MINI-SYMPOSIUM: RESPIRATORY VIRUSES – PART I

Paediatric
Respiratory Reviews

The classification of viruses infecting the respiratory tract

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Summary Following the boom in respiratory virology in the 1960s, species of rhinoviruses, coronaviruses, enteroviruses, adenoviruses, parainfluenza viruses and respiratory syncytial virus were added to influenza and measles viruses as causes of respiratory tract infection. In restricted patient groups, such as the immunocompromised, members of the family of herpesviruses including herpes simplex, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus and human herpes virus 6 have also been associated with respiratory disease. This list of pathogens was extended last year with the discovery of a novel virus, the human metapneumovirus. More than 200 antigenically distinct viruses have been documented as causes of sporadic or epidemic respiratory infections in infants, children and adults. However, this varied and diverse group can be divided among six distinct families. Understanding some of the basic biology of these families gives an insight into possible strategies for diagnosis, control and therapy.
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INTRODUCTION

Against a background of competing and incompatible classification systems, the International Committee on Nomenclature of Viruses (ICNV) was established in 1966 to establish a universal taxonomic scheme.[1] One of the most important principles embodied in the scheme was to develop the idea that viruses should be grouped according to their shared properties. A second was to focus on the nucleic acid genome as the primary criterion for classification. Viral genomes take a variety of forms: DNA or RNA, double stranded or single stranded, segmented or not, plus (+) or minus (−) polarity, circular or linear. The smallest genomes have a few thousand nucleotides (kb) containing genes for a structural protein and a few essential enzymes. Other larger viruses have genomes of more than 200 kb that include complex regulatory elements. The overall taxonomic system identified four characteristics to be used in the classification of all viruses:

- nature of the nucleic acid in the virion;
- symmetry of the capsid;

- presence or absence of an outer lipid envelope;
- dimensions of the virion and capsid.

With the advent of readily available automated nucleic acid sequencers, it is now possible to obtain very precise information about viral genomes. Sequence data means that quantitative measurements of relatedness can be made with the construction of accurate family trees using specialist software. In fact, the rapid evolution of RNA viral genes, in particular, may enable investigators to differentiate between lineages in an outbreak and help to identify chains of infection at the level of the individual host.

In formal taxonomic usage, the first letters of the virus family, sub-family and genus names are capitalised and the terms are printed in italics. Species designations are not capitalised or italicised. The following represents an example of the full formal taxonomic terminology: order – *Mononegavirales*; family – *Paramyxoviridae*; sub-family – *Paramyxovirinae*; genus – *Respirovirus*; species – parainfluenza virus type 3. Comparative sequencing data identifies genotypes or clades. The many criteria used to group viruses into this hierarchical classification system may be found on the International Committee on Taxonomy of Viruses (ICTV) website.[1] For informal vernacular usage, virus family, sub-family and genus are written in lower case,

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for example, "the picornavirus family" and the "enterovirus genus".

SYSTEMATIC REVIEW

Paramyxoviridae

This family of viruses includes some of the most successful respiratory pathogens causing epidemics of major medical and veterinary importance (Table 1). Their genome consists of a single strand of negative (−) polarity RNA in a linear arrangement of around six genes. These viruses must carry RNA polymerase in the viral particle because the incoming viral RNA can neither be translated nor copied by the cellular machinery. All these viruses have an envelope with surface projections surrounding the nucleocapsid. The projections are the major protective antigens and induce neutralising antibodies and resistance to infection.

Mumps and particularly measles virus infections continue to cause high levels of morbidity and mortality in developing countries but have been largely controlled by vaccination elsewhere. The means to control infections caused by respiratory syncytial virus (RSV), parainfluenza viruses (PIV) and the human metapneumoviruses (hMPV) are the focus of much current research.

The molecular virology of RSV and the PIVs is well understood and forms the basis on which to build a vaccine-development programme.[2,3] The genome of RSV encodes 10 sub-genomic messenger RNAs (mRNAs). These mRNAs are translated into 11 known proteins: four nucleocapsid proteins – nucleocapsid N protein, phosphoprotein P, large polymerase subunit L, and transcription elongation factor M2-1; three transmembrane envelope glycoproteins – fusion F protein, attachment G protein and a small hydrophobic SH protein; two non-structural proteins – NS1 and NS2; a matrix M protein; and a RNA regulatory factor M2-2. The G and F glycoproteins are the major protective antigens and induce RSV-neutralising antibodies and resistance to infection. The order of the genome for RSV appears as: 3'-NS1-NS2-N-P-M-SH-G-F-M2-L-5'.

