Viruses and the Future – Promises and Problems

CHAPTER 22

- Clouds on the horizon – emerging disease
- What are the prospects of using medical technology to eliminate specific viral and other infectious diseases?
- Silver linings – viruses as therapeutic agents
- Why study virology?
- QUESTIONS FOR CHAPTER 22

The study of viruses in the past century has provided many insights that have been successfully exploited in the development of modern biology and medicine. Virology is now a "mature" discipline, and while much detail remains to be elucidated, there is no doubt that the basic processes of virus replication, infection, and pathology are understood. The ever-accelerating pace of technology will only serve to facilitate the study of new viral variants and provide new information on old problems.

While technology has provided many benefits to our studies and our lives, especially to those of us in the "technologically developed" (European, American, East Asian) countries, which hold economic sway at the beginning of the twenty-first century, we should be sophisticated enough to know that exploitation of our understanding of nature is a mixed blessing. Technology has bred many problems. These problems are different in scope and impact from those facing the world at the beginning of the twentieth century, but loom large, especially to those in less economically favored portions of the globe. Viruses are part of the problem and may provide part of the solution.

Clouds on the horizon – emerging disease

The ravages of the "new" disease AIDS, caused by an emergent virus, HIV, has generated much concern, controversy, and public debate. It is not the first virus to cause concern, and will not be the last. Despite its success in garnering media attention, HIV is nowhere close to being near the top among infectious diseases in threats to human welfare. The most formidable viral challenge to our society in the 20th century was caused by influenza!

The Spanish influenza worldwide pandemic in the last year of World War I killed 20 million people worldwide, and more than 600,000 in the United States alone. Many of its victims were young people in the prime of life—those who might be expected to be the least affected by such a disease. The large spike in infectious disease mortality caused by this virus is clearly evident in the statistical display of mortality rates due to infectious disease shown in Fig. 22.1.

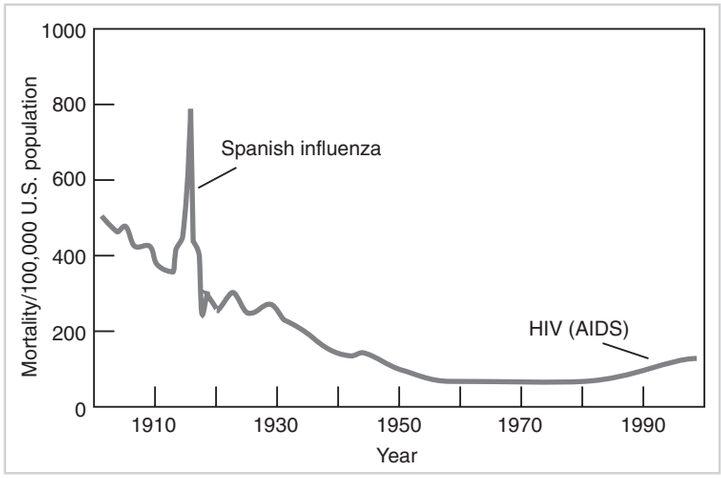


Fig. 22.1 Mortality from infectious diseases in the United States between 1900 and the present. The data show a continuing decline associated with improvements in public health measures and antiseptic methods in hospitals through the end of World War II. The already low rate was then further lowered by the use of antibiotics. The two increases seen are due to the 1918 Spanish influenza worldwide pandemic and the onset of HIV-induced AIDS in the early 1980s. The rate may continue to increase as antibiotic-resistant bacterial strains become established in the population. (Data are from the US Centers for Disease Control and Prevention.)

The lethality of the Spanish influenza was in part due to its causing hemorrhaging and extensive cell damage in pulmonary tissue, much like the more recent hantavirus infections seen in the winter of 1994 in the American Southwest. Victims essentially drowned in their own body fluids. Why did this Spanish influenza virus cause so much damage to the lungs while earlier and subsequent strains of flu did not? This question is an important one, and it has been suggested that perhaps the virus was a direct infection from an avian strain instead of passing through pigs, as is the usual route of infection. As discussed in Chapter 16, such transmission was recently reported for a strain of avian influenza A in Hong Kong. Another possibility is that it encoded an unusual gene or combination of genes responsible for its devastating virulence and unique (for influenza) patterns of pathogenesis.

In an attempt to answer such questions, US Army researchers isolated RNA from preserved lung tissue of victims of the disease and used reverse transcriptase to make cDNA copies. This was then subjected to polymerase chain reaction (PCR) using a number of primer sets that are known to be specific for various strains of flu mRNAs present now. The basic methods of carrying out reverse transcription (RT)-PCR were described in Chapter 11, and perhaps surprisingly, the method worked! Scientists found that the Spanish influenza was not an avian strain, but a previously unknown strain of swine influenza! Despite this, they have not found any particular features of the genes they detected that would explain the Spanish strain's exceptional virulence.

It is not known whether this strain of flu is truly "extinct" or whether it is lurking in some porcine influenza reservoir that could be tapped to generate a new, lethal outbreak. If more concern about such a possibility is necessary, the direct transmission of avian influenza from chickens to humans seen in Hong Kong in the winter of 1997–98 should provide further impetus, since that virus targeted young people and has had a relatively high mortality rate compared to other current strains. Luckily, this outbreak does not seem to be associated with the appearance of a variant of the avian flu that can spread directly between people, but most epidemiologists consider the occurrence as a portent of future risk. Clearly, while we consider flu a mild, inconvenient disease, it can show itself to be a formidable threat!

People tend to respond to the problem most apparent and nearest at hand, and thus, emerging diseases only take center stage when a crisis looms. Hopefully, the medical and scientific community is a bit more discerning in its evaluation of potential risks. The impact of HIV infection and AIDS has been profound and extremely disquieting. If there is anything at all salutory about the appearance of this virus in the long run, it may be that it has reinforced the certain knowledge that infectious disease is not conquerable; it is only controllable, and such control is expensive.

