1 Introduction
Basic Virology

History of Viruses

The existence of viruses was first suspected in the nineteenth century when it was shown that filtered extract of infective material, when passed through filters small enough to stop all known bacteria, could still be infectious, and hence the word 'virus' (Latin for poisonous liquid) was first introduced. However, viral diseases such as smallpox and poliomyelitis had been known to affect mankind for many centuries before that.

Subsequent to the discovery of the existence of viruses, the next major step in elucidating their role in human disease was the invention of electron microscopy, followed by cell culture and now molecular techniques to detect the presence of viruses in infected material. Many new viruses have been discovered in the past two to three decades, but it was the discovery of human immunodeficiency virus (HIV) in 1983, the etiological agent of the AIDS epidemic, that brought clinical virology to the forefront as a significant speciality. There was a concerted effort by the scientific community to identify the causative agent of AIDS and then by the pharmaceutical industry for drug development. This was followed a few years later by the discovery of hepatitis C and hepatitis E viruses. Since then, molecular technology (e.g. cloning and sequencing) has been applied to rapidly identify new viral threats, such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and SARS coronavirus 2 (SARS-CoV-2).

The availability of rapid and sensitive molecular diagnostic techniques and effective antiviral drug therapy means that patients can now be treated in real time. Almost all physicians and healthcare workers have to deal with the consequences of viral infections. The aim of this book is to demystify virology, provide sufficient information to enable the reader to deal with day-to day virus-related problems and achieve the most rapid diagnosis and beneficial treatment.

To do this we must first understand some basic principles of virology.

Viral Taxonomy

Unlike all other organisms, viruses have either DNA or RNA genomes (never both). Viruses are classified in *families* on the basis of their genome (RNA or DNA) and whether it is single or double stranded (SS or DS). SS RNA viruses are further split on the basis of whether they carry a negative (−RNA) or a positive (+RNA) strand as this affects their replication strategy. As a rule of thumb *all DNA viruses except those belonging to Parvoviridae are DS and all RNA viruses except those belonging to Reoviridae are SS.*

Other features of viruses to take into account are their size and shape, and the presence or absence of a lipid envelope, which some viruses acquire as they bud out of

cells. RNA viruses generally tend to be enveloped and have outer proteins (required for attachment to the cell surface) projecting out of this lipid envelope.

The viral genome is packaged within a nucleoprotein (capsid), which consists of a repetition of structurally similar amino acid subunits. The viral genome and the capsid are together referred to as the nucleocapsid. The viral nucleoprotein or capsid gives the virus its shape (helical or icosahedral).

Virus Replication

Viruses are obligate intracellular pathogens and require cellular enzymes to help them to replicate. Unlike bacteria, which replicate by binary fission, viruses have to 'disassemble' their structure before they can replicate. The steps of viral replication can be broadly divided into:

- attachment
- cell entry
- virus disassembly or uncoating
- transcription and translation of viral genome
- viral assembly and release.

The structure of the viral genome dictates the steps in its replication cycle.

Attachment

The first step in the replication cycle is the attachment of the virus particle to the cell surface. To do this, different viruses use specific cellular receptors on the cell surface, which are therefore very specific in the cell type that they can infect – this gives them their 'cell tropism' and is important in disease pathogenesis (i.e. why some viruses only affect certain organs). Influenza viruses use the haemagglutinin protein to attach to the sialic acid–containing oligosaccharides on the cell surface. Viruses may use more than one cell receptor, for example HIV uses the CD4 receptor to attach to the CD4-T-helper cells, but it also uses a chemokine receptor CCR5 as a co-receptor. It is now believed that most viruses use more than one receptor on the cell surface in a sequential binding process.

Cell Entry

Viruses may enter the cell directly by endocytosis or, for enveloped viruses, by fusion of their lipid envelope with the cell membrane.

Virus Disassembly or Uncoating

Before the virus can replicate, the viral genome has to be exposed by removal of the associated viral proteins. This is usually mediated by the endocytosed viral particle merging with cellular lysosomes; the resulting drop in pH dissociates the viral genome from its binding protein.

Transcription and Translation of the Viral Genome

How the virus replicates is dictated by the structure of its viral genome.

