Section 1 – Individual viruses

Introduction to 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 passed through filters small enough to stop all known bacteria could still be infectious, and hence the 'virus' (Latin for poisonous liquid) concept was first introduced. However, viral diseases such as smallpox and poliomyelitis had been known to affect mankind since many centuries before this.

Subsequent to the discovery of viruses, the next major step in elucidating their role in human disease was the invention of the electron microscope, followed by cell culture and now molecular diagnostic 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) (the virus responsible for acquired immunodeficiency syndrome (AIDS)) in 1983 and the explosion of the AIDS epidemic that brought clinical virology to the forefront as a significant specialty. Millions of dollars have been spent by pharmaceutical companies in discovering drugs to treat AIDS; a by-product has been that our understanding of virus replication and pathogenesis has improved substantially and this has resulted in new antiviral drugs becoming available to treat other viral infections.

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, and the aim of this book is to demystify virology and to provide sufficient information to enable the reader to deal with day-to-day virus-related problems.

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

Viral taxonomy

Viruses have either an RNA or DNA genome (never both) and are classified *in families* on the basis of their genome (RNA or DNA) and whether it is single or double stranded (SS or DS). Single-stranded 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 (see below). As a rule of thumb *all DNA viruses except those belonging to Parvoviridae are double stranded and all RNA viruses except those belonging to Reoviridae are single stranded* (see Table 1).

Table 1. *Classification of human viruses.*

Family	Example viruses	DNA/ RNA	DS/ SS	Enveloped	Chapter
Poxviridae	smallpox, cowpox, monkey pox, orf, molluscum contagiosum viruses	DNA	DS	Yes	21
Herpesviridae	herpes simplex viruses types 1 and 2 (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpes viruses 6, 7 and 8 (HHV 6, 7 and 8)	DNA	DS	Yes	3, 4, 10, 12
Adenoviridae	adenoviruses	DNA	DS	No	1
Papovaviridae	papilloma and polyoma viruses	DNA	DS	No	19
Hepadnaviridae	hepatitis B virus	DNA	DS	No	7
Parvoviridae	human parvovirus B19	DNA	SS	No	20
Reoviridae	rotaviruses	RNA	DS	No	25
Picornaviridae	enteroviruses, rhinoviruses, hepatitis A virus	+RNA	SS	No	5, 6, 24
Caliciviridae	hepatitis E virus, noroviruses	+RNA	SS	No	9, 17
Coronaviridae	coronaviruses	+RNA	SS	Yes	27
Flaviviridae	hepatitis C virus, yellow fever virus	+RNA	SS	Yes	8
Togaviridae	rubella virus	+RNA	SS	Yes	26
Paramyxoviridae	parainfluenza viruses, respiratory syncytial virus (RSV), measles virus, mumps virus	−RNA	SS	Yes	15, 16, 18, 23
Orthomyxoviridae	influenza A and B viruses	−RNA	SS	Yes	14
Rhabdoviridae	rabies virus	−RNA	SS	Yes	22
Filoviruses	Ebola virus	−RNA	SS	Yes	2
Bunyaviridae	hantavirus, Crimean–Congo haemorrhagic fever virus etc.	−RNA	SS	Yes	2
Arenaviridae	Lassa fever virus	−RNA	SS	Yes	2
Retroviridae	human immunodeficiency virus (HIV), human T-cell lymphotrophic virus (HTLV)	+RNA	SS	Yes	11, 13

Other features taken into consideration 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, e.g. haemagglutinin (HA) of influenza A virus.

The viral genome is packaged within a nucleoprotein (*capsid*) which consists of a repetition of structurally similar amino acid sub-units. The viral genome and the capsid are together referred to as nucleocapsid. The viral nucleoprotein or capsid gives the virus its shape (helical or icosahedral). Table 1 shows the classification (with examples) of human viruses.

Virus replication

Viruses are obligate intracellular pathogens and require cellular enzymes to help them 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, and viral assembly and release.

Attachment

The first step in the replication cycle is the attachment of the virus particle to the cell surface. To do this specific viruses use specific cellular receptors on the cell surface and therefore are very specific in the cell type that they can infect – this gives them the 'cell tropism' and is important in disease pathogenesis (i.e. why some viruses affect certain organs only). Influenza viruses use the haemagglutinin (HA) 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 viral genome

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

- Viruses containing SS +RNA use their +RNA as 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 a 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 a +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, see below.
- 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 the cellular process 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.
- Single stranded DNA viruses are first converted into double stranded, and then mRNA is transcribed as for the 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 the cell membrane. Enveloped viruses use the latter mechanism and acquire their lipid envelope at this stage. Viral enzymes such as the neuraminidase (NA) of influenza viruses (which acts on the sialic-acid bond on the cell surface to release the infectious virus particle) may be required for the viruses released via budding.

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 factors

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 tend to be limited to infections of those systems). Furthermore certain cells may regulate the expression of viral genes and some viruses can code for tissue-specific enhancers to stimulate transcription of viral genes in certain cells.