Modern molecular biology has suggested direct approaches toward treatment of HIV-infected individuals; some are outlined in Chapter 8. These may reverse some or all of the ravages of AIDS. For example, a San Francisco periodical dedicated to tracking AIDS in that city's large and affluent gay community reported in the late 1990's a week without any death from AIDS for the first time since the mid-1980s. Still, as evident in Fig. 22.1, HIV is responsible for the first general rise in mortality rates due to infectious disease since the great flu pandemic of 1918, and present therapy strategies will have little effect on the impact of HIV worldwide in the foreseeable future. Even when controlled, HIV and AIDS will be endemic diseases, and only the relatively inefficient mode of passage between individuals is proof against a more major impact.

As ominous as AIDS is — a progressive disease with awful consequences — it may well not be the greatest threat to our well-being from viruses in the next decade or so. The honor of that place may go to a positive-sense strand RNA virus in the flaviviridae family — hepatitis C virus (HCV). The widespread occurrence of this virus in the general population has only become appreciated with development within the last decade of effective screening tests for its presence in the blood of individuals and of blood stored for transfusion. It is now known that HCV infections account for 40% to 50% of all chronic liver disease.

Currently, screening for HCV has reduced transmission of the virus through blood supplies to a negligible level, but screening also reveals that there is a high incidence of the virus in healthy individuals, many of whom have never had a transfusion. Similarly, although the virus can be spread by shared needle use among injection drug users, and possibly by venereal routes, many HCV-positive individuals have no history of either drug abuse or high-frequency casual sexual encounters. Clearly, other routes of transmission are effective.

It is not known how important or extensive future problems will be from HCV infections. It is known that most individuals who are HCV positive are healthy and show no signs of liver dysfunction. Further, acute infection with the virus is often cleared with no sequelae. Unfortunately, there is a small but significant proportion of chronically infected individuals who go on to develop cirrhosis and other critical liver damage. In addition, there is a strong statistical correlation between these complications and the development of hepatocarcinoma in some of these afflicted individuals. The fear is that if the unknown mode of spread of the virus is efficient and difficult to control, the reservoir of chronic HCV carriers is already great enough to provide a significant health risk to the entire population.

Further study and the development of effective vaccines may obviate any dangers threatened by HCV infections, but the general problem illustrated by this virus is clear. The use of transfusion, the occurrence of reservoirs of unknown pathogens within human populations, and the increasing interaction between populations and individuals within these populations will consistently pose threats to our public health. We cannot appreciate the risk engendered by a virus and its transmission until that virus is identified and screened, and such identification and screening is only going to be directed at the known threat. It is impossible to know them all before the fact.

Sources and causes of emergent virus disease

The Centers for Disease Control and Prevention list many factors that contribute to the emergence of a novel viral disease. They term these "enabling" factors. Of particular importance is the potential evolution of novel infectious strains of virus due to coinfection of the same individual. This is the sine qua non of influenza epidemics, but genetic recombination and reassortment have been observed in many mixed infections with viruses in the laboratory, and a rare, nonhomologous recombination could take place at any time. Indeed, this must be the source of so many genes of host origin that are maintained in various viruses and clearly provide a great survival advantage to the virus.

Other enabling factors include breakdown of public health measures due to social and economic disruptions such as war and depressions. Such disruptions occur sporadically throughout the world, and there is no evidence that the rate of occurrence is in decline.

Concentration of people with shared lifestyles can also be an important enabling factor. Certainly, HIV infection and AIDS are not more confined to the subset of the homosexual community habituated to casual sex with large numbers of partners than they are confined to injection drug users, but the concentration of these populations in small urban areas has served an important role in the virus's establishment in American and European populations. In addition, the virus has established itself in female "commercial sex workers," especially in Southeast Asia and in urban areas of Central Africa.

The global economy also has served as an important enabling factor for establishment of viral disease. For example, the Asian Tiger mosquito has moved to California and the United States in the stagnant water inside worn-out rubber tires that were sent to these places for recycling. The viral diseases spread by this vector are already on the rise and will continue. Another example of a virus that has established a new geographical range due to modern communication is West Nile virus, an agent of arboviral encephalitis. As mentioned earlier, this virus, previously found in Africa and the Middle East, is now well established throughout the United States. The exact details of its arrival are not clear at this time.

The application of intensive and invasive agricultural methods has led to severe habitat disruption in Africa, South America, and parts of Asia. As discussed in Chapter 16, these are important factors in the sporadic outbreak of arenavirus and filovirus (Marburg and Ebola viruses) infections.

One must also be aware that periodic disruptions in typical weather patterns can have an important role in enabling new virus infections. Unusually wet, warm winters led to the emergence of hantavirus in the American Southwest as its natural rodent hosts proliferated and interacted with human populations. It is virtually certain that this has happened before, because Navajo and Ute indigenous knowledge warns against approaching wild rodents during such periods of unusual weather.

Obviously, technology in the form of efficient transportation and effective but invasive medical procedures also have a role in fostering the establishment and spread of novel viral diseases. Technology can have a more direct role in emergence of novel virus infections. Canine parvovirus appeared as a significant pathogen for young dogs only in 1978, and genetic and other virological analyses clearly show that the virus is closely related to and derived from feline panleukopenia virus. While there is no complete answer as to how this abrupt change in host range occurred, a strong case has been made that the canine pathogen had its origin in the development and early use of a vaccine strain of the feline parvovirus. Thus, the maintenance of vaccine strains of virus is not without documented risks.

The threat of bioterrorism

On September 11, 2001, our world changed irreversibly. The terrorist attacks on the World Trade Center and the Pentagon, along with the foiled attempt at flying a plane into the White House brought home in a frightening way modern cities' vulnerability to a determined and ruthless foe. In the wake of these events it has become all too clear that biological weapons may also be the choice of those wishing to attack the United States or any other nation.

The Center for Disease Control and Prevention lists three classes of agents, all of which include viruses. Of most concern is Class A, which includes agents of viral hemorrhagic fever (Ebola/Marburg, Lassa, and Machupo), and smallpox virus (variola major). Class B includes agents of viral encephalitides (the various equine encephalitis viruses, for example). Class C includes emerging viruses such as Sin Nombre virus.