- Viruses containing SS +RNA use their +RNA as messenger RNA (mRNA) and utilize the cell's ribosomes and enzymes to translate the information contained in this

+RNA to produce viral proteins. One of the first proteins to be produced is RNA-dependent RNA polymerase, which then transcribes viral RNA into further RNA genomes. These viruses, because they can subvert the cellular system for their own replication, do not need to carry the information for the initial replication enzymes within their genome.

- Viruses containing SS −RNA need to convert it first to +RNA strand, which is then used as an mRNA template for translation or direct transcription to the genomic −RNA. They therefore need to carry a viral-specific RNA-dependent RNA polymerase.
- DS RNA viruses have to first convert the −RNA strand of the DS RNA into a complementary +RNA to be used as mRNA. The +RNA strand of the DS RNA acts as a template for viral genome replication. These viruses also need to carry the RNA-dependent RNA polymerase to initiate the first steps of viral replication.
- Retroviruses are unique SS +RNA viruses. Instead of using the SS +RNA as an mRNA template, the RNA is first transcribed into complementary DNA by an RNA-dependent DNA polymerase in a process called reverse transcription (hence the name – retro = reverse). The normal transcription is always from DNA to RNA. Further transcription then occurs as for other SS DNA viruses.
- DNA virus mRNA is transcribed from the DS DNA viruses in a similar fashion to cellular DNA replication. These viruses can therefore completely depend upon cellular processes to replicate. The genome of these viruses (e.g. cytomegalovirus (CMV), Epstein–Barr virus (EBV)) needs to carry information to code for the virus-specific proteins only. Regulatory proteins and those required for viral DNA synthesis are coded early on and the later proteins are generally structural proteins.
- SS DNA viruses are first converted into double stranded, and then mRNA is transcribed as for DS DNA viruses.

Viral Assembly and Release

Before the virus particle can be released, its proteins and genome have to be assembled within the cell as a 'viral package'. This process may require the cell to alter viral proteins by glycosylation, etc. Viral release may occur either through cell death or through viral budding from cell membrane. Enveloped viruses use the latter mechanism and acquire their lipid envelope at this stage. Viral enzymes may be required for the viruses released via budding (e.g. the neuraminidase of influenza viruses acts on the sialic-acid bond on the cell surface to release the infectious virus particle).

Immune Response to Viruses

Immune response can be divided into two types of responses:

- Innate immunity: innate immune response is the first immediate response to an insult/injury including viral infections. It can be defined as a system of rapid immune responses that are present from birth and not specific to a particular microorganism.
- Adaptive immunity: immunity that develops when the immune system responds to a foreign substance or microorganism for the first time. Unlike the innate immune response, which is immediate, the adaptive immune response may take days or weeks to develop. It is specific for the microorganism and results in immune memory so the

host is able to prevent disease in the future by mounting a quick immune response when exposed to the same pathogen. Adaptive immunity lasts for a long time or may be lifelong.

Innate and adaptive immunity are not mutually exclusive but are complementary defence mechanisms, with defects in either system affecting the host mechanism.

Innate Immune Response

These host defence mechanisms are evolutionarily found in all multicellular organisms, and expressed in humans as conserved elements. There is a vast array of physical, cellular and chemical defence involved in this response. The first line of defence is physical barriers like skin, mucous membrane and mucus, and cilia, which may trap microorganisms. The cellular component is comprised of phagocytic cells (e.g. neutrophils, monocytes and macrophages), which engulf the microorganism and destroy it. In addition, natural killer (NK) cells, a type of T-cell, plays a role in both innate and adaptive immunity. They play a major role in the destruction of cells infected by viruses. Infected cells are destroyed by the release of perforins and granzymes (proteins that cause lysis of target cells) from NK cells, which induce apoptosis (programmed cell death). Chemical defence involves chemokines, cytokines and interferon, which are secreted by the cells of the innate immune system and help regulate the immune response as they do for adaptive immunity.

Adaptive Immunity

The adaptive immune response is aided by the actions of the innate immune response. The adaptive immune response acts by recognition of specific 'non-self' antigens. It is composed of a cell-mediated response effected through T-cells, and antibody-mediated response, mediated through B-cells, which differentiate into plasma cells to produce antibodies.

