokinetic properties and toxicities thus are easier to compare to each other and ultimately combine into multi-agent cocktails. However, the mechanism of action may vary from RNAse H or RISC mediated degradation of the targeted RNA or steric inhibition of RNA function. The objectives of our antiviral program have been to exploit the broad understanding of RNA-based therapeutics for antiviral activity with a common chemical type, the phosphorodiamidate morpholino oliogmer (PMO) and their enhanced derivatives. In this way the mechanism of action is common to all agents, which is steric blockade of RNA function.

Studies reported by Zamecnik and Stevenson introduced the first approach to identification of an antisense antiviral agent (Zamecnik and Stephenson 1978; Stephenson and Zamecnik 1978). They used a 13-mer targeted to Rous sarcoma virus. Since the Rous sarcoma virus pioneering efforts, antiviral RNA based therapeutics have involved multiple oligomer chemistries with a variety of different mechanisms of action.

Chemical approaches to oligomers directed to HIV have been plentiful. A nonionic methylphosphonate oligonucleotide targeted to the splice acceptor site of HIV tat inhibited splicing of viral RNA (Sarin et al. 1988) inhibiting syncytia formation and p24 synthesis at 3uM concentration. Poor aqueous solubility limited the utility of the methylphosphonate chemistry. Phosphoramidate chemistry was investigated for inhibition of the splice-donor and splice-acceptor of HIV tat Agrawal et al. 1988) and were more potent but these agents were cytotoxic and poorly water soluble. Phosphorothioate chemistry targeting HIV-rev (Matsukura et al. 1987) and HIV-tat were shown to be effective in inhibiting HIV replication, were not cytotoxic and were very soluble. Further, the HIV-rev phosphorothioate oligodeoxynucleotide was stable *in vivo* with an acceptable pharmacokinetic profile (Iversen et al. 1994). A 25-mer phoshorothioate called GEM91 targeting the initiation site of HIV-gag was evaluated in clinical trials (Agrawal 1998) but the trials were discontinued. I focused on phosphorodiamidate morpholino oligomer chemistry which is both stable and net-neutral in charge at physiological pH.

Single Stranded RNA Viruses with Positive Sense (ssRNA(+)) These viruses are the most simple in terms of genome size, number of potential translated viral proteins, their genomes are all linear and they enter the cell ready for translation. The design of steric blocking RNA-based therapeutics involves preventing translation, disrupting RNA secondary structure and masking recognition sites for RNA dependent RNA polymerase (RdRp). The targeting of either the 5′-terminus and the first

ORF-AUG are active. Further, efficacy in animal challenge studies is observed with high fidelity when the most effective agent identified in vitro is employed.

The family *Astroviridae* with six different human astroviruses (HuAstV) responsible for 2–17% of all gastroenteritis. We found the 5′-terminus[1] to be the most effective site to target. This was also the optimal site for *Caliciviridae* including vesivirus (VeV; Martin-Alonso et al. 2005; Stein et al. 2001), Norovirus (NoV; Bok et al. 2008), and feline Calicivirus (FCV; Smith et al. 2008); the *Flaviviridae* including Dengue (DEN; Kinney et al. 2005; Holden et al. 2006), and West Nile Virus (WNV; Deas et al. 2007; Zhang et al. 2008), *Arteriviridae* including agriculturally important Equine Arterivirus (EAV; van den Born et al. 2005), and porcine respiratory and reproductive virus (PRRSV; Zhang et al. 2006a, b); and the *Togaviridae* exemplified by Venezuelan Equine Encephalitis Virus (VEEV; Paessler et al. 2008). The family *Coronaviridae* revealed a new active target site in the transcription regulatory sequence (5'-CGAAC-3′) in both mouse hepatitis virus (MHV; Neuman et al. 2004) and the Severe Acute Respiratory Syndrome virus (SARS; Neuman et al. 2005). The *Picornaviridae* active target site involved a highly conserved sequence in the internal ribosomal entry site (IRES) in polio virus (PV; Stone et al. 2008), foot and mouth disease virus (FMDV; Vagnozzi et al. 2007), and the coxsackievirus (CVB3; Yuan et al. 2006).

Single Stranded RNA Viruses Negative Sense (ssRNA(−)) These viruses are generally more complex with respect to genome size, number of potential translated viral proteins, and multiple genome segments. The genome must be replicated prior to translation of viral proteins. The design of steric blocking RNA-based therapeutics is similar to the positive sense RNA genomes in that targets involve preventing translation and masking recognition sites for RNA dependent RNA polymerase (RdRp).

