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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

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

(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.

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

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,

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.

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

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

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.

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