The most immediate concern for many public health agencies is smallpox virus. As discussed earlier, with the eradication of wild virus by the massively successful WHO program, few people receive vaccination against this agent. As a result, stocks of vaccine are being built up and plans are being put in place for necessary measures to take in case of dispersion of smallpox within the population. The existing stocks of the virus, scheduled to be destroyed, are now being pressed into service to study the efficacy of vaccination protocols as well as potential antiviral treatment strategies.

It is sadly true that biotechnology, just as chemical technology, can be directly applied to human destruction, and stockpiles of biological weapons can be readily made. Although such weapons might be considered to be the last resorts of "rogue states" or terrorists, it is well to remember that the US CIA released the bacteria *Serratia marcescens* from a submarine outside of San Francisco in the 1950s to test methods for spread of biological weapons. While this bacteria is usually considered a harmless one, a high incidence of infections among the elderly and infirm was noted by public health officials at the time. Only after the Freedom of Information Act was enacted was the reason for this unusual outbreak determined.

This is not to argue that technology is evil. Technology in the form of efficient screening, effective countermeasures, and development of new medical products and procedures such as blood substitutes for transfusion is vital in controlling the threats posed by pathogenic microorganisms, whether from the environment or as the result of a deliberate release. Still, there is little likelihood that all or even most can be eliminated by technology. Certainly, HIV, HCV, and other as yet unknown viruses will still exist in the human ecosystem, even in the setting of effective and inexpensive modes of treatment and control. And all of our technology cannot guard against the inhuman (and arguably psychotic) decision of any one group to rain destruction on another through the use of biological agents.

What are the prospects of using medical technology to eliminate specific viral and other infectious diseases?

The discovery and exploitation of antibiotics following World War II led to unreasonable euphoria. Experts widely claimed and the lay population believed that infectious bacterial diseases were no longer threats, and that viral diseases would soon be conquered and eliminated.

This has not happened, and with some notable exceptions, will not happen. Misuse of antibiotics, coupled with the ability of pathogenic bacteria to transmit drug resistance horizontally via plasmids, has led to an increasing incidence of multiple drug-resistant strains of bacteria being detected in hospital laboratories. If there ever was the chance of eliminating bacterial infections, it is long past.

Most viral infections will not be eliminated for different reasons. In order for a virus disease to be eliminated, the virus must have a human or readily controllable reservoir, it must be able to induce an effective and lasting immune response, and there must be an effective vaccine against it. The success of eliminating smallpox shows that given these conditions, eradication of a disease is possible. Presently, workers with the World Health Organization have targeted polio and measles for future eradication. Some are predicting that this can be accomplished during the first decade of the twenty-first century, and it is certainly within the realm of possibility provided the world's economy remains relatively stable and world politics does not continue its current swing into irrationality, xenophobia, and self-destruction!

The promise is notable, but what of other viral diseases? What of HIV, cytomegalovirus, and the panoply of viruses that either establish persistent infections, effectively counter the immune response, have a nonhuman reservoir, or cause diseases that are more expensive to control than to live with? The answer is obvious. Viruses, like bacteria and parasites, will continue to be our life partners.

Silver linings – viruses as therapeutic agents

We have briefly described laboratory uses of viruses and viral genes. Further, we have identified some values of recombinant viruses in biotechnology such as the development of recombinant virus vaccines (see Chapter 8). These uses will increase as the need for them arises, and the sophistication and sensitivity of the methods of using them will increase in concert.

One of the most promising uses of current knowledge of virology and viral replication is in the area of gene transfer. The idea is simple: to construct (engineer) viruses that have no pathological properties, but retain their ability to selectively interact with and introduce their genes into specific cells and tissues. Another important area of study is the development of viruses specifically engineered to eliminate diseased or malfunctioning cells in the host.

Viruses for gene delivery

A number of laboratories are actively investigating the potential uses of adenoviruses, adeno-associated viruses (AAVs), several different retroviruses, and HSV as agents for gene delivery to specific tissues. Currently promising candidates are listed in Table 22.1.

Two basic approaches are currently under intense study and development. The first approach is to engineer a virus from which all pathogenic genes or elements are eliminated, but that can effectively express a therapeutic gene. The second is to produce a virus expressing the appropriate gene but that is unable to replicate by itself (a replication-deficient virus). A replication-deficient virus must be grown with the aid of a helper virus, or in a complementing cell. In principle, the methods for the generation and production of such viruses follow those outlined in Chapter 14.

Table 22.1 Viral vectors for gene therapy.

Vector	Maximum insert size (kbp)	Comments
Retrovirus (e.g., Moloney MLV)	7.0–7.5	Will only infect growing cells and is used for ex vivo delivery. DNA inserts into genome, with possible insertional mutagenesis. In spite of this, the duration of expression is short.
Lentivirus (HIV-1)	7.0–7.5	Newly developed system. Vector can infect both dividing and nondividing cells and can be used both ex vivo and in vivo. DNA inserts into genome, with possible insertional mutagenesis. Duration of expression is long.
Adenovirus	~30	Vector can be used both ex vivo and in vivo. There is no integration of DNA and therefore transient expression. Problem of immune reaction is severe.
Adeno-associated virus based	3.5–4.0	Vector can be used both ex vivo and in vivo. Integration can take place, leading to long-term expression. It is difficult to prepare large amounts of vector.
Herpesvirus	40–50	Vector is a potential delivery system for neurological tissue. Episomal latent infection is established, leading to the possibility of long-term expression.

As an example of the potential use of such a therapeutic virus, consider an individual with a genetic disease caused by the mutation of a gene whose function is required for normal functioning of a specific cell or tissue. Such a function might be the ability to produce insulin in cells of the pancreas, or it might involve a factor necessary for the maintenance of healthy neurons in an aging individual. Infection of the appropriate cells with a retrovirus containing this gene could lead to its integration in the provirus and continued expression. Alternatively, injection of a replication-deficient HSV containing a therapeutic gene into the brain of a person susceptible to early-onset Alzheimer's disease might lead to expression of a remedial protein from a latently maintained genome.

