# 1

# Introduction to Virology

The COVID-19 pandemic caused by the virus SARS-CoV-2 has greatly increased interest in virology. The following chapters build on that interest.

Chapter 1 provides an overview of virology, including discussions of the detection of viruses. Chapter 2 focuses further on those discussions, and assays to specifically measure infectious virus particles are presented. Chapter 3 includes discussions of some aspects of molecular biology, important in considering the replication of viruses and the mechanisms of antiviral medications. Discussion of immunology, important in considering host mechanisms to control virus infections, follows in Chapter 4. Chapter 5 discusses viral pathogenesis, particularly infection of the nervous system. Chapters 6 and 7 discuss viral and immune-mediated illnesses of the nervous system. Chapter 8 discusses experimental neurovirology, and Chapter 9 looks at possible future aspects of virology and neurovirology.

## What Are Viruses?

Viruses are very simple organisms that may infect people, animals, plants, and even bacteria. Specific viruses infect only specific types of animals, plants, and bacteria. Most of the known viruses do not infect humans. Recent epidemics and pandemics due to viruses such as human



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immunodeficiency disease virus (HIV), influenza virus, Ebola virus, Zika virus, West Nile virus, recently SARS-CoV-2, and most recently mpox virus (formerly monkeypox) have emphasized human viral infections. Many people are concerned about the development of new viruses that might infect humans. Viruses are not inhibited by antibiotics, which inhibit bacteria, but they may be inhibited by antiviral medications. Recent antiviral medication development has emphasized treatment of HIV infections, the cause of acquired immunodeficiency disease syndrome (AIDS).

Viral infections may be asymptomatic. That is, testing the blood serum of some people shows the presence of antibody to a virus, indicating prior infection, although those people do not have a clear history of clinically apparent infection by the virus.

## Are Viruses Alive?

Are viruses living organisms? This will depend in large part on how one defines living organisms. Discussion of this point starts here with viruses and later moves to discussion of even simpler atypical agents.

Some may consider viruses as living organisms, although many would not. An initial conclusion is that infections may be caused not only by bacteria and fungi, which are living organisms, but also by viruses and atypical agents, which are not living organisms.

Viruses consist of a nucleic acid (RNA or DNA) core and viral proteins, and some (including SARS-CoV-2) have a lipid envelope containing additional viral proteins, such as the "spike" proteins of SARS-CoV-2. Viruses are classified as being either RNA viruses (SARS-CoV-2, polio, mumps, measles, influenza, HIV) or DNA viruses (herpes simplex, chickenpox, smallpox, papilloma). Some DNA viruses contain a single strand of DNA (these viruses are uncommon) or they contain double-stranded DNA (more common). RNA viruses contain double-stranded RNA (these viruses are uncommon) or they contain a single strand of RNA

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(more common). The viral RNA or DNA nucleic acid (the viral genome) is the substrate for viral reproduction and the synthesis of new viral RNA or DNA. The viral genome is also the blueprint for the synthesis of viral proteins.[1]

Viruses can readily reproduce. If one starts with 100 infectious virus particles and places them on living cells that are susceptible to them and keeps them at body temperature, in a few days there will be many more virus particles. Important words in this sentence include "infectious" (not all virus particles are infectious); "living cells" (viruses can only reproduce in living cells); "that are susceptible to them" (specific viruses only infect certain cell types, for example, skin cells but not blood cells, and cells from some animals, for example, mice or humans, but not others). Viruses are obligate intracellular organisms and only grow (replicate) inside of cells.

Bacteria such as rickettsia are also obligate intracellular organisms. However, unlike viruses they may be considered as living organisms, in large part because they replicate by fission. Viruses may be thought of as reproducing but not living organisms. The concept of agents that reproduce but are not living organisms is discussed further in considering atypical agents such as prions.

Infection of specific cell type(s) by a virus is sometimes termed the tropism of that virus. This is somewhat implied for SARS-CoV-2 when discussing it as causing respiratory infections. Later discussion includes poliomyelitis virus, which causes clinical polio by infecting specific cell types in the spinal cord. Some viruses have narrow tropisms, and some infect many cell types.

Many virus particles are not infectious. In Chapter 2 (viral plaque assay), discussion of how infectious virus particles may be measured (counted) is presented. Infectious and noninfectious virus particles may appear similar when examined by electron microscopy (discussed below). Polymerase chain reaction (PCR) technology, which is commonly used to detect viral nucleic acid, does not differentiate between infectious and noninfectious virus.

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Reports in the news stating that SARS-CoV-2 can "live" for several days on hard surfaces (for example, a bench) are probably not accurate, in that the investigators did not determine the presence of infectious virus. It is assumed that when people discuss "live" virus, they mean infectious virus. However, the reports that live virus was detected usually relied on PCR methods that detected part of the virus (some of its RNA) but not the entire viral RNA genome. The entire viral genome is necessary for virus replication. As noted above, the genome of an organism is all of the nucleic acid, DNA or RNA, by which a virus reproduces copies of itself.

PCR technology is introduced below and discussed in detail in Chapter 3.

## Virus Infection and Replication

Viruses can only reproduce in living cells, and they use the cellular biochemistry to reproduce. In describing clinical or experimental situations wherein virus numbers are increased, the terms "reproduce," "grow," "replicate" will be used interchangeably. The fact that viruses only grow in living cells is one reason it has been difficult to develop antiviral medications: inhibition of viral growth may also inhibit the functions of the cells in which they are growing.

Most investigations of virus infections of cells have been performed in cell culture, in living cells cultivated in the laboratory, that is, in vitro. Cell culture is discussed further in Chapter 2. In vivo studies refer to those in intact living individuals (animals or people).

Viruses get into cells (necessary if the virus is to reproduce) by an active process. Viruses do not simply infect all cells that they contact. Typically, part(s) of the virus (for example, a specific protein on its surface) binds to a specific cell protein on the cell surface. If cells do not have the specific protein "recognized" by the virus protein, the virus will not bind to the cell, will not enter the cell, and will not infect the cell. Some cells of the human upper respiratory tract have the cell surface

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protein to which, for example, SARS-CoV-2 spikes bind. These viral spikes can be visualized (by electron microscopy) on the surface of SARS-CoV-2 particles. The receptor on human cells to which SARS-CoV-2 spikes bind is the human cell surface angiotensin-converting enzyme (ACE) protein.