We investigated 11 different targets in measles virus (MeV), a member of the *Paramyxoviridae,* finding the translation initiation start site of N the optimal target (Sleeman et al. 2009). Studies with the human respiratory syncytial virus (hRSV) found the translation site for L to be most active (Lai et al. 2008). The *Orthomyxoviridae* studies investigated influenza A virus probing each of the 8 viral segments finding translation of PB1 active as well as the 5'terminal of vNP for H3N2 (Ge et al. 2006) and H3N8 (Lupfer et al. 2008) but a combination of targets was required in animal studies with a high pathogenic viral H7N7 isolate (Gabriel et al. 2008). More extensive influenza A studies revealed a new target, the M-segment splice donor site. This target was evaluated in phase I clinical trials.

The antisense platform technology has limitations in targeting viral sequences. Inhibiting virally encoded proteins or blocking viral replication by interfering with the polymerase does not always work. The *Arenaviridae* family proved difficult. We

[1] Collaborative studies with Drs. David Matson and Anne Campbell at Eastern Virginia Medical School identified the most effective target which involves the highly conserved sequence at the 5′-end of the 5'-UTR.

experienced some success with Junin and Lymphochoriomeningitis virus (LCMV) in cell culture observing 4 $\log_{10}$ reductions in viral titer with a PMO targeting the highly conserved viral genome terminus. However, we failed to provide survival benefit to guinea pigs challenged with either Junin or Lassa virus. We then tried the mouse challenged with LCMV and failed again but we observed hemorrhagic disease in the mouse, a severe consequence of infection that mimics the worst aspects of arenaviral infection. We decided to try targeting host genes to mitigate disease in the mouse and found targeting IL-17 provided a survival benefit (Schnell et al. 2012). The success in the face of failure puts targeting host genes at the center of attention for dealing with emerging infectious diseases.

The antisense platform technology represents an excellent approach to rapid drug discovery for emerging infectious disease. Rapid discovery demonstrated repeatedly but of course, the cost of advanced development is daunting. The application is best for immediate treatment of index cases, close contacts, and healthcare workers.

An Unfavorable Climate for Antiviral Therapeutics

We have an 800-pound gorilla in the room and everyone in the room considers it someone else's problem. The current course is to ignore the majestic creature until it starts tearing limbs from people and then the consensus is to kill the gorilla. Agencies like the World Health Organization (WHO), the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) can assemble highly skilled personnel and can confer with some of the greatest minds in the world. Unfortunately, they are all aware of the potential problem that an emerging pandemic is likely to take us by surprise. Significant speculation that a single-stranded RNA virus will emerge killing tens of million people, costing hundreds of billions of dollars, and changing the course of human history.

There is a high probability that the virus will be influenza A (H5N1 or H7N9) that will jump from a reservoir population of birds and establish human-to-human transmission. The pandemic will be a global event by the time an effective vaccine is available. Neuraminidase (NA) inhibitors as therapeutic and prophylactic agents in the setting of pandemic influenza A (FLUA) were called in to doubt in the past decade (Michiels et al. 2013; Jefferson et al. 2014). Indeed, 100% of isolates in the 2008/2009 A/H1N1 pandemic were found to be resistant to adamantanes, and resistance to oseltamivir (Tamiflu; OSL) was observed in virus recovered from individuals taking OSL therapeutically or prophylactically (Dharan et al. 2008; CDC MMWR 2009), while effect on duration of shedding was not impacted. A recent outbreak of an influenza A (H7N9) virus caused 137 cases and 45 deaths in China revealed a novel NA mutation R292K resulting in high level resistance to OSL (Wang et al. 2014). Therapeutic options for treatment of individuals with complicated influenza A are severely limited, perhaps no options. Pandemic strains such as A/H1N1pdm09 carried significant morbidity and mortality, particularly in those

who had not experienced H1-strain influenza in their lifetime. We are also witnessing more rapidly emerging highly pathogenic avian influenza strains that are resulting in human infection; some such as A/H5N1 and A/H7N9 appear to becoming more efficient in person-to-person transmission, and reports suggest OSL resistance develops during treatment (de Jong et al. 2005; Lam et al. 2015). We are not ready for an outbreak of avian flu or any other emerging single-stranded RNA virus. Why not?

An estimate for the time and cost to develop a new drug is 10 years and $1 billion. The commercial use of a drug for an emerging infection is hard to estimate since by definition when you start development the infection has not emerged. Most of us would be unlikely to use our retirement savings to invest in a drug development project with no reliable way to expect a return on our investment. It is a poor business model. When you consider there may be hundreds of emerging infectious diseases each times 10 years and $1 billion each the task is daunting. On which disease should we focus?