An example of the ability of a replication-deficient engineered HSV to deliver a reporter gene to neurons in an experimental animal is outlined in Fig. 22.2. In the experiment shown, the bacterial β-galactosidase gene under the control of a retrovirus long terminal repeat (LTR) was introduced into the HSV genome to replace the essential immediate-early gene α4. The recombinant virus was isolated by screening for virus unable to replicate in normal cells but able to replicate in cells that contained and expressed a copy of the HSV α4 gene.

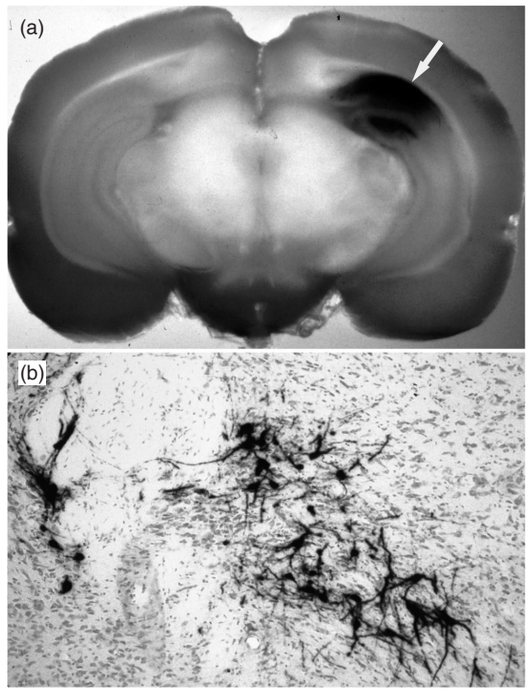


Fig. 22.2 Infection of a rat brain with an "engineered" replication-deficient HSV bearing the bacterial β-galactosidase gene as a marker. An amount of virus ($\alpha 4^-$ virus able to form 10^6 plaques on complementing cells) was injected into the hippocampus of male Sprague-Dawley rats. *a.* After 4 days, the rats were killed and the brain sectioned, fixed with paraformaldehyde, and then stained with X-gal, which forms a blue precipitate in the presence of the enzyme. *b.* A 40× magnification of the area indicated by the arrow in *a.* The cells were counterstained with another dye (pyrodin Y). Note that the virus is concentrated in pyramidal neuronal cells and their dendritic processes. See Plate 9 for color image. (Photographs courtesy of D. Bloom.)

Such viruses were further screened for their ability to express the reporter enzyme by infecting cells in the presence of the substrate *X-gal* (5-Br-4Cl-3-indolyl-β-galactoside), which is colorless but is converted into an insoluble blue dye when cleaved by β-galactosidase. The virus was injected into the hippocampus of rats. After 4 days, tissue was isolated and the presence of the enzyme indicating virus infection and enzyme expression was assayed. The concentration of virus in neuronal cells is quite evident.

There are many complications to this approach. First, a significant number of cells that can express the therapeutic gene effectively must be infected. This means the virus either must replicate in the host, which could lead to immune elimination, or must be injected at a very high titer, which is technically quite difficult. One further problem is that when making such stocks, there is always the danger of generating a replication-competent recombinant virus by recombination between the viral and helper cell genome.

Another and more general problem in using viruses to deliver genes for continued expression is that many viruses maintain themselves in the host by limiting their own gene expression as a way to avoid immune responses. This limiting of expression could (and usually does) lead to loss of expression of the therapeutic gene shortly after infection and its incorporation into the cell.

A third problem specific to many retrovirus vectors is that they must infect dividing cells that would then need to colonize the tissue of interest. This is very difficult to do in adults.

There are ways around these road blocks. For example, some retrovirus vectors use the HIV integrase. This allows them to effectively infect nondividing cells. The therapeutic gene could be controlled by an inducible promoter so that it can be "turned on" only when needed. Recombination can be virtually eliminated if the complementing genes used are not homologous to the virus.

There is, however, one danger to the use of viruses for gene therapy that cannot be fully controlled. This danger is the possibility of inadvertently transferring a contaminating gene or genes along with the therapeutic ones. Of course, appropriate standards of purity and safety will go a long way to reducing such a danger, but it can never be eliminated. A survey of the relationships between viral genes clearly demonstrates that viruses can borrow genes from cells or from other viruses by

rare nonhomologous recombination events. If the resulting virus has a strong replication advantage and is pathogenic, it could lead to a novel disease of unpredictable severity.

This problem could be eliminated if the genes mediating the specific tissue tropism of the virus were engineered into another delivery vehicle. One possibility might be a manufactured enveloped particle with viral glycoproteins interactive with a desired cellular receptor on the surface and the desired gene or genes within.

Using viruses to destroy other viruses

The latent stage of infection with AAV is briefly discussed in Chapter 17. This virus requires a helper for replication, but can integrate and maintain a latent state in cells that it infects without the helper virus. Then, when these cells are infected with an adenovirus, the reactivation of AAV can lead to reduced yields of the infecting pathogen. There has been some semi-serious discussion of adapting this feature of parvoviruses to the development of antiviral strains. Of course, it is not clear how the host immune response to this antivirus defense will be overcome.

Another approach toward using viruses to combat other viruses is to take advantage of the fact that HIV requires a biochemical "handshake" between the CD4 receptor on the surface of the cell it infects and a second co-receptor such as CXCR4 (see Chapters 6 and 20). An HIV "missile" being developed by several laboratories here and in Europe is based on a recombinant vesicular stomatitis virus (VSV) that contains genes for both the CD4 and CXCR4 proteins but lacks its own surface protein that interacts with VSV receptors. Thus, this engineered virus can attach to any membrane that contains the HIV envelope glycoprotein, since it interacts with CD4 and CXCR4. This will include HIV, but significantly, also cells infected with HIV that express the envelope glycoprotein (gp120) on their surface.

Preliminary tests demonstrate the ability of the engineered virus to kill HIV-infected cells in culture. However, like all such approaches, this one may be obviated by the host generating an immune response to the therapeutic virus. The fact that this virus only expresses cellular glycoproteins on its membrane, however, suggests that an immune response may not generated.

Why study virology?