At the sub-species level, there are two antigenic groups of RSV divided on the basis of their reactions with panels of monoclonal antibodies.[4] Isolates of Groups A and B circu-

Table 1 The *Paramyxoviridae* (human viruses only).

Order	*Mononegavirales*	Species
Family	*Paramyxoviridae*	
Sub-family	*Paramyxovirinae*	
Genus	*Respirovirus*	PIV1, PIV3
	Rubulavirus	Mumps virus, PIV2, PIV4a, PIV4b
	Morbillivirus	Measles virus
Sub-family	*Pneumovirinae*	
Genus	*Pneumovirus*	RSV
	Metapneumovirus	Human metapneumovirus

PIV, parainfluenza virus; RSV, respiratory syncytial virus.

late concurrently during most epidemics. Reverse transcription polymerase chain reaction (RT-PCR) of part of the G gene followed by restriction enzyme digestion analysis is a rapid technique that has been used by Cane et al. to screen samples to obtain an estimate of the genomic variability within an epidemic.[5] Each epidemic was found to be made up of a number of different genotypes and could be considered as an aggregate of separate concurrent epidemics. Genotypes circulating in the UK are very similar to those found in other parts of the world.[6] There is evidence that certain genotypes are more pathogenic than others.[7]

The genetic maps of the PIVs have some similarity to that of RSV encoding N, P, M, F and L proteins that have distantly related functional counterparts to the same proteins in RSV. However, the human PIVs lack NS1, NS2, SH and M2 proteins, and the PIV P gene gives rise to additional accessory proteins. The two protective antigens of the PIVs are the HN (haemagglutinin-neuraminidase) attachment protein and the F protein. The order of the genome for PIV3 appears as: 3'-N-P/C/D/V-M-F-HN-L-5'.

It has been technically more difficult to develop vaccines against viruses that replicate solely on mucosal surfaces. Efforts have focused on developing live-attenuated intranasally administered RSV and PIV vaccines.[8] Historically, vaccines against these two viruses, based on inactivated virus or purified proteins, have been associated with more severe disease in vaccinees; live vaccines appear to induce immunity without disease enhancement. The attenuated viruses are currently designed in vitro by using reverse genetics technology, where infectious virus is produced entirely from cDNA. Pre-determined changes in the nucleotide sequence can be introduced into infectious virus by way of the cDNA intermediate.[9] By using this technology, it may also be possible to create a chimeric vaccine strain that has genes, for example, for several different paramyxovirus F proteins.

Recently, van den Hoogen et al. reported the isolation of hMPV from nasopharyngeal aspirates taken from young children in the Netherlands.[10] Data from that report suggest that hMPV is similar to RSV, in that infection occurs during the winter months and is common in childhood. They subsequently showed that there is a high percentage of sequence identity with avian pneumovirus serotype C (APV-C), the aetiological agent of respiratory disease in birds.[11] APV belongs to the *Metapneumovirus* genus, which together with the *Pneumovirus* genus constitutes the *Pneumovirinae* sub-family (Table 1). The classification of the two genera is based primarily on the gene constellation; metapneumoviruses lack non-structural proteins NS1 and NS2 and the gene order is different from that of the pneumoviruses (RSV) shown above: APV, 3'-N-P-M-F-M2-SH-G-L-5'.

The relationship between hMPV and APV-C is as close as that between the two sub-groups of RSV-A and -B. However, this relationship is not reflected in the host range, since APV infects birds in contrast to hMPV. Sequence analysis of other cultured hMPV isolates suggest that

different stable genotypes exist. Subsequent studies with isolates from Quebec, Canada propose the existence of two distinct lineages similar to the situation with RSV-A and -B.[12]

Orthomyxoviridae

This family of viruses include the three genera, *Influenza virus A, B* and *C*. The genome consists of a single strand of (−) RNA segmented into eight fragments that code for 10 proteins. The RNA is protected by a closely associated nucleoprotein (NP) forming a helical structure around it called the nucleocapsid. The NP is a type-specific antigen and occurs in one of three forms, which provide the basis for the classification of human influenza viruses into A, B and C.