Cell-mediated response: T-cells are derived from hematopoietic stem cells in bone marrow. T-cells are activated when they encounter a foreign antigen, which is recognised by the unique antigen-binding receptors on their membrane, known as the T-cell receptor (TCR). This process requires the antigen presenting cells (APCs; usually dendritic cells, but also macrophages, B-cells, fibroblasts and epithelial cells). APCs express proteins called major histocompatibility complex (MHC) on their surface, which are classified into MHC class I (present on the surface of almost all nucleated cells) and class II (found on the cell surface of cells of the immune system). The foreign antigen complexed with the appropriate MHC on the APCs is presented to the TCR for activation of T-cells. This stimulates the T-cells to differentiate, primarily into either cytotoxic T-cells (CD8+ cells) or T-helper (Th) cells (CD4+ cells). CD8+ cytotoxic T-cells are primarily involved in the destruction of viral-infected cells. Clonal expansion of cytotoxic T-cells produces effector cells which release substances that induce apoptosis of target cells. Most effector cells die after clearance of infection, but a few of these cells remain as memory cells.

CD4+ Th cells have no cytotoxic or phagocytic activity but they 'mediate' the immune response by directing other cells to perform these tasks and regulate the type of immune response that develops by releasing cytokines that influence the activity of many cell types, including the APCs that activate them.

Antibody-mediated response: This is effected via B-cells which also arise from the hematopoietic stem cells in the bone marrow. Like T-cells, they also carry unique antigen-binding receptors on their cell surface, but unlike T-cells, which require APCs, the B-cell antigen binding receptor can directly bind to foreign antigens that it recognises. Once activated, the B-cells undergo proliferation and differentiate into antibody-secreting plasma cells or memory B-cells which are long lived and can be called upon to eliminate an antigen quickly on re-exposure, by producing the appropriate antibodies. Five major types of antibodies are produced: IgA, IgD, IgE, IgG and IgM. These antibody classes can be further subdivided according to their functionality (e.g. to fix complement, opsonisation (coating of antigen) for destruction, neutralisation of viruses, etc.).

Both the cell-mediated and antibody-mediated responses are interdependent on each other and act in unison to clear infections. T-helper cells secrete cytokines that help the B-cell multiply and direct the type of antibody that will be produced subsequently. Some cytokines, such as IL-6, help B-cells to mature into antibody-secreting plasma cells. The secreted antibodies bind to antigens, flagging them for destruction through complement activation or promotion of phagocytosis by the cells of the immune system, etc.

Viral Pathogenesis

Viral pathogenesis can be described as the process by which the virus interacts with its host to produce disease. As this is a process which involves virus–host interaction, both viral and host factors have a bearing on the pathogenesis of viral disease.

Viral Tropism

The disease manifestation depends upon the organs infected, which in turn depends upon viral tropism. The ability of viruses to infect only certain cell types due to the presence of specific viral receptors on the cell surface has already been discussed. Other factors that affect this tropism are the route of viral entry (e.g. viruses that infect through the respiratory or genital route generally tend to be limited to infections of those systems).

Viral Spread

The mechanism of viral spread is important in pathogenesis. Up to a million potentially infectious particles can be produced as a result of sneezing. The smaller the particle size the more likely it is to escape the mechanical trapping barriers within the respiratory system. The lipid envelope of the enveloped viruses can be easily stripped by detergents or 70% alcohol; such viruses can be easily destroyed in the environment. Only those viruses that can resist the acidity of the stomach can cause gastrointestinal infections. Enteric viruses that spread by the faecal-oral route need to be acid resistant to escape destruction by gastric juices, which may have a pH as low as 2.

Many viruses cause only localised infection as they are unable to spread. Viruses that spread further afield from the infecting site may use virus-encoded proteins to direct their transport within the cell in a way that enhances their spread via blood or along nerves (e.g. polio and rabies viruses). Other viruses, such as CMV, EBV and HIV, are carried by infected blood cells to distant parts.

Measles virus, varicella-zoster virus (chickenpox) and rubella virus all spread via the respiratory route but cause systemic infections. These viruses have a transient ‘primary

viraemia' just after infection to lodge in the reticuloendothelial system. The virus replicates there for a period of time (incubation period) without causing disease symptoms. This is followed by a second longer phase of viraemia (secondary viraemia) when the infection is spread to the target organs to manifest the disease symptoms.

Viral Persistence

Many viruses cause persistent infection, which can be latent, as in herpes virus infection, or chronic, as in hepatitis B virus infection. Many persistent/chronic viral infections can induce malignancies and this is discussed further in Chapter 46.