We finish with the same question that was posed in Chapter 1: Why study this complex subject, and how much of this detail can be used in everyday life? For students who use this text as a foundation for further study in medicine or biology, important concepts and details will be reinforced many times and the question is a nonstarter. But we submit that students who never touch this subject again can still profitably remember a few things.

The first is that viruses and all microorganisms, whether pathogenic or benign, are important members of the biosphere and have an important impact on our daily and future activities. This impact goes both ways.

Second, virology is biology "writ small." The principles studied here apply to all biological sciences. Remembering that the field *is* complex, even if the complexities themselves are forgotten, will go a long way to maintaining that healthy skepticism required of a citizen in a technologically complex world to inflated claims and counterclaims.

QUESTIONS FOR CHAPTER 22

1 What factors may account for the sporadic emergence or reemergence of human viral diseases?

2 What are the essential differences between the following strategies designed to deal with viral diseases:

a Development of a vaccine

b Development of an antiviral drug

3 Viruses are used as gene delivery systems in attempts to modify the genetic information of cells or tissues. What features of viruses make them good candidates for this technology? What features of viruses make this a difficult approach?

PART IV Discussion and Study Questions

1 Inhibition of protein synthesis in a cell can occur as a direct result of a virus infection or as a result of an infected cell being in an antiviral state due to interferon treatment. The following table describes data obtained for various viral infections (they are not all the same virus). Indicate whether the observed alteration of protein synthesis is a direct result of a virus or is the result of the antiviral state in the cell induced by interferon. Be careful! There may be effects that are true for both! Indicate your predicted results by placing a checkmark in the appropriate square.

Protein synthesis inhibited because	Directly a result of the virus?	A result of the antiviral state induced by interferon?
Cap structures are endonucleolytically removed from host mRNA in nucleus		
RNase L is activated in the cytoplasm of the cell		
Protein synthesis initiation factor eIF-2 is phosphorylated		
Protein synthesis initiation factor eIF-4F is proteolytically degraded		

2 For most DNA genome viruses, gene expression is classified as either "early" or "late."
a What event of the viral life cycle is used to distinguish early from late expression?
b For the following kinds of viral genes, indicate whether you would expect early or late expression.

Viral gene	Class (early or late)
Capsid protein	
DNA polymerase	
Inhibitor of host transcription	
Lytic enzyme	

3 Sin Nombre virus is the causative agent of the adult respiratory distress syndrome that is transmitted by deer mice in many regions of the western United States. From your knowledge of the family in which these viruses are classified, fill in the following table of properties.

Property	Data for Sin Nombre virus
Sense of the RNA genome	
Number of genome segments	
Presence or absence of a virion-associated polymerase	
Site of replication in the cell	

4 The senioritis variant of spring fever virus (SpFV-4) is now at peak expression in a local epidemic at the University of Arizona. Even the most ambitious premedical student seems to be susceptible. However, while wandering through the nearly vacant science library, you come upon a study room with a group of seniors who are intently working behind an immense pile of books. As you watch, it becomes clear from observing their behavior that this group has not been infected. You convince them to donate some of their cells to your laboratory for further study. Remember that you have already determined SpFV-4 to be a member of the newly defined *Procastinovirus* genus of the family Orthomyxoviridae.

The following table of data compares the properties of the susceptible cells you have been using to grow the virus and the resistant cells you obtained from the uninfected study group:

Experiment to examine	Results for SpFV-4 Infection of Susceptible cells	Resistant cells
Virus attachment	Viral particles found attached to surface receptors in normal numbers	Viral particles found attached to surface receptors in normal numbers
Virus entry	Viral particles observed within endosomes in normal numbers	Viral particles observed within endosomes in normal numbers
Viral gene expression	Viral gene expression taking place in the cell nucleus; viral mRNAs in the cytoplasm	No viral gene expression in the nucleus; no viral nucleic acid present in the nucleus or cytoplasm

a Based on these data, what step in SpFV-4 infection do you think is blocked in the case of the resistant cells from the study group?

After interviewing the members of the study group you find the following common characteristics of the members:

- They all take the bus to campus each morning.
- They all eat breakfast together each day in Louie's Lower Level, a student union restaurant.
- They are all biochemistry majors.

b Which of these behaviors suggests a possible origin for the resistance to SpFV-4?

c How would you begin to identify the source of the resistance?

5 For each of the following viruses, describe the most likely *normal route* of entry into the host cell, based on the described experimental observations.

a La Crosse encephalitis virus: At neutral pH (pH 7) the envelope of the virus particle does not fuse with a cell membrane, but at acidic pH (pH < 5) the envelope of the virus particle fuses with a cell membrane.

b Sendai virus: The envelope of the virus particle fuses with a cell membrane at either neutral or acid pH.

6 You have discovered that Mardi Gras virus (MGV) has a genome structure that most closely resembles members of the Bunyaviridae family (three single-stranded RNA segments). However, the symptoms of disease associated with MGV (behavioral abnormalities) are unlike any produced by the other members of the family, all of which cause either encephalitis or a hemorrhagic syndrome (except for the lone plant virus member). In spite of this, you wish to determine whether MGV has other molecular properties that would warrant inclusion into this family, possibly as the prototype member of a sixth genus.

a Complete the following checklist that you are preparing for members of your laboratory. In each case, if MGV is a member of the Bunyaviridae, predict the result you would expect when your laboratory investigates each property.

Molecular property	If MGV is a member of the Bunyaviridae
Number of membrane glycoproteins	
Mechanism of 5′-cap addition to viral mRNA	
Possible strategies for gene expression from the S RNA	
Site of virus maturation in the host cell	

b On a Tuesday afternoon, while you are away from the laboratory (teaching your virology class), one of your graduate students drops a vial containing MGV. The vial shatters on the desktop. Your technician, another graduate student, and an undergraduate student are present. The following Friday, none of them is in the laboratory. You discover that they are all at O'Malley's Tavern and therefore have been infected with MGV. What is the most likely route of infection in the laboratory accident?

c Given this accidental "experiment," is it likely that MGV is an arbovirus? Why or why not?