The outer layer of the influenza viruses is composed of a lipid bilayer acquired as the virus buds through the host cell's cytoplasmic membrane. Two important glycoproteins are inserted into the membrane giving the virus a spiky appearance under the electron microscope. The first of these glycoproteins is haemagglutinin (HA), which functions in attachment of the virus to receptors on the surface of respiratory cells. The second glycoprotein radiating from the surface of the virus particle is the neuraminidase (NA), which is involved in the release of newly formed particles from the surface of infected cells.

In addition to the viruses that infect humans, influenza A also infects animal species such as birds, pigs and horses. The most plausible theory for the origin of novel pandemic strains is that the new sub-types are in fact re-assortant viruses resulting from double infection of a single host cell; this is possible because the genome is made up of discrete segments. Major antigenic shift is not seen in influenza B or C because these viruses only infect humans. The antigenic differences of the HA and NA glycoproteins provide the basis of the classification of influenza sub-types. The practical importance of this classification is that no cross-immunity is conferred between different sub-types and the antigenic differences are vital for monitoring epidemics across the world and in vaccine production. Currently, a total of 15 distinct HAs have been recognised among the influenza A viruses.[13]

Antigenic differences also occur in the NA antigen of which nine sub-types have been identified among humans and avian species. It is technically possible to refer to a laboratory influenza isolate as either A or B, followed by the place of isolation, the laboratory number, and the year of isolation; followed by the sub-type designation in parentheses. Thus the influenza virus A/Hong Kong/1/68 (H3N2) signifies an influenza A virus isolated from a patient in Hong Kong in 1968 that was of sub-type H3N2.

The most recent sub-types affecting humans occurred in Hong Kong, where 18 cases of H5N1 were recorded, six of whom died. This influenza A sub-type was transmitted from chickens; however, human-to-human transmission was not documented.[14] Two more recent cases of H9N2 in the same region once again show the potentially devastating consequences of avian influenza sub-types triggering a pandemic of human influenza.[15]

The control of influenza, among high-risk groups, has depended on annual vaccination using inactivated vaccine formulations with current epidemic type A (two strains) and type B (one strain). Intranasally administered, live attenuated vaccines may be more acceptable for children and provide longer lasting immunity. Studies are currently underway to assess the additional benefit of these vaccines over inactivated vaccine in high-risk populations. Two approved anti-viral drugs, known as neuraminidase inhibitors, are available for treatment and prevention of both influenza type A and B infections in older children and adults.[16,17] Zanamivir is given by inhalation and oseltamivir is given orally. Controversy surrounds the use of these drugs because clinically significant side-effects are not tolerable in the treatment of mild self-limiting infections, and treatment should be initiated within 24–36 h of initial symptomatology. The rational use of neuraminidase inhibitors depends to a large extent on the availability of rapid diagnostic tests.[18]

Point-of-care testing (near-patient testing) is available in kit-form utilising enzyme-immunoassay-based technology. Tests are performed at room temperature with no specialist equipment by non-laboratory personnel. These assays will detect virus antigen in 15–30 min but with a sensitivity and specificity range from 60 to 95% and 52 to 99%, respectively. RT-PCR is the most sensitive diagnostic test allowing the use of minimally invasive specimens. However, these tests take hours to complete in their current format and are not likely to play a role in the decision to initiate anti-viral therapy or to institute control of infection procedures.

Picornaviridae

The picornavirus family are a diverse group of human pathogens, including enteroviruses and rhinoviruses that together constitute the most common causes of infections of humans in the developed world (Table 2). The genome is a single strand of (+) sense RNA that encodes a single "polyprotein" of between 2100 and 2400 amino acids. This polyprotein is cleaved in a highly specific manner by proteases to produce both structural molecules and proteins involved in viral replication. The non-enveloped capsid consists of a densely packed icosahedral arrangement of protomers, each consisting of four polypeptides; VP1, 2, 3 and 4, all derived from cleavage of the original protomer VP0. The viral particle is small (hence pico-RNA), 27–30 nm in diameter, while the length of the genome is ∼2500 nm and consequently tightly packed into the capsid.[19]

The human rhinoviruses include more than 100 serotypes in two main groups based on their cellular receptors. Over 90% of rhinovirus serotypes use the intracellular

Table 2   The *Picornaviridae* (six other genera not shown).