To date, there has not been evidence of an effect of ACE inhibitors or ACE receptor blockers, commonly used to treat hypertension, on COVID-19, caused by SARS-CoV-2.

## Transfection

Although the matching of a viral protein and a cell receptor protein noted above are the usual means by which viruses bind to cells, following which viral nucleic acid is introduced into the cells, mention should be made of a process by which viral nucleic acid is directly entered into cells. Termed "transfection," this is an experimental process by which viral nucleic acid is directly introduced into cells.[2] By this process, it is possible to have viral nucleic acid enter cells without the more typical infection sequence of events. Viral transfection procedures have been used in investigations of viral transformation of cells to investigate possible viral causes of cancer.

Viruses are thought to cause cancer by having their nucleic acid inserted into the DNA of the host cells. Viruses that may cause cancer in humans include Epstein-Barr virus, hepatitis B virus, hepatitis C virus, human herpesvirus type 8, human papillomavirus, and human T-lymphotropic virus type 1. In addition, HIV, an RNA virus, may cause AIDS, and people with this illness are at increased risk for several types of cancer, related to their immunosuppression.

## Infection

Returning to consideration of the process of viral infection of cells, investigations have focused on cell surface proteins. Cell surface proteins to which viruses bind vary very much among cell types (muscle cells,

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skin cells, and blood cells) of an individual and among cells of different animals. Similarly, the proteins on the surface of viruses vary greatly. When there is a match, that is, when the virus protein (for example, the spikes on SARS-CoV-2) are able to bind to protein on the cell surface (for example, ACE), the virus enters and infects the cell.

Once inside the cell, there must also be a match whereby the viral nucleic acid (RNA in the case of SARS-CoV-2) can interact with and use the cellular biochemistry to reproduce itself. Virus-infected cells are usually destroyed during this process. However, some viruses establish latent infections, whereby destructive viral effects may be slight. These cells appear to continue their usual functions, and the virus is maintained in the cell. However, in most instances, virus-infected cells are destroyed.

Infections in which the infected host cells are destroyed are termed "lytic infections" (the infected cells are lysed) to differentiate them from latent infections in which the infected cells appear not to be damaged. Some viruses, for example, HIV and herpes simplex virus (HSV), cause both lytic and latent infections (Chapter 5).

Some viruses infect bacteria, and the occurrence of such viral infections has led to considerations of viruses as therapeutic agents. For example, bacteriophages, viruses that specifically infect bacteria, have been considered as therapeutic agents, using viruses to destroy bacteria.

In further consideration of "therapeutic" viruses, oncolytic viruses, viruses that infect and kill cancer cells, have been considered. And viruses have been used to transport DNA genes into individuals with genetic illnesses. These "therapeutic" viral options are discussed later in this chapter and in Chapter 9.

## Infectious Virus vs Living Virus

Viruses are not alive, and it is probably not an important discussion point; rather, whether a virus is infectious or not is the important point. It is stretch to conclude that viruses are ever "alive," and most investigators would probably conclude they are not.

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The important question is whether a virus is infectious. The reports that SARS-CoV-2 can "live" on surfaces for days, that is, viral RNA was detected by PCR (polymerase chain reaction) methodology, do not enhance understanding of whether it is infectious. However, they may be important in considering SARS-CoV-2 epidemiology, and the spread of the virus (Epidemiology is discussed below).

## Detection of Virus by PCR

It is possible to use PCR technology to detect the DNA of DNA viruses, and, with slightly increased complexity, the RNA of RNA viruses. PCR techniques greatly increase the amount of target nucleic acid, for example, SARS-CoV-2 RNA, facilitating its detection. PCR methodology is more thoroughly discussed in Chapter 3.

PCR studies of SARS-CoV-2 virus RNA almost always investigate the presence of only part of the viral RNA genome. And even if the entire viral RNA were present (the entire genome), it would not necessarily mean that infectious virus was present – for infectious virus to be present, the protein spikes in the viral envelope and other viral factors would also need to be present. These are not detected by PCR methodology.

By analogy, the presence at a site of all of the DNA of a human cell (the entire human genome) would not necessarily indicate that a living cell is present.

## PCR Data and Its Interpretation

Polymerase chain reaction technology has been the most widely used technology in reports of SARS-CoV-2, noting the presence of viral RNA. Many sites have been sampled, including throat swabs (nasopharyngeal swabs) and park benches. The possible presence of all of the viral nucleic acid could be investigated by PCR methodology, but most PCR studies only determine the presence of a small amount of viral nucleic acid to

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conclude that the virus is present. In the case of SARS-CoV-2, PCR has usually been performed to detect 2 or 3 of the probably 29 RNA genes of the virus.

The presence of SARS-CoV-2 RNA by PCR in a swab – from a nasopharyngeal or from a park bench swab – is the data (the result of a test) that indicates that the viral genome was present (at least part of the viral genome was present). The interpretation of that data, whether it indicates the presence of infectious virus, would remain to be determined.

After a digression to discuss the interpretation of data, for example, PCR data, methods other than PCR to detect viruses is then presented.

## Data vs the Interpretation of Data: Results vs the Interpretation of Results

Relative to the detection of SARS-CoV-2 RNA and whether that suggests infectious virus is present, a brief discussion is presented to consider data versus the interpretation of data. For example, if viral RNA is present (data), is infectious virus present (interpretation of the data)? One could directly determine the presence of infectious virus (data), as in Chapter 2, but when PCR methods are used to detect virus, positive results (data) require an additional step to consider whether infectious virus is present.

The detection of SARS-CoV-2 RNA by PCR technology in a throat or nasopharyngeal swab of an individual very likely does indicate the presence of infectious virus. The PCR detection of the viral RNA in such swabs (data) does not prove the presence of infectious virus, but it can very reasonably be concluded that it indicates the presence of infectious virus (the interpretation of the data). Since the throat or nasopharyngeal swab was from a living individual and includes human throat or nasal

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epithelial cells where the virus likely grew, it is very reasonable to conclude that infectious virus is present.