Spread

The mechanism of viral spread is significant 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. Only those viruses that can resist the acidity of the stomach can cause gastrointestinal infections. Enteric viruses that spread by a 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 a localized 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 (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 (chickenpox) virus 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 (lymph nodes and spleen). 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. 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 localized infection, as in the case of herpes simplex virus, or may spread along the nerves, as in 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 histocompatibility complex (MHC) class 1 receptors on the cell surface; (3) by integrating in the viral genome and replicating with the cells e.g. HIV, hepatitis B virus (HBV).

Viruses and cancers

Many viruses can induce malignancies and this is discussed further in Chapter 44.

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 is dependent on virus and host factors. The host factors are discussed below. 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 response

Disease manifestations may be the direct result of infection or may be immune mediated as a result of the host immune response to the infection. Hepatocellular damage in HBV infection is a result of destruction of infected hepatocytes by the cytotoxic T-cells. In influenza, most of the symptoms are mediated by interferon produced in response to the infection. Human immunodeficiency virus induces immunodeficiency by destroying the helper T-cells (CD4 cells) of the cell-mediated immune system.

Environmental factors

Some of the viral routes of spread (e.g. respiratory and faecal–oral route) require the viruses to remain stable in a defined environment for a period of time before they can initiate infection. Enteric viruses need to be able to withstand the acidic pH of the stomach before they can reach the intestine to establish infection. For the enveloped viruses, the viral proteins responsible for attachment to the cells are on the outside of the lipid envelope. As this lipid envelope is easily stripped by detergents or 70% alcohol, such viruses can be easily destroyed in the environment. Non-enveloped viruses, such as enteroviruses and noroviruses, are much harder to destroy.

Conclusion

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, 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.

1 Adenoviruses

The viruses

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

Epidemiology

Route of spread

There are 51 different serotypes of adenoviruses (each designated by a number) 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.

Prevalence

Adenoviruses are very prevalent in the UK. 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. 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.

Incubation period

2–5 days.

Infectious period

Patients are infectious while they are symptomatic.

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

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.	Virus culture	Indicates adenovirus infection. Particular serotypes can be diagnosed by neutralization assays.
	Bronchoalveolar lavage fluid.	PCR	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
	Nasopharyngeal aspirates.	Immunofluorescence test on nasopharyngeal aspirates (takes less than 2 hours)	Indicates adenovirus infection.
		EIA	Indicates adenovirus infection.
Conjunctivitis	Conjunctival swab in virus transport medium.	Virus culture	Indicates adenovirus infection. Particular serotypes can be diagnosed by neutralization assays.
		PCR	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
Diarrhoea and vomiting	Faeces.	PCR	Indicates adenovirus infection. Type-specific primers can be used to distinguish between different types of adenoviruses.
		Rapid test devices	Indicates adenovirus infection.
		Electron microscopy	Indicates adenovirus infection.

PCR, polymerase chain reaction; EIA, enzyme-linked immunosorbent assay.

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 infected 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.

Immunocompromised patients

Organ transplant recipients, especially children, infected with respiratory adenoviruses can have measles-like symptoms. 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.

Laboratory diagnosis

Several laboratory methods and clinical specimens can be used to diagnose adenovirus infection. See Table 1.1.

Management

Treatment

There is no antiviral treatment for immunocompetent persons. Bone marrow transplant recipients can experience severe and fatal infections, and can be treated with cidofivir (see Chapter 50).

Prophylaxis

There is no prophylaxis available.

Infection control

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

2 Arboviruses and haemorrhagic fever viruses

Haemorrhagic fever viruses

Haemorrhagic fever viruses are viruses that cause outbreaks of severe or fatal infections with haemorrhagic symptoms, principally in the tropics. These infections are occasionally imported into the UK and other countries outside the tropics, usually causing disease in individual persons, but occasionally resulting in clusters of cases of those infections with person-to-person spread. Since there are several different viruses with different geographical distributions, animal vectors and symptoms, these details have been collated in Table 2.1 to aid differential diagnosis. Knowledge of the outbreaks occurring in different parts of the world and the recent travel history of returning travellers is very important for initial clinical diagnosis. Malaria should always be considered in the differential diagnosis. If haemorrhagic fever is suspected patients should be initially cared for in the highest security isolation rooms available, and immediately transferred to a specialist facility designed to care for cases with haemorrhagic fever once malaria is excluded. No special infection control precautions are required for hantavirus and dengue virus infections.

Although dengue fever is the most common of these viral infections to be imported into the UK, the haemorrhagic form of the disease is relatively rare.

Specimens for diagnosis

EDTA blood for virus culture, or polymerase chain reaction (PCR) and clotted blood for specific IgM antibody. In the UK all diagnostic tests are carried out, according to the Advisory Group on Dangerous Pathogens (ACDP) guidelines, in a category 4, high-security facility.

Lassa fever

Lassa fever virus is an arenavirus. *Incubation period* is 1–3 weeks. Initial symptoms include fever, retro-sternal pain, sore throat, back pain, vomiting, diarrhoea, conjunctivitis, facial swelling, proteinuria and mucosal bleeding. Clinical diagnosis is often difficult because symptoms of Lassa fever are so varied and non-specific. Eighty per cent of people have mild or asymptomatic infection; 20% have severe multisystem disease; 15–20% of hospitalized patients die, but the overall death rate is about 1%. In West Africa 100000–300000 infections occur per year with 5000 deaths. There are a number of ways the virus can be transmitted to humans. Virus can be *transmitted* by