Family	*Picornaviridae*	Species	Serotype
Genus	*Enterovirus*	Human enterovirus A	10 coxsackievirus A serotypes
		Human enterovirus B	6 coxsackievirus B serotypes
			33 echovirus serotypes
		Human enterovirus C	11 coxsackie A serotypes
		Human enterovirus D	Enterovirus 68,70
		Human enterovirus E	Poliovirus 1-3
Genus	*Parechovirus*	Parechovirus 1	Echovirus 22
		Parechovirus 2	Echovirus 23
Genus	*Rhinovirus*[*]	Human rhinovirus A	18 rhinovirus serotypes
		Human rhinovirus B	3 rhinovirus serotypes

[*] Many rhinovirus serotypes have not been allocated to a species.

adhesion molecule (ICAM-1) as the cell receptor for attachment. Approximately 10% of the serotypes attach by the low-density lipoprotein (LDL) receptor. A review of rhinoviruses is found in the article by Savolainen in this issue.

Many enterovirus (EV) infections are accompanied by non-specific, usually mild respiratory illness. A review of EV-associated respiratory illness found that 45% of cases presented with upper respiratory tract infections, 13% with respiratory distress, 13% with pneumonia and 12% with otitis media; others presented with bronchiolitis, croup and sore throat.[20]

Initially, EVs were allocated four species on the basis of their pathogenesis in humans and in experimental animals; poliovirus (PV), coxsackievirus A and B (CVA and CVB) and echovirus (E). Through the use of serotype specific sera, enteroviruses were further classified into 64 serotypes (e.g. PV1-3; CV A1-24; CV B1-6; E1-33). Biological and sequence analysis has indicated that two picornaviruses, echovirus 22 and 23, have a number of important features that distinguish them from all other picornaviruses. They have highly divergent sequences and show major differences in capsid proteins. On this evidence, E-22 and E-23 were classified in a new genus, *Parechovirus*, where they were designated human parechovirus 1 and 2, respectively.

With the development of molecular biological techniques, the means of classifying and identifying EVs changed from serotyping assays to genome analysis. In 1999, the re-classification of EVs on the basis of the nucleotide and deduced amino acid sequences was approved by the International Committee on Taxonomy of Viruses (ICTV), after which the 64 EV serotypes in the genus *Enterovirus* were grouped into five species, human EV A–E inclusive. Ishiko et al. have developed a rapid and reliable method based on RT-PCR and sequencing of the VP4 region to place clinical isolates into one of the five species.[21] EVs, like other RNA viruses, have a high mutation rate because of the lack of proofreading activity during genome replication. It is estimated that approximately one mutation is generated per newly synthesised genome and that EVs have a mutation rate 1 million times higher than that of the human genome. Ishiko et al. state that this higher mutation rate is advantageous for phylogeny-based classification because it enables detailed molecular epidemiological studies even using a short region of the genome (207 base pairs of complete VP4).

Apart from the huge public health impact of the poliovirus vaccination programme, control and therapeutic strategies for EV infections are otherwise sparse. Immunoglobulin has been used prophylactically and therapeutically against EV infections in neonates and immunocompromised patients with anecdotal reports of clinical success. The anti-viral drug pleconaril has broad activity against members of the picornavirus family. In a double blind, placebo-controlled trial, 180 patients aged 14–65 years received either 200 mg of pleconaril three times per day, or placebo. Those receiving pleconaril had a 2-day shorter duration of headache and a 2-day faster resolution of all symptoms of aseptic meningitis.[22] The question remains as to whether the benefits of such a drug are worthy of widespread use.[18] However, the control of picornavirus infections is most likely to be achieved by the anti-viral approach rather than by vaccination. X-ray crystallographic studies have revealed very precise three-dimensional structures of the picornavirus capsid and the way they interact with cell receptors. On the basis of such studies, drugs such as pleconaril have been designed to block viral attachment to host cells; other similar drugs will follow.

Coronaviridae

A small number of epidemiological studies suggest that the human coronaviruses are second only to the rhinoviruses as causes of the common cold, accounting for up to 35% of mild upper respiratory tract infections in adults.[23] The human coronaviruses are the most difficult of the human respiratory viruses to detect in the laboratory; they do not grow well in cell culture and are not detected in most routine diagnostic virology laboratories.

Coronaviruses were first isolated 37 years ago in organ cultures of human embryonic trachea or nasal epithelium

and in primary human kidney cell cultures.[24] The majority of human coronaviruses that have been cultured in vitro since then are serologically related to one of two reference strains, 229E and OC43 (OC standing for organ culture). Molecular analysis has shown that these two prototype viruses differ substantially and represent different species, group 1 species and group 2 species, respectively.