However, it is not likely that the same detection of SARS-CoV-2 RNA from a park bench using PCR technology could be similarly interpreted. It is very unlikely that intact living throat or other human cells are present in the bench-positive swab. Even if the whole viral RNA genome were detected (not usually determined in most PCR studies), it could not be concluded that infectious virus is present. To be infectious, SARS-CoV-2 particles would need viral proteins and lipid, in addition to RNA.

Second, some PCR-testing results raise another interesting issue of data interpretation – whether all SARS-CoV-2–positive PCR nasopharyngeal swab results, which do suggest the presence of infectious virus (data), should be interpreted as indicating that the clinical illness COVID-19 is present (interpretation of data). Some people with SARS-CoV-2–positive swabs do not have clinical evidence of any illness, and illness is usually considered to exist in individuals with symptoms. Despite not having symptoms, these PCR-positive individuals (data) are usually counted as cases of COVID-19 (interpretation of data). The issue of SARS-CoV-2–positive throat swabs in asymptomatic individuals and whether this should be interpreted as indicating the presence of illness (COVID-19) is further considered below in discussions of epidemiology.

## Data and the Interpretation of Data

In science and medicine there is data (results) and the interpretation of the data. PCR results indicating the presence of SARS-CoV-2 RNA in a nasopharyngeal swab from an individual are reasonably interpreted as indicating the presence of infectious virus. The detection of that the same piece of viral RNA on a park bench should likely not be similarly interpreted.

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# Methods to Detect Viruses

To return to methods to detect viruses, in addition to PCR, multiple other methods exist for the detection of viruses.

## THE DETECTION OF VIRAL DNA OR RNA

Multiple molecular methods have been used to detect viral DNA or RNA as means to identify the specific causes of viral infections. Recently, PCR methods have been the most popular. Historically, PCR was preceded by Southern (DNA) and northern (RNA) methods – which were primarily used in research studies. Newer methods that might supplant PCR are being developed.

Most recent are metagenomic next-generation sequencing (mNGS) methods to determine the presence of the nucleic acids of viruses and other pathogens.

These molecular biology methods rely on the concepts of complementary DNA and RNA, and secondly on the hybridization of complementary DNA and RNA.

As discussed in Chapter 3, specific sequences of DNA will hybridize (bind to) other specific sequences of DNA to which they are complementary. They will also bind to complementary sequences of RNA. If a specific DNA sequence (the probe) is labeled, when it binds to (hybridizes with) a specific viral DNA or RNA (the target), the label will provide evidence for the presence of the specific target DNA or RNA virus.

### Southern and Northern Blot Hybridization to Detect Viral DNA and RNA, Respectively

The first of the blot hybridization techniques to be developed was the use of a labeled DNA probe to detect target DNA, described by Edwin Southern. Therefore, the technique has often been described as a "Southern blot" study. When DNA probes were subsequently used to

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**Table 1.1 Blot hybridization**

| | Southern blot | Northern blot |
|---|---|---|
| **Target*** | **DNA** | **RNA** |
| Procedure | Electrophoresis of the target in an agarose gel** | |
| Additional step | Denaturation of DNA*** | None |
| Blot of gel after electrophoresis | DNA or RNA in the gel is transferred to a nylon membrane | |
| Probe | Single-stranded DNA complementary to the DNA or the RNA target, labeled with a radioactive or chemical tag**** | |
| Detection of target | Sites of radioactive signal or of chemical color on the membrane where the probe which was complementary to the target and therefore hybridized to the target indicates the location of the target DNA or RNA***** | |

* Tissue containing the target DNA or RNA is homogenized and prepared.

** DNA and RNA fragments move at different speeds in the gel, based on their sizes.

*** DNA is double stranded (the double-stranded helix), discussed in Chapter 3. For study, the DNA is denatured – the double strands are separated into two single strands. RNA is single stranded, and so this step is not needed.

**** A single-stranded fragment of DNA (probe) that is complementary to single-stranded DNA or RNA (target) will hybridize (bind to) the target.

***** Complementation and hybridization are discussed in Chapter 3.

detect RNA, the technique was termed a northern blot study (Table 1.1). In recent years, PCR methodology has replaced blot hybridization in many situations. The former is considerably less labor intensive than the latter.

In many ways, the concepts of Southern and northern blot hybridization are similar, whereby labeled DNA probes are used to bind to complementary target DNA (Southern) or RNA (northern).

DNA hybridization that detected human polyoma virus DNA in the brain of a patient with the illness progressive multifocal leukoencephalopathy (PML) is shown in Chapter 7.

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## THE DETECTION OF VIRAL PROTEINS (ANTIGENS)

As discussed in Chapter 4, antibodies bind to specific proteins (antigens). If such antibody is labeled, when it binds to a specific viral antigen, the label will provide evidence for the presence of the virus. Multiple variations on this principle exist.

### Immune-Mediated Detection of Virus Protein

Common methods used in studies to detect virus infection are variations on immune-mediated methodology – using an antibody probe to bind to a viral target. Monoclonal or polyclonal antibody (discussed in Chapter 4) probes can be used. Antibody (labeled in one of many ways) that binds to virus antigen(s) will indicate the presence of the virus.[3,4]

Prior to the use of molecular nucleic acid methods to detect viruses, clinicians and investigators often used antibody-based methods. These relied on the specificity of an antibody, which was coupled with a label. The label served as a means to visualize the location of the antibody when it bound to a virus-infected cell. The presence of the label could be used to detect a virus infection (yes-no answer), including the cellular site of the virus within an infected organ. Semiquantitative results could be estimated from the intensity of the label.

The keys to immunodetection methods are the specificity of the particular antibody (the probe) used to bind to the antigen (the target). Second is the type of label attached to the antibody to identify its location.

## IMMUNOHISTOCHEMISTRY

The antibody probe to be used is labeled so that it can be detected when it binds to the virus protein antigen (target), for example, in the cells of a biopsy. The cells of the biopsy would then be examined under a

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microscope to evaluate the localization of the labeled target. This use of labeled antibody is known as the direct method.

More commonly used is the indirect method. Here, unlabeled antibody, for example, antibody raised in a rabbit, to a virus (primary antibody) is used to bind to virus protein antigen in a biopsy. Then a second antibody is used to bind to the primary antibody. The second antibody is labeled, and the label is detected by microscopy. This use of an unlabeled primary antibody against a virus and then use of a labeled antibody to the primary antibody is termed the "indirect method."