Consider the Ebola drug AVI-7537. The Ebola therapeutic project began in 2004 following a laboratory accident at USAMRIID. We identified three compounds each with activity and when combined we observed unprecedented efficacy in a lethal challenge primate animal model (Warfield et al. 2006). Hundreds of experiments over the next 3 years optimized these agents (Swenson et al. 2009). We were able to obtain research grants from the Transformational Medical Technologies (TMT) division of the Defense Threat Reduction Agency (DTRA) within the Department of Defense (DOD) and we completed proof of efficacy studies (Warren et al. 2010). This led to submission of an investigational new drug application (IND) to the Food and Drug Agency (FDA) and phase I safety and tolerability studies were conducted I healthy human volunteers (Heald et al. 2014). After 11 years of continuous effort, we completed key aspects related to the FDA approval process under the "Animal Rule" and we streamlined our treatment to a single agent, AVI-7537 (Warren et al. 2015). However, shortly before the Ebola outbreak in Western Africa, the US government "budget sequester" cancelled our project and the most advanced therapeutic on the planet was not deployed to treat those infected during the outbreak. As the outbreak continued, we found no viral resistance of AVI-7537 unlike the monoclonal antibody therapy in use (Khiabanian et al. 2014). AVI-7537 sits on a shelf, a political casualty and an unfavorable business model.

Environmental Viral Reservoirs

The global virosphere may contain up to 10^{31} virus/virus-like particles (Suttle 2005), the greatest reservoir of genetic diversity. The Earth's atmosphere transports viruses all over the planet. Viruses are found in soil at 1.5×10^8 to 6.4×10^8 particles per gram of dry soil (Kimura et al. 2008). The surface oceans carry approximately ten million viral particles in each milliliter of seawater, most of which are bacteriophages (phage). The small viral particles are easily carried into the upper

atmosphere by up drafting winds. Bacteria are deposited from the atmosphere at a rate of 0.3 to 8 x 10^7 per meter each day and viral deposition rates are 9–461 times greater (Reche et al. 2018). These phages influence bacterial lifecycles and play a role in natural energy and nutrient cycles fundamental to life on Earth. The dynamics of phage-bacterial evolution drive changes in photosynthesis, phosphate, and nitrogen balance (Breitbart 2012). Human accidental release of radioactive waste (discussed in Chap. 2) and disposal of chemicals including potent antiviral and antibacterial compounds (discussed in Chap. 7) may alter the eco-evolutionary dynamics producing unanticipated environmental consequences.

Conclusion

The objective of this chapter has been to provide convincing evidence that infectious disease is the most significant threat to human health. The focus has been on viral infections because they rely on host ribosomes to produce their proteins, recent emerging infections have been from single-stranded RNA genome viruses, and replication of RNA viruses is error prone. Pandemic infections have accompanied the rise of human civilization frequently re-occurring leaving a lasting imprint on human history punctuated by profound loss of life. Emerging infections become endemic with an annual death toll. Each decade brings a new onslaught of emerging infectious agents. We are surprised again and again but have never prepared for these inevitable catastrophies. The long-term consequences often remain unrecognized and are always inconvenient such as cancer, cardiovascular disease and immune associated diseases that threaten our health. Reliance on clusters of clinical symptoms in the face of diverse and non-descriptive viral infection symptoms is a foolhardy form of crisis management. Infectious disease will certainly continue to pose the most significant threat to human health in the age to cell phones, artificial intelligence, and global commerce.

Rapid replication of viral genomes combined with low fidelity polymerases provide the foundation for an unending source of new emerging infectious agents. These traits also make viral genomes sensitive to environmental contaminants in a way that may expand probabilities for zoonosis. Infectious disease as part of our environment is not appreciated. The study of infectious disease is not a part of the curricula of students in environmental science/management. Textbooks in in environmental studies do not include chapters in infectious disease. The integration of research at superfund sites focused on chemical contamination with infection and zoonosis would result in valuable insights into threat analysis.

Viruses with RNA genomes lack sequence proofreading quality control during replication. The cumulative mutations in their genomes limits the genome size to under 30,000 bases. Essentially, a larger genome would evolve out of existence, so called catastrophe evolution. The limited genome size makes these viruses exceptionally resilient to a changing environment. The virus must economize by combining functions. This means evolution and resilience are the same thing in the RNA genome viruses. A unique insight is that in human evolution is restricted to the DNA genome and resilience limited to RNA, as it is in the RNA genome viruses.

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