In latency, the virus lies dormant. The mechanisms of latency are not understood very well, but the virus reactivates from time to time to cause localised infection, as in the case of herpes simplex virus causing cold sores, or may spread along the nerves, as in the case of varicella-zoster virus (shingles). In chronic infection the virus replicates and continues to cause damage. Viruses are able to persist to cause chronic infection: (1) by escaping the immune system by constantly mutating, e.g. HIV; (2) by downregulating the host immune system, e.g. CMV, which codes for proteins that reduce the expression of major MHC class 1 receptors on the cell surface; and (3) by integrating in the viral genome and replicating with the cells, e.g. HIV and hepatitis B virus.

Viral Virulence Factors

Viral virulence is defined as the amount of virus required to produce disease or death in 50% of a cohort of experimentally infected animals. This virulence depends on virus and host factors. Viral virulence determinants are often viral surface proteins. Viruses can also induce apoptosis (genetically programmed cell death) or block apoptosis, depending upon the best strategy for its continued replication and spread.

Host Factors

Disease manifestations may be the direct result of infection or may be immune-mediated as a result of the host immune response to infection. The aplastic anaemia in parvovirus B19 infection is due to destruction of the red blood cells by the virus, whereas the rash is immune-mediated. Hepatocellular damage in hepatitis B infection is a result of destruction of infected hepatocytes by the cytotoxic T-cells. In influenza and Covid-19, most of the symptoms are mediated by the interferon and interleukin pathways as a result of the host response to the virus. Human immunodeficiency virus induces immunodeficiency by destroying the helper T-cells (CD4 cells) of the cell-mediated immune system.

Conclusion

The study of viruses is providing insight into many cellular mechanisms. Understanding of the steps in the viral replication cycle has enabled many 'designer' antiviral drugs (such as the influenza A virus neuraminidase inhibitor drug, oseltamivir) to be manufactured. It is hoped that this brief introduction to basic virology will enable the reader to understand some of the underlying mechanisms that are relevant to the subsequent chapters in this book, and help the reader to make the most of the information contained within.

Section 1 Individual Viruses

Chapter 1

Adenoviruses

The Viruses

Adenoviruses are double-stranded DNA viruses and belong to the family Adenoviridae.

Epidemiology

Route of Spread

There are more than 50 different serotypes (each designated by a number) of adenoviruses and several disease syndromes associated with different serotypes. Respiratory adenoviruses are spread by the respiratory route. Enteric adenoviruses (adenovirus 40 and 41) are spread via the faecal-oral route, and adenoviruses causing conjunctivitis are very infectious and spread by direct contamination of the eye. Adenovirus infections in humans are generally caused by adenoviruses types B, C, E and F.

Prevalence

Adenovirus infections affect all ages. Respiratory adenovirus infections occur every year in the community, causing outbreaks in persons of all ages, often in children in schools and other institutions throughout the year. Severe disease is rare in people who are otherwise healthy. Adenovirus infection accounts for up to 10% of respiratory infections in children. Most cases are mild.

Enteric adenoviruses are a cause of sporadic diarrhoea and vomiting, mainly in young children, throughout the year. Although they cause small outbreaks, usually in community settings, they are not associated significantly with large outbreaks of diarrhoea and vomiting in hospitals and cruise ships.

Adenoviruses associated with conjunctivitis occur sporadically, often associated with clusters of cases. They happen throughout the year, and outbreaks can occur particularly in winter and spring, when they may spread more quickly in closed populations such as in hospitals, nurseries, long-term care facilities, schools and swimming pools.

A total of 75% of viral conjunctivitis cases are due to adenovirus infection.

Incubation Period

2–14 days.

Infectious Period

Patients are infectious while they are symptomatic. Spread occurs mainly when an infected person is in close contact with another person. This may occur by the faecal–oral route, airborne transmission or small droplets containing the virus. Less commonly, the virus may spread via contaminated surfaces.

At-Risk Groups

Immunocompromised persons, who often have prolonged carriage of the virus, especially in enteric infections.