Although requiring a second step, the indirect method has often been the preferred technique, since the labeled secondary antibody could be used in multiple studies. For example, to study various antigens, as long as rabbit antibody against each is used as the primary antibody, the same labeled secondary antibody (for example, made in a goat against rabbit antibody) could be used.

Early immunohistochemistry studies of this type utilized a fluorescent label (and fluorescence microscopy) to localize the site of binding of the primary antibody. Subsequently, other methods of labeling were developed, including peroxidase-antiperoxidase and avidin-biotin labeling. The underlying principle of these immunohistochemistry methods is the detection of antigen, such as viral protein in cells by the binding of known specific antibody.

An example of peroxidase-antiperoxidase immunohistochemistry identification of viral antigen (protein) is seen in Figure 1.1.

A variation on the immunohistochemical detection of virus is to first grow virus that might be present in cell culture, and then detect that virus by immunohistochemistry. For example, virus from a nasopharyngeal swab would be grown in cell culture. Virus that grows in the cell culture would then be definitively identified by an immunohistochemistry method as above.

For all immunohistochemical procedures, antibody specific for an virus in question must be used.

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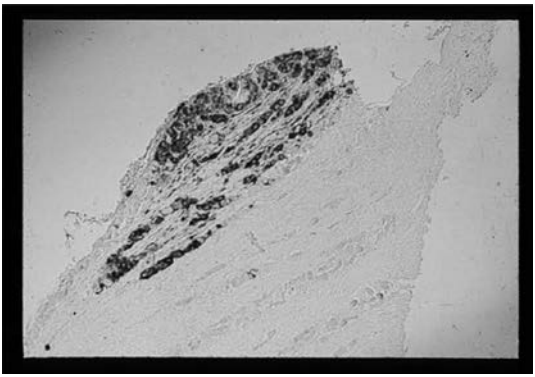

Figure 1.1 Peroxidase-antiperoxidase detection of virus. Presence of herpes simplex virus antigen in a mouse trigeminal ganglion is indicated by the dark label in multiple neurons and supporting cells. The neurons are of the first division (the ophthalmic division) of the trigeminal ganglion.

## ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Although more commonly used to detect and quantitate amounts of antibody, for example, antibody in the serum of patients (Chapter 4), with slight technical modifications, enzyme-linked immunosorbent assay (ELISA) testing can be used to detect antigen, such as virus.

In brief, to detect virus, known antibody to the virus would be bound to the well of a plastic plate. A liquid preparation of the unknown clinical sample to be investigated (for example, a nasopharyngeal swab in saline) would be placed on that. If specific virus is present in the throat swab sample, it will be bound to (be captured by) the specific antibody bound to the plastic plate. Then known, labeled, antibody to the virus would be added. The presence of the label of the second antibody would be evidence of the virus.

For the detection of viral antibody (Chapter 4) rather than to detect viral antigen, known viral antigen would be bound to the plastic plate.

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Serum from a patient would be added, and binding of antibody (primary antibody) present in the patient's serum will occur. A secondary labeled antibody would then be used to determine the presence and the amount of the primary antibody.

## LATERAL FLOW TECHNOLOGY

This immune methodology is possibly best known from its use as a pregnancy test: labeled antibody to human chorionic gonadotropin detects the presence of this hormone in the urine of pregnant women. Lateral flow technology is usually considered to give a yes-no answer. Similar methodology has been adapted to determine the presence of other antigens, with recent emphasis on the detection of SARS-CoV-2. Stability of the antibody in the kit to the virus is most important.

Lateral flow methodology can be used in home test kits to investigate nasal swabs for the presence of viral protein (antigen). Nasal swab material can be tested for SARS-CoV-2 protein (antigen), with results being provided in a very short time. Appropriate sample preparation is important, and there are likely to be more false negatives with this methodology than by other methods. On the other hand, it is very convenient.

## WESTERN BLOT

After the electrophoretic separation of the viral proteins and blotting, viral antigen may be detected with labeled specific antibody to the viral antigen. Continuing with geography and building on the Southern and northern blot terminology, when antibody is used to detect antigen in a blot after gel electrophoresis, the technique has often been termed a western blot study. Probe antibody may be polyclonal or monoclonal. As above, a primary antibody is typically used to bind to the target antigen or antigens, and a labeled secondary antibody used to bind to the first antibody.

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## NOMENCLATURE OF BLOT STUDIES

As noted above, the procedure by which target DNA fragments are separated by gel electrophoresis and after transfer to a blot are detected by use of labeled DNA probes was developed by Edwin Southern. The procedure, therefore, has often been termed a Southern blot study. Shortly thereafter, a similar procedure was used to separate RNA fragments which after blotting were probed with labeled DNA probes. With slight tongue in cheek, this procedure was termed a northern blot study. Subsequently, protein studies were termed western blots. And there are southwestern blots (DNA-protein) and northeastern blots (RNA-protein).

## VISUALIZATION OF VIRUS BY ELECTRON MICROSCOPY

Although viruses are very small, they can be seen by electron microscopy. Viruses are usually measured in terms of nanometers.

1 inch = 2.54 centimeters (cm)
1 cm = 10 millimeters (mm)
1mm = 1,000 micrometers (μm)
1 μm = 1,000 nanometers (nm)

Therefore, 1 inch = 25,400,000 nm

The size of poliomyelitis virus is about 30 (~30) nm, human herpes simplex virus is ~150 nm, and SARS-CoV-2 is ~130 nm. For comparison, a red blood cell is ~8 μm (8,000 nm). The largest human virus is the smallpox virus, which is ~400 nm. Some giant viruses have been reported, up to 1,500 nm in size. However, there is no evidence that such giant virus, sometimes termed "girus," infects humans.

A single virus particle is often referred to as a virion. The term does not indicate whether the particle is infectious or not. Polyoma

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virus particles seen by electron microscopy the brain of a patient with progressive multifocal leukoencephalopathy (PML) are shown in Figure 6.1 in Chapter 6.

### VIRUS CAN BE GROWN AND QUANTITATED IN CELL CULTURE: THE PLAQUE ASSAY

Other than by the growth of infectious virus in cell culture, the above methods do not definitively indicate the presence of infectious virus. Methods to measure amounts of infectious virus are discussed in Chapter 2.