7 You have created two strains of *E. coli*, genetically engineered to express λ bacteriophage proteins under certain conditions. In each case, the λ gene has been inserted into the bacterial DNA such that expression of the gene is under control of an inducible promoter (the promoter and operator sequence from the lac operon). In each case, assume that the uninduced cell behaves exactly as a normal *E. coli* cell would with respect to λ.

a The first strain is an *E. coli* cell with the gene for the λ cro protein, placed downstream from a copy of the lac promoter and operator. The cell is placed in the presence of IPTG, an inducer of the lac operon. Describe what will happen in such an induced cell to the λ bacteriophage in each of the following cases. Justify your answer with respect to control of the λ life cycle:

- The cell is infected with bacteriophage λ.
- The cell contains a λ provirus as a part of its DNA.

b The second strain is an *E. coli* cell with the gene for the λ cI protein, placed downstream from a copy of the lac promoter and operator. The cell is placed in the presence of IPTG, an inducer of the lac operon. Describe what will happen in such an induced cell to the λ bacteriophage in each of the following cases. Justify your answer with respect to control of the λ life cycle:

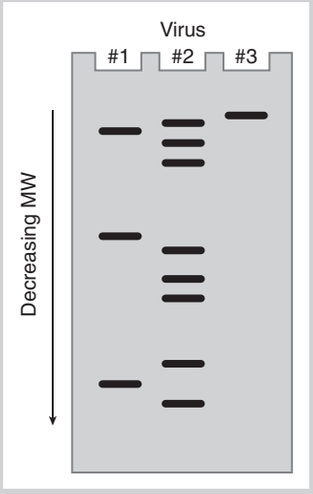
- The cell is infected with bacteriophage λ.
- The cell contains a λ provirus as a part of its DNA.

8 Messenger RNAs in eukaryotic cells are monocistronic. Viruses have evolved a variety of schemes for growth within this environment, given this constraint. Explain *briefly* (a short phrase will do) how each of the following viruses express all of the necessary viral proteins within their host cell.

a Poliovirus

b Vesicular stomatitis virus

9 In searching through the freezer in your advisor's laboratory, you discover a box containing three vials of material. In the bottom of the box you find three labels that had fallen from the three now unlabeled vials. The labels read as follows: "influenza virus type A," "La Crosse encephalitis virus," and "vesicular stomatitis virus." Your advisor sets you the task of identifying which of the vials contains which virus. You grow each virus in cell culture and prepare virions of each with the genome radiolabeled with phosphorus 32. After isolation of the genomic RNAs, you display them by electrophoresis in an agarose gel. The autoradiogram from this experiment is shown below.



Based on these data, indicate which of the vials originally had which of the labels found in the box. Use the table and indicate which label belongs on which vial.

Vial label	Virus no.
Influenza virus type A	
La Crosse encephalitis virus	
Vesicular stomatitis virus	

10 Methods are available for removing the nuclei from eukaryotic cells in culture. These enucleated cells can still carry out their cytoplasmic metabolic functions for a period of time. Predict which of the following viruses would be able to grow in such enucleated cells (assume that the enucleated cells can survive and metabolize long enough for a virus life cycle to be completed).

Virus (Family)	Growth in enucleated cells?
Poliovirus (Picornaviridae)	
Influenza virus (Orthomyxoviridae)	
Human immunodeficiency virus (Retroviridae)	
Vesicular stomatitis virus (Rhabdoviridae)	

11 A physician is treating a patient who is having recurring outbreaks of herpes simplex virus type 2 (HSV-2) infection (herpes genitalis). She has decided to administer the drug acyclovir (acycloguanosine) to this patient during such outbreaks. The patient has a degree in molecular biology, but no training in virology. He asks the physician to explain the *mode of action and safety* of acyclovir to him.

a What should she tell him are the two reasons why acyclovir works specifically against HSV-2–infected cells?

b The physician has explained that these recurring outbreaks are due to an established latent infection in the basal ganglia. The patient wonders if the acyclovir treatment will cure him of this latent infection. Should the physician answer yes or no *and* what reason should she give for her answer?

12 Human immunodeficiency virus (HIV) is a member of the *Lentivirus* genus of the family Retroviridae and is the causative agent of AIDS. In designing drugs that will inhibit this virus, it is important that the drug in question target a specific viral function. For each of the drug types listed below, predict what stage of the HIV virus cycle will be blocked.

a an inhibitor of the viral protease, such as saquinavir;
b a yet to be developed inhibitor of the viral integrase;
c AZT and related compounds; and
d a theoretical inhibitor of the viral protein Rev.

13 You have been called in as a virological consultant in the case of the voles living at the site of the Chernobyl reactor. These rodents are apparently thriving in the midst of the radioactive waste and are mutating at a rate 10 times greater than normal. Your hypothesis is that viruses that might be vectored by these rodents could also mutate at a more rapid rate than normal. In a preliminary investigation, you have isolated viruses from this population of voles. You have discovered a virus from these rodents that you have tentatively named the Chernobyl vole virus (CVV). The following table lists some of the features of this virus that you have determined:

Virus feature	Result for CVV
A Virion	Enveloped, with two membrane glycoproteins
B Genome	ssRNA, negative sense, three segments
C Virion-associated RNA polymerase	Present
D mRNA synthesis	Nuclear cap scavenging to begin mRNA synthesis
E Disease	Causes encephalitis in voles, with a case-fatality rate of about 15%

a Which of these features (A through D) would justify inclusion of CVV into the Bunyaviridae family (points will be deducted for features listed that *do not* justify this inclusion)?
b Which, if any, of these features make CVV different from other members of the family Bunyaviridae?
c Which, if any, of these features make CVV different from members of the *Hantavirus* genus of the Bunyaviridae?

14 Three of the strategies found in the translation of RNA genome viruses in eukaryotic cells are: (i) monocistronic mRNAs translated into a polyprotein; (ii) monocistronic mRNA translated into a single protein; and (iii) the use of overlapping reading frames to produce two proteins from one mRNA. Indicate which of these strategies is employed for each of the following viruses. Indicate your choices by either a "Yes" or a "No."