Clinical

Symptoms

- Respiratory adenoviruses cause a range of respiratory symptoms from mild coryza to pneumonia. Clinical symptoms include fever, cough and sore throat due to pharyngitis and tonsillitis. Some infections are asymptomatic. It is difficult to differentiate adenovirus infection from other respiratory virus infections symptomatically, although adenoviruses, unlike influenza viruses, do not usually produce myalgia. Some adenoviruses can also cause a maculopapular rash. Rarely, death occurs due to disseminated adenovirus infection.
- Enteric adenoviruses cause diarrhoea, vomiting and fever, particularly in children less than 2 years of age. The diarrhoea lasts for an average of 8 days (range 3–11 days), longer than diarrhoea caused by rotaviruses.
- Ocular adenoviruses cause conjunctivitis with red, sore injected conjunctiva. It is a very infectious condition and scrupulous infection control procedures are necessary to prevent spread, particularly by the direct contact route. Large outbreaks have been reported. One famous outbreak called 'shipyard eye' occurred in a shipyard in the north of England, when metal workers were treated for metal slivers in their eyes. Contaminated eye instruments were blamed for transmitting the virus.
- In the spring of 2022, the UK Health Security Agency identified a growing cluster of acute hepatitis cases in children under the age of 10 years with no association with travel and hepatitis viruses A–E. Of the children, 66% had adenoviruses (adenovirus 41) detected, and a case control study confirmed this association. Some children had severe infection, necessitating liver transplantation. Adenovirus-associated viruses were also found in the majority of cases. Several different factors are thought to be associated with this outbreak, which was not a point source outbreak, with several adenovirus 41 lineages detected.

Immunocompromised Patients

Organ transplant recipients, especially children, infected with respiratory adenoviruses can have measles-like symptoms (e.g. measles-like rash and conjunctivitis but no Koplik's spots). Bone marrow transplant recipients can experience severe or fatal infection. Enteric adenoviruses can cause prolonged symptoms and viral excretion in transplant recipients, especially children. Many paediatric centres therefore follow their high-risk bone marrow transplant recipients with regular laboratory screens for adenovirus infection.

Table 1.1 Laboratory diagnosis of adenoviruses

Clinical indication	Specimens	Test	Interpretation of positive result
Respiratory symptoms	Nose and throat swab in virus transport medium. Bronchoalveolar lavage fluid. Nasopharyngeal aspirate.	NAAT	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
Conjunctivitis	Conjunctival swab in virus transport medium.	NAAT	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
Diarrhoea and vomiting	Faeces.	NAAT	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
		Rapid test devices	Indicates adenovirus infection.

Adenoviruses may cause myocarditis, meningoencephalitis or hepatitis in immunocompromised people.

Laboratory Diagnosis

Several laboratory methods and clinical specimens can be used to diagnose adenovirus infection (Table 1.1). Nucleic acid amplification techniques (NAAT), like polymerase chain reaction (PCR), are the method of choice (although virus culture, immunofluorescence and electron microscopy can be used if available).

Figure 1.1 shows an electron micrograph of a group of adenoviruses.

Management

Treatment

Aerosolised ribavirin can be given to children with bronchiolitis and intravenous ribavirin can be given (under expert advice) to immunocompromised children. Bone marrow transplant recipients can experience severe and fatal infections and can be treated with cidofivir (see Chapter 54).

Prophylaxis

There is no prophylaxis available. Currently, there is no adenovirus vaccine available to the general public, but a vaccine is available for the United States military for Types 4 and 7. US military personnel are the recipients of this vaccine because they may be at a higher risk of infection because of closer prolonged contact.

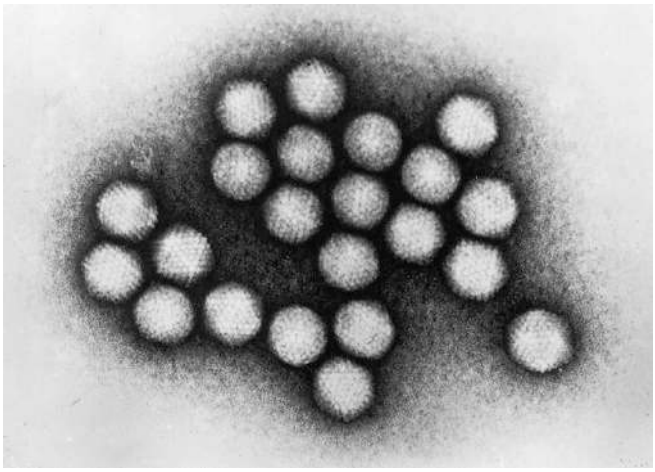

Figure 1.1 A group of negatively stained adenoviruses (courtesy of CDC)

Infection Control

All adenovirus infections are infectious and patients should be isolated whenever possible, especially when in the same ward as immunocompromised patients.