With all methods the problems of false negatives (virus is there but goes undetected) need be considered. False positives (virus is not there but the test reads out that it is) is usually a lesser problem.

## Antibody to Viruses

Viral proteins have often been discussed as their being viral antigens. Such antigens often lead to the production of antibody by individuals infected with the virus. Antibodies are made by infected individuals as a reaction by their protective immune systems. These antibodies are present in convalescent immune serum (or plasma) of people after they recover from infection or after immunization with a vaccine.[5]

When multiple immune cells in humans respond to an infection or to vaccine immunization by making antibody, they make polyclonal antibody. The viral proteins to which the immune cells respond are complex (for example, SARS-CoV-2 spikes), and so the parts of the spike to which one immune cell responds may be different from another, even if they are both responding to the viral spike. The specific part of the spike protein to which an immune cell responds is termed the "epitope."

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Each of the many immune cells that respond to an infection makes a single type of antibody to the epitope it recognized. Therefore, convalescent immune blood would be expected to contain multiple antibodies made by multiple immune cells (polyclonal antibody). In the laboratory, antibody from a clone of a single cell can be developed, termed "monoclonal antibody."

When antibody is therapeutically administered to patients (termed "passive immunization"), antibody is not expected to persist in the individual. Long-term antibody, however, will persist after recovery from infection or after vaccine immunization (termed "active immunization").

The immune system is discussed further in Chapter 4.

## Antiviral Medications

In addition to the use of antibody to inhibit viral infections, antiviral medications have been considered. As noted above, it has been difficult to develop antiviral medications because viruses grow only within living cells, and inhibiting viruses at those sites might also damage the cell.

Several ant-viral medications in the category of nucleoside analogs have been investigated, including remdesivir, molnupiravir, and nirmatrelvir, which are being tested in the treatment of SARS-CoV-2 infections. Antiviral medications and nucleoside analogs are discussed further in Chapters 3 and 8.

## The History of Virology

With the foregoing background, the history of virology can be considered. It might date to the third century BC, based on smallpox-like lesions (rash) on the face of several Egyptian mummies. And the atrophic leg of a figure on an Egyptian stele from as early as 1500 BC has been thought to indicate poliomyelitis virus infection. Of course, at those times and

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for many, many years, there was no concept of viruses (or bacteria) as causes of disease. The concept of the germ (bacterial) theory of disease did not develop until the third quarter of the nineteenth century through the work of Louis Pasteur and Edward Koch. Consideration of disease causation by particles smaller than bacteria did not develop until later, through the work of Dmitri Ivanovsky and Martinus Beijerinck. The latter is credited with coining the word "virus." Carlos Finlay and Walter Reed described the first human virus infection, yellow fever, in 1900 (Chapter 7).

Several recent pandemics have been caused by viruses, including AIDS caused by HIV.

Subsequent to the widespread influenza virus pandemic of 1918–1919, the largest influenza virus pandemics were those of 1957–1958 (Asian flu) and 1968–1969 (Hong Kong flu). The COVID-19 pandemic caused by a novel coronavirus, probably bat-related (discussed below), has been very much in recent news.

The first well-described viral pandemic was the influenza pandemic of 1918–1919. As is the case with many respiratory illnesses, infections likely started as an infection of the upper respiratory tract and in more severe cases included the lower respiratory tract. The upper respiratory tract includes the nose, pharynx, and larynx, and the lower respiratory tract includes the lungs. That pandemic has been estimated to have killed more than 50 million people, possibly ~5% of the population of the world. Many people probably died of bacterial infection superimposed on the viral infection. Antibiotics, to say nothing of antiviral medications, were not available.

In the COVID-19 pandemic, ~0.05% of the population of the world is thought to have died.

For a historical comparison, the Black Death in 1348–1352, caused by the bacterium *Yersinia pestis*, killed ~50% of the population of Europe.

Influenza virus is a single-stranded RNA virus. Influenza types A, B, and C infect humans, with the great majority being due to influenza type

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A. Vaccines exist, but the virus often changes slightly year to year, which may decrease the effectiveness of any one vaccine.[6]

In discussing the SARS-CoV-2, the cause of the COVID-19 pandemic, it was noted previously that protein spikes are present on the outside of each virion (virus particle). For influenza virus, proteins are also on the surface of the virus particles, and these have been well characterized. The two primary influenza virus proteins, which are targets of influenza vaccines, are the neuraminidase (N) and the hemagglutinin (H). Since these virus proteins often change over time, they receive numbers in accord with the change. For example, influenza A may be described as being $H_1N_1$ or $H_7N_9$. Vaccines are changed to keep in step with changes in the virus.

The 1918 influenza pandemic was caused by influenza A virus ($H_1N_1$), the 1957 influenza pandemic by influenza A ($H_2N_2$), the 1968 influenza pandemic by influenza A ($H_3N_2$), and the 2009 influenza pandemic by influenza A ($H_1N_1$).

Human coronavirus epidemics include the severe acute respiratory syndrome (SARS) in 2002–2003 and the Middle East respiratory syndrome (MERS) in 2012. The coronaviruses that caused SARS, MERS, and COVID-19, along with two other human coronaviruses that cause the common cold are members of the *Betacoronavirus* genus.

Epidemics are less widespread than are pandemics.

The HIV pandemic, starting in ~1980, continues as a worldwide problem. Other viral outbreaks in recent years have included those due to Ebola virus, Lassa fever virus, several arboviruses (dengue virus, Zika virus, yellow fever virus, West Nile virus, and chikungunya virus), and measles virus (discussed in Chapters 6 and 7).

Vaccines have been developed to treat (to prevent) viral and other infections. The history of vaccines may start with the smallpox virus vaccine of Edward Jenner (1796) and the rabies virus vaccine of Louis Pasteur (1885). Vaccines are further discussed in Chapter 4.

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## Antigenic Shift and Antigenic Drift

Many viruses, particularly some RNA viruses, show major (antigenic shift) or minor (antigenic drift) changes year to year. As discussed in Chapter 3, mutations occur spontaneously due to nucleic acid (DNA or RNA) changes. Those that are deleterious to the replication of the virus will obviously not be passed on to new virus. Those that increase viral infectivity or rate of virus replication may lead to increased infection. However, there is complexity in that increased infection in one animal host may not have the same effect in another animal host, and an altered virus may enhance immune responsiveness of the infected individual.