The coronavirus genome is a (+) strand RNA of extraordinary size and complexity. It is composed of approximately 30 kb and it is the largest known autonomously replicating RNA.[25] Another unique feature of these viruses is that two-thirds of the genome is devoted to encoding proteins involved in the replication and transcription of the viral RNA. These (+) strand RNA viruses have evolved a different mechanism of mRNA synthesis, one that allows structural and non-structural proteins (generally needed in greater and lesser quantities, respectively) to be separately produced. The latter are expressed from full-length (+) strand genomic RNA, while structural proteins arise from five or more sub-genomic mRNAs. A nucleocapsid protein (N) surrounds this viral nucleic acid.

These medium-sized viruses (60–200 nm) are pleomorphic and have an outer lipid envelope, which is dissolved by ether. Under the electron microscope, the particles have club-shaped surface projections or spikes (S) giving them an appearance reminiscent of a crown (Latin *corona*, crown). Group 2 species (OC43) also contain an additional envelope protein, the haemagglutinin-esterase (HE) protein that remarkably has 30% sequence identity with the equivalent protein in *Influenzavirus C*. S and HE proteins induce virus-neutralising antibodies and consequently could be the likely components of a putative sub-unit vaccine.

Perhaps it is the general unavailability of routine diagnostic tests that have restricted the demand for control of these virus infections by vaccination or by the use of antiviral drugs. The recent advent of molecular amplification tests and the growing use of real-time PCR will give a better appreciation of the levels of morbidity in children and other vulnerable groups. An RT-PCR-hybridisation test has been developed by Vabret et al. that amplified a section of the gene coding for the smaller membrane (M) protein of 229E and OC43. Respiratory specimens (202 sputum and 146 nasal aspirates) were tested with this system and 12 (3%) coronaviruses were detected. The authors concluded that such a test would be useful for investigating the role of human coronaviruses in infections of the respiratory tract.[26]

Adenoviridae

In contrast to the coronaviruses, many members of the adenovirus family are readily propagated in routine laboratory cell culture systems. By the mid-1950s, it was established that these viruses were associated with acute respiratory infections and could maintain low-level persistent infections in lymphatic tissue.

The adenovirus genome is a linear double-stranded DNA molecule containing 30–35 kb pairs coding for at least 10 different structural polypeptides. The viral particles are comparatively small (80 nm) non-enveloped icosahedrons, which are stable to low pH, bile and proteolytic enzymes. The molecular biology of these viruses is understood in great detail. Unlike the RNA respiratory viruses that have been discussed so far, viral DNA replication proceeds with a much higher level of fidelity. Viral DNA polymerases possess an intrinsic exonuclease, which preferentially excises mismatched nucleotides from duplex DNAs. Recent experiments have shown that mutations that impair the exonuclease activity of the DNA polymerase greatly increase the rate of mutation of the viral genome.[27]

The classification of human adenoviruses is now based on nucleotide sequence differences between genomes from distinct viruses. These viruses also display a characteristic G–C content in their DNA which has been exploited by using the enzyme SmaI that cleaves specifically at 5′ CCC GGG. By running the digested DNA on agarose slab gels, it is possible to obtain patterns unique to each of six species (Table 3). This technique provides an efficient means of identification and classification of unknown strains. Other methods to differentiate the six species include G–C%, haemagglutination patterns, oncogenicity and serotype.[28]

Adenovirus infections have, for many years, been successfully controlled among young military recruits in the USA, using live enteric-coated oral vaccines against serotypes 4 and 7. Their use was associated with a 50–60% reduction in overall respiratory illness and a 95–99% reduction in adenovirus-specific illness rates in recruits.[29] The use of this or similar vaccines among the paediatric population has not been investigated. However, these observations show the potential public health benefit of using such a crude vaccine preparation.

Of greater concern are the 5–20% of adenovirus infections that occur following stem cell transplantation with an associated mortality of up to 50%.[30] Low lymphocyte count was the most significant predictor of adenovirus infection, and its recovery was in turn significantly delayed in patients receiving CAMPATH in vivo. Cidofovir, a broad-spectrum anti-DNA viral agent, has been shown to be of therapeutic benefit in life-threatening adenoviral disease in such patients.

Table 3   The *Adenoviridae*.

Family	*Adenoviridae*	Species (number of serotypes)
Genus[*]	