Virus: Family	Monocistronic to polyprotein	Monocistronic to single protein	Overlapping reading frames
Poliovirus: Picornaviridae			
Sindbis virus: Togaviridae			
Vesicular stomatitis virus: Rhabdoviridae			
La Crosse encephalitis virus: Bunyaviridae			

15 An experiment is designed to test the effect of infecting a cell with two different viruses. You wish to examine the effect of the coinfection on viral protein synthesis in the cell. Assume that in each case the host cell is susceptible to each virus and could support the growth of either virus alone. Predict which of the two viruses will predominate or whether both will be normal with respect to viral protein synthesis in each case below *and* give a *brief* reason in defense of your choice.

First virus	Second virus	Protein synthesis by which virus?	Why?
Adenovirus	Poliovirus		
Vesicular stomatitis virus	La Crosse encephalitis virus		
Influenza virus	Poliovirus		
Adenovirus	Herpesvirus		

16 The table below has three viruses that have been considered in detail in this text: bacteriophage T4, poliovirus, and vesicular stomatitis virus. The table also includes events that may be steps in the assembly of one or more of these viruses. Place a "Yes" or a "No" in the table to indicate which of the events is associated with the assembly of which virus.

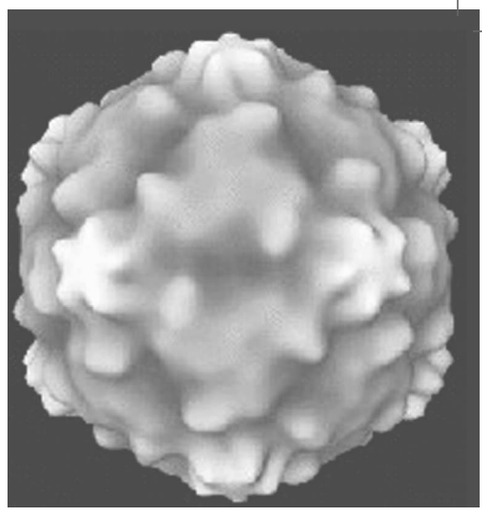
Event	Bacteriophage T4	Poliovirus	Vesicular stomatitis virus
Capsids or nucleocapsids are assembled before the insertion of the genome.			
Capsids or nucleocapsids have a helical symmetry.			
Final maturation of the particle requires proteolytic cleavage of one of the capsid or nucleocapsid proteins.			
New viral particles are released by budding from the surface of the cell.			

17 Both poliovirus (Picornaviridae) and Rous sarcoma virus (Retroviridae) have RNA genomes that are positive in sense. However, replication of the genome of these viruses differs drastically. In the table below, indicate which feature applies to the replication of the RNA genomes of these viruses. Write "Yes" if the feature applies or "No" if the feature does not apply.

Replication feature	Poliovirus	Rous sarcoma virus
Replication requires the use of a host cell tRNA as a primer.		
Replication requires the use of a viral protein as primer.		
Replication results in the conversion of ssRNA to dsDNA.		
The *final product* of replication is progeny single-stranded, positive-sense RNA.		

18 For each of the control points given below, identify at least one virus that can alter the cell such that it grows out of control and might result in a tumor. In each case, state how the virus changes the cell.

a Growth hormone/receptor
b G protein
c Tyrosine kinase
d Transcriptional regulator
e Tumor suppressor



Additional Reading for Part IV

The best place to begin research and further reading on a specific virus or aspect of a specific virus is in the general reference:

• Webster, R.G., and Granoff, A., eds. *Encyclopedia of Virology*. New York: Academic Press, 1994.

More detailed information and specific recent citations of experimental articles and recent reviews can be found in:

• Fields, B.N., and Knipe, D.M., eds. *Fundamental Virology*, 3rd edn. New York: Raven Press, 1996.

• Fields, B.N., and Knipe, D.M., eds. *Virology*, 4th edn. New York: Raven Press, 2002.

• Flint, S.J., Enquist, L.W., Krug, R.M., Racaniello, V.R., and Skalka, A.M. *Principles of Virology*. Washington: ASM Press, 2000.

Detailed aspects of pathogenesis of virus infections, again organized as a group of specific reviews by individual experts, is covered in:

• Nathanson, N., ed. *Viral Pathogenesis*. Philadelphia: Lippincott-Raven, 1997.

The Cold Spring Harbor Press publishes many books that contain detailed reviews of many aspects of the molecular biology of viruses and cells. Specific titles of interest to virologists include:

• Coffin, J.M., Hughes, H.H., and Varmus, H.E. *Retroviruses*. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1997.

• DePamphilis, M.L., ed. *DNA Replication in Eucaryotic Cells*. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1996.

The ASM Press also publishes a large number of books of great use to molecular biologists, biomedical research workers, students, and the like. One recent title that contains chapters covering viruses discussed in this section is the following:

• McCance, D., ed. *Human Tumor Viruses*. Washington, DC: ASM Press, 1998.

A very thorough (definitive, actually) discussion of the numerous genetic switches occurring during the replication of bacteriophage λ and the biochemical basis of these switches can be found in:

• Ptashne, M. *A Genetic Switch: Gene Control and Phage λ*. Palo Alto: Blackwell Scientific Publications and Cell Press, 1986.

Virus resources on the Internet

As noted in the introduction to this text, the Internet provides a very effective resource for the most recent information about specific viruses and recent publications concerning them. It is important, however, to keep in mind that many such Internet sites are not formally reviewed, or edited by any single group or body of experts, and addresses change; therefore, the reliability of any given site address or "factoid" within that site is subject to independent verification.

Some important sites for searching journals and publications include the following:

American Society for Microbiology (ASM):
http://www.asmusa.org/
Cold Spring Harbor Press:
http://www.cshl.org/books/new-hmpg.htm
Journal of General Virology: http://vir.sgmjournals.org/
Journal of Virology: http://jvi.asm.org/
Nature: http://www.nature.com/
Science: http://www.sciencemag.org/
Scientific American: http://www.sciam.com/index.html
The National Library of Medicine and government resources: http://www.ncbi.nlm.nih.gov/
Virology: http://www.apnet.com/www/journal/vy.htm

An increasingly large number of Internet sites are devoted to individual virus topics related to virus replication such as oncology and cell transformation. The following sites should be useful for beginning a study of any given virus.