Influenza A may change its H and N and be resistant to antibodies that had been raised against its prior H and N forms. The changes are random and are manifest when the changes happen to enhance viral infectivity or replication. Subsequently, these viral advantages may be lost as the host adapts. For some RNA viruses such as influenza, such viral changes may be a clinical problem. Fortunately, measles virus, which like influenza virus is also a single-stranded RNA virus and which in the past often caused severe illness, does not appear to make these changes.

The year-to-year variation of influenza virus, in which there may be clinical significance in the changes, is more the exception than the rule among viruses. Emphasized is that virus variants occur during infections by random changes in the RNA or DNA genome of the viruses. Viral variants are common, although such variations are more common with RNA than with DNA viruses.[7]

Multiple variants have been described for SARS-CoV-2, with reports noting that one variant or another may be more infectious or more pathogenic. Emphasized has been whether variants are inhibited by currently available vaccines.

In considering yearly influenza, the usual question is whether the influenza virus of the year is susceptible to the vaccine previously

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administered. The jury is still out on the clinical importance of SARS-CoV-2 variants, and a conservative viewpoint is probably most reasonable. The clinical importance of some variants and their control with present vaccines is not yet clearly known.[8,9]

At the time of writing this book, more than 15 SARS-CoV-2 variants and subvariants have been described, most recently BA.5.

As discussed in Chapter 4, vaccines against SARS-CoV-2 virus emphasize immune reactivity to the surface "spikes" of the virus. Unless those change, similar to the changes that occur in the hemagglutinin (H) and neuraminidase (N) of influenza virus, vaccines are likely to be effective against SARS-CoV-2 variants. Since different RNA and non-RNA vaccines focus on different antigens, some have said it may make sense for "booster" shots in the future to be of vaccines other than the vaccine the individual initially received.

## Evolution of Viruses

Before discussing the evolution of viruses, brief mention will be made of the possible effects of viruses on evolution. One could speculate on dinosaur viruses and viruses of other extinct animals and hypothesize a possible viral role in extinctions.

Somewhat less speculatively are endogenous retroviral elements (DNA copies of RNA viruses) that are present in animal cells, including humans. They are further discussed in Chapter 6. These elements likely were important in the evolution of cells, and one may consider their possible role in human evolution. These elements exist at present as parts of human cells and play a role in cellular functions. Some have speculated on their being responsible for some human illnesses.

Since viruses can only live (replicate) in living cells, some have reasonably thought that although they seem very primitive, they must have developed after living cells developed. Possibly they developed as pieces of the RNA of bacteria or other early organisms. It is generally

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thought that early life was RNA based, and only later in evolution did the DNA basis that we know today develop.

Because viruses are either RNA or DNA, if viruses developed from primitive organisms, it might suggest two such events, one for RNA viruses (developing from RNA-based organisms) and later one for DNA viruses (developing from DNA-based organisms). That might then require concluding that the panoply of currently existing RNA viruses developed from some primordial RNA virus, and DNA viruses from some similar early DNA virus. Alternatively, there may have been multiple virus from RNA events and also virus from DNA events. Lastly, it is interesting to speculate on the possibility that present DNA viruses evolved from a primordial RNA virus.

Clearer than this speculation is the likelihood that some viruses of animals have evolved to infect humans. An example of this is the likelihood that measles virus developed from rinderpest virus, a virus of cattle. It is thought that measles virus developed ~1,000 to 500 BC in conjunction with cattle farming. In the instance of rinderpest, it is likely a new virus developed, that measles virus developed from rinderpest virus. It is not thought that humans are infected with a variant of rinderpest but rather a new virus, measles virus. Interestingly, rinderpest is currently thought to have been eliminated from cattle. Measles virus is also closely related to canine distemper virus.

An interesting area of speculation might be the time of origin of smallpox virus, which probably developed from a rodent pox virus. When Europeans first came to America, there was devastating loss of life from smallpox, suggesting that Native Americans had very little resistance to the virus. While not at all immune to the virus, Europeans had more resistance than did Native Americans. Second, America was populated by travelers coming across the Behring Strait about 20,000 years ago. Together, these observations might be taken to hypothesize that smallpox virus developed in Europe/Asia/Africa less than 20,000 years ago.

In other instances, rather than the evolution of new human viruses, viruses of animals have on occasion infected humans, and this occurs

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at the present. For example, avian influenza and swine influenza virus may infect humans. Such viruses have a mixture of human influenza and swine or avian influenza genes. One could argue whether such a virus is variant of an animal virus or is a new virus. Random mutations in influenza virus that increase infections, for example, in avian hosts, may not increase infections in humans.

Several viral zoonoses, viral infections of animals that are transmitted to humans, are discussed in Chapter 6.

Changes in viruses to facilitate viral infection of cells not previously infectable by the virus may occur by spontaneous mutation of specific viral genes and by recombination. As noted below, in discussing the origins of SARS-CoV-2, this gain of function is sometimes enhanced in the study of viruses. Viral recombinants may also occur spontaneously during infection. In recombination events, the genes of a virus are mixed with the genes of another virus and a novel, "hybrid" virus is produced.[10,11]

Lastly, it is noted that viruses have been synthesized from "off the shelf" chemicals, the best example of this being poliomyelitis virus.[12]

Poliomyelitis virus is an RNA virus. Since the entire sequence of the viral RNA genome was known, adding RNA nucleotides to a framework until the entire RNA sequence was constructed was possible. Infectious poliomyelitis virus was thus made de novo. The argument in favor of this process is that greater understanding of viral functions will be obtained. Philosophically, this might lend support to the conclusion that viruses are not alive. Ethically, one could question the de novo construction of infectious virus on safety grounds.

## Origin of SARS-CoV-2 Virus

Where did the SARS-CoV-2 virus come from? The answer of course is not definitively known.[13,14] What appears to be true is that the first infections appeared in Wuhan, China. Second, the Wuhan Institute of Virology was

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probably the most active coronavirus laboratory in the world, and it had stored many bat coronavirus isolates. Third, coronavirus isolated from an individual in Wuhan in December 2019 had more than 96% similarity with a bat coronavirus from a cave in China.

Important is that it has not been difficult to adapt coronaviruses to grow in human cells, done in order to more fully study them.[13] This is sometimes termed "gain of function."

The above is circumstantial evidence relative to the COVID-19 pandemic. A reasonable hypothesis is that bat coronavirus was altered so that it grew well in human cells in order to be more completely studied. The virus then escaped from the Wuhan laboratory by accidentally infecting laboratory personnel, who then accidentally infected others in Wuhan.

## Epidemiology

Given the importance and recent emphasis on epidemiology in considering viral infections, a brief discussion of some aspects of epidemiology follows.

In the recent past, epidemiology was emphasized in considerations of viral infections and autoimmune illnesses of the nervous system, including influenza virus vaccine as a cause of Guillain-Barré syndrome, West Nile virus of meningitis/encephalitis, and Zika virus infection as a cause of microcephaly.

More recently, epidemiology has been brought to the forefront in considerations of COVID-19. For example, PCR testing of nasopharyngeal swabs for SARS-CoV-2 RNA in asymptomatic individuals is primarily an epidemiological issue. The primary goal in these studies is not to care for and treat the individuals (they are asymptomatic) but to follow and alter the transmission of SARS-CoV-2 infection in the population.

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While the goals of epidemiology and of clinical medicine are largely the same, to minimize human illnesses, including viral infections, their means and points of emphasis sometimes differ and are interesting to consider.[15]

In very brief, epidemiology emphasizes groups of people, and clinical medicine emphasizes individuals. Of course, if treatment is found to be effective in epidemiological studies of groups of individuals, that would be very important for individuals. Similarly, insights gained from evaluating individual patients may lead to formulating epidemiological studies.

In many ways, these approaches are complementary. Epidemiological studies noted the importance of blood pressure control in populations of people to decrease stroke and heart disease. At present, when a patient sees a clinical medicine physician, blood pressure measurement and means to control blood pressure are greatly emphasized. Epidemiological studies also noted the relationship between smoking and lung cancer, and this is currently a major point of emphasis in clinical medicine.

Preludes to these epidemiological studies were the clinical observations that elevated blood pressure seemed important as a cause of stroke and heart disease. And initial clinical observations that there seemed to be a relationship between cigarette smoking and lung cancer led to epidemiological studies to investigate the relationship.

Early clinical observations of the COVID-19 pandemic suggested the importance of age on survival, and this has been important in epidemiological investigations. However, even if one discusses differences based on age, it would remain necessary to determine what about age is important. Impaired immune responsiveness of the very young and the very old is often cited when considering infectious illnesses.[16,17]

Several of the many points important in considerations of epidemiology are briefly discussed here.

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## PROBABILITY AND STATISTICS

How would one investigate the importance of age, of pregnancy, or of ethnicity and COVID-19? A general start might be to compare groups of people, to determine whether people with the trait being studied differ in illness severity than do others. The goal would be to determine the probability that that the trait is important in illness progression.

Many studies in the past emphasized the probability (the *p*) that smoking caused lung cancer. Others have investigated the *p* of an antiviral medication curing a patient with an illness. The *P* is a means to predict the future, albeit not with 100% accuracy.

Common to both epidemiology and clinical medicine is the use of statistics to estimate the *P*, the likelihood that smoking will cause lung cancer in an individual, and the likelihood that the antiviral medication will cure the patient. The accurate determination of the *P* is the quest, but because of the many variables in determining disease occurrence, severity and cure, statistics need be used.

A significant *P*, indicating benefit of a medication may be thought of as follows. If a medication is more than 1,000-fold more likely to produce a beneficial effect than is no treatment (or treatment with a placebo), the *P* is significant.

So, $P < .001$ indicates the likelihood, the probability of a beneficial effect occurring simply by chance is less than 1 in 1,000. If on testing the medication, the $P < .001$ it would be reasonable to conclude that the beneficial effect (B) after treatment (T) is not simply due to chance.

Almost any treatment may seem to be effective, because some people will do well with no treatment. If 10 people out of 100 do well with no treatment, how many out of 100 would need to do well to demonstrate the benefit of a particular treatment? Appropriate statistical methods may provide the answer.

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## PLACEBO AND NOCEBO EFFECTS

Many issues come up in treatment studies, and statistics may be useful in attempting to determine the *P*. Placebo and nocebo occurrences are two.

Efficacy of treatment is most easily determined in double-blinded, placebo-controlled trials. In determining the *P* of medication efficacy, such trials are the gold standard. When neither the patient nor the treating physician knows whether the patient has received the medication being tested (active medication) or a placebo ("sugar pill"), efficacy may most clearly be determined.

Interestingly, in many studies, the symptoms of some patients receiving the placebo improve – termed the "placebo effect" This is particularly an issue in testing medications to treat pain, where improvement with the placebo may occur in about 30% of treated individuals. Many are aware of reports of individuals who were injured but did not report pain – because of circumstances at the time. This may be thought of as an effect of the mind. The placebo effect may be related to such a mind effect. In testing the efficacy of a medication, the P of improvement need be significantly greater than that of the placebo.

The nocebo effect is the flip side of the placebo effect, and in some ways, it is even more interesting, in terms of a mind effect. In trials where a beneficial placebo effect may be noted in some, others may develop adverse side effects to the placebo, the sugar pill, termed the "nocebo effect."[18] Since the administration of all medications is predicated on a risk:benefit ratio, nocebo-related side effects (risks) may have an effect on whether a medication is used.

Typically, placebo and nocebo effects are thought to be related to information given to patients at the time of clinical trials. Patients in trials are informed as to the possible benefits of the medication being tested (including the improvement of symptoms) and potential adverse side effects of the medication being tested. In such trials, patients do not know

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whether they received the active medication being tested or the placebo, but patients usually believe that they received the active medication.

## PCR TESTING OF ASYMPTOMATIC PEOPLE

In the past, clinicians would likely have performed PCR testing to determine the possible presence of an infectious agent in patients with suggestive symptoms. If the patient did not have symptoms such as fever, congestion, cough, shortness of breath, headache, such PCR testing would likely not have been performed – in the pre–COVID-19 world. This has changed with COVID-19, in large part by epidemiological concerns of the spread of infection.

In terms of screening for SARS-CoV-2 virus, epidemiologists emphasized PCR testing of nasopharyngeal swabs in large numbers of people, including people without clinical symptoms of illness. Goals included understanding how the virus spreads among people and plans to isolate virus-positive people from others to limit disease transmission.

However, the data has also led to an interesting conclusion that may have widespread future impact. As discussed above, SARS-CoV-2, PCR-positive individuals have often been considered as being cases of COVID-19, including individuals who were asymptomatic. Should individuals who are SARS-CoV-2, PCR-positive but who are asymptomatic be considered as COVID-19 cases? Possibly not.

However, it is not so simple. It is well known that people shed various types of viruses and may be asymptomatic. Some are treated for the asymptomatic infection. If they are treated, should they not be thought of as cases of infection?

For example, people with asymptomatic genital herpes simplex virus (HSV) infection may be treated with medications such as acyclovir (Chapters 5 and 8). The medications do not eliminate their infections, and they are treated primarily to prevent their transmitting the virus to

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others – an epidemiological concern. They may be considered as cases of HSV infection, even though they are asymptomatic.

All people who shed virus, including those that are asymptomatic, may be thought of as having at least a low-level virus infection. Virus is being produced in infected cells, and probably those cells are being destroyed – lytic infection is present (Chapter 5). Treatment of virus-positive but asymptomatic individuals will likely receive much future discussion. This blurs the distinction between epidemiology and clinical medicine.

# Therapeutic Viruses and Atypical Agents

This introductory virology chapter will close with an introduction of unusual viruses and virus-like atypical agents.

## VIRUS VECTORS

Some viruses have been used as a means to treat human genetic illnesses. Such viruses have been modified to incorporate specific human DNA genes and are then used as vectors to bring this DNA into human cells. These viruses are also modified to decrease their disease-causing capability. Virus vectors are further discussed in Chapters 3 and 9.

## BACTERIOPHAGE

Interestingly, some viruses can infect, reproduce in, and thereby kill bacteria. Such virus, termed "bacteriophage" (or "phage"), has been considered as possible treatment for some bacterial infections. A specific

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type of bacteriophage would need be used to treat a specific bacterial infection. It would be important to use selective bacteriophage, so that "good" bacteria are not destroyed. The same selective concept is considered in the development of antibiotics. Bacteriophage therapy has taken a back seat to antibiotic use in treating human bacterial infections, but it may be of real value in the future; viruses may be used to control animal and plant pathogens.[19] For example, AgriPhage is a bacteriophage approved for agricultural use in the United States.

It should also be noted, however, that bacteriophages may have deleterious effects. Specific bacteriophages that infect diphtheria and cholera bacteria may lead to those bacteria producing toxins that are the mark of clinical diphtheria and cholera. Specific bacteriophage contribute the genes for those toxins, without which the bacteria do not produce the toxins.

## ONCOLYTIC VIRUSES

Viruses have also been considered as possible therapeutic agents in the treatment of cancer. Varied types of such oncolytic viruses have been studied. A type of herpes simplex virus mutant termed "thymidine kinase negative" (TK neg.) is one such viral mutant that has been studied. This viral mutant grows well in dividing cells (presumably by using the high levels of nucleosides and cellular replication enzymes that are present in these types of cells) but not in nondividing cells (which have low levels of nucleosides and replication enzymes). Some investigators have studied TK neg. mutants for their potential in treating cancer, where rapidly dividing cells are present.

Since neurons (nerve cells) are nondividing cells, TK neg. mutants of this type have also been used to investigate infections of neurons (Chapter 8). The use of oncolytic viruses is further discussed in Chapter 9.

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## ATYPICAL AGENTS

While the emphasis of this volume is on viruses and viral illnesses, discussion of atypical "virus-like" agents is included. Among these unusual agents are endogenous retroviral elements, virusoids, viroids, and prions. The importance of these agents relates in part not only to illnesses they may cause but also to human biology and speculatively to evolution. At the beginning of this chapter, the question was raised as to definitions of living organisms and whether viruses were living organisms. If it can be questioned as to what type of organism viruses are, the difficulty in categorizing the atypical agents is even more difficult.

Endogenous retroviral elements are RNA retroviruses, which as DNA proviruses have become part of the DNA of cells, including human cells (introduced above, in the section Evolution of Viruses). Their functions are part of human biology and biochemistry. It is possibly reasonable to conclude that while their incorporation in cells was a type of infection in the very distant past, at present any disease related to them is more in the category of a metabolic illness than infection.

Mitochondria, another type of endogenous element, is discussed in Chapter 3.

Virusoids are pieces of RNA that cannot replicate, unless they receive biochemical aid from viruses which coinfect cells with the virusoids. And viroids are even more primitive, and they require biochemical aid from the cells that they infect (Chapter 6). Are these primitive entities from which viruses evolved, or did they develop subsequent to the development of viruses and cells, upon which they are dependent for replication?[20]

Lastly are prions, "infectious" proteins that contain neither RNA nor DNA. Amazingly, prion diseases can not only be inherited (genetically transmitted) but are also infectious, and "replicate" by causing normal protein to become abnormal (Chapter 7).[21]

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# The Future of Virology

Important for the future of clinical virology are the new mRNA techniques to develop vaccines. The use of RNA techniques to develop vaccines (discussed in Chapter 4) will hopefully keep pace with any new viruses that appear. RNA vaccines may also be of use in bacterial and in other infections. Might they also be useful in the treatment of cancer?

Also, the great progress in antibiotic development in the last quarter of the twentieth century may well be followed by similar progress in antiviral medication development. The development of effective antivirals, probably first dating from the development of acyclovir (Chapter 8) and given a big boost by the development of antivirals to treat HIV (Chapter 3), will very likely grow as the result of COVID-19. Remdesivir, molnupiravir, ritonavir, and nirmatrelvir are among the antivirals being tested in the treatment of SARS-CoV-2 infections (Chapter 3).

The future of virology is more thoroughly considered in Chapter 9.

Next, Chapter 2 returns to more traditional virology, with emphasis on the means to measure infectious virus particles by the viral plaque assay.

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