General virus information and databases

http://www.tulane.edu/~dmsander/garryfavweb.html
http://life.anu.edu.au/viruses/welcome.htm
http://www.virology.net/Big_Virology/BVHomePage.html
http://www.virology.net/ATVnews.html
http://life.anu.edu.au/viruses/welcome.htm
http://www.urmc.rochester.edu/smd/mbi/VirtLec.html
http://www.epa.gov/microbes/index.html
http://www.wadsworth.org/databank/viruses.htm
http://www.diseaseworld.com/

Specific virus sites include

Adenoviruses
http://www.virology.net/Big_Virology/BVDNAadeno.html
http://www.cdc.gov/ncidod/dvrd/revb/respiratory/eadfeat.htm
http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/01000000.htm
http://www.stanford.edu/group/virus/adeno/adeno.html

Arenaviruses
http://www.tulane.edu/~dmsander/WWW/335/Arboviruses.html
http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/03000000.htm
http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/arena.htm
http://gsbs.utmb.edu/microbook/ch057.htm

Bacteriophages
http://www.asmusa.org/division/m/M.html

Baculoviruses
http://www.baculovirus.com/
http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/06000000.htm

Bunyaviruses
http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/11000000.htm
http://www.bocklabs.wisc.edu/ed/bunya.html

Calicivirus
http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/12000000.htm
http://hgic.clemson.edu/factsheets/HGIC3720.htm
http://www.iah.bbsrc.ac.uk/virus/caliciviridae/index.html

Coronaviruses
http://www-micro.msb.le.ac.uk/335/Coronaviruses.html

Filoviruses
http://www.cdc.gov/ncidod/diseases/virlfvr/virlfvr.htm
http://www.bocklabs.wisc.edu/ed/ebolasho.html
http://www-micro.msb.le.ac.uk/335/Filoviruses.html

Flaviviruses
http://www.tulane.edu/~dmsander/WWW/335/Arboviruses.html
http://www.cdc.gov/ncidod/dvbid/westnile/index.htm
http://www.cdc.gov/ncidod/diseases/hepatitis/c/index.htm
http://www-personal.usyd.edu.au/~sdoccett/fact/dengue.htm
http://www.cdc.gov/ncidod/dvbid/yellowfever/index.htm

Geminivirus
http://www.bocklabs.wisc.edu/ed/fauquet.html

Hantavirus
http://www.cdc.gov/ncidod/diseases/hanta/hps/index.htm
http://www.bocklabs.wisc.edu/ed/hanta.html
http://www.science.mcmaster.ca/Biology/Virology/16/index.htm

Hepadnavirus
http://www.globalserve.net/~harlequin/HBV/pathogen.htm

Hepatitis delta virus
http://www.hepnet.com/oops.html

Herpesvirus
http://darwin.bio.uci.edu/~faculty/wagner/main.html
http://home.coqui.net/myrna/herpes.htm
http://www.ihmf.org/
http://www.uct.ac.za/depts/mmi/stannard/herpes.html
http://www.science.mcmaster.ca/Biology/Virology/19/CYTOG.HTM
http://www.cdc.gov/ncidod/diseases/ebv.htm

Human immunodeficiency virus
http://www.bcm.tmc.edu/neurol/research/aids/aids1.html
http://www.urmc.rochester.edu/smd/mbi/grad/hiv297.html

Human T-cell leukemia virus
http://www.kufm.kagoshima-u.ac.jp/~derma/atll.html

Oncogenes
http://www.rerf.or.jp/eigo/radefx/mechanis/q2.htm
http://www.cancerresearch.org/immonco.html

Oncornavirus
http://www.oncolink.upenn.edu/
http://www-micro.msb.le.ac.uk/335/Trans2.html

Orthomyxoviruses
http://www.cdc.gov/ncidod/diseases/flu/fluvirus.htm
http://www.uct.ac.za/depts/mmi/stannard/fluvirus.html
http://www-micro.msb.le.ac.uk/335/Orthomyxoviruses.html

Papillomaviruses
http://hpv-web.lanl.gov/

Papovaviruses
http://www-micro.msb.le.ac.uk/335/Papovaviruses.html

Paramyxoviruses
http://www-micro.msb.le.ac.uk/335/Paramyxoviruses.html
http://www.cdc.gov/ncidod/dvrd/revb/index.htm

Picornaviruses
http://www.cbs.dtu.dk/services/NetPicoRNA/
http://www.iah.bbsrc.ac.uk/virus/picornaviridae/
http://www-micro.msb.le.ac.uk/335/Picornaviruses.html
http://www.unmc.edu/Pathology/Myocarditis/
http://vm.cfsan.fda.gov/~mow/chap31.html

Poxviruses
http://www-micro.msb.le.ac.uk/335/Poxviruses.html
http://www.uct.ac.za/depts/mmi/jmoodie/pox2.html

Reoviruses
http://www.bocklabs.wisc.edu/Reovirus.html
http://www.iah.bbsrc.ac.uk/virus/Reoviridae/
http://www.cdc.gov/ncidod/dvrd/revb/

Retroviruses
http://www-micro.msb.le.ac.uk/335/Retroviruses.html
http://www.retrovirus.info/
http://medstat.med.utah.edu/WebPath/TUTORIAL/AIDS/AIDS.html

Rhabdoviruses
http://www-micro.msb.le.ac.uk/335/Rhabdoviruses.html
http://www.hhs.state.ne.us/epi/epirabie.htm

Rhinoviruses
http://www.bocklabs.wisc.edu/Rhinovirus.html

St Louis encephalitis virus
http://www.vicioso.com/Health/disease/encephalitis/SLE.html

Togaviruses
http://www.ictvdb.iacr.ac.uk/ICTVdB/73020001.htm

Viral Hepatitis
http://www.cdc.gov/ncidod/diseases/hepatitis/index.htmch-groups/MES/vide/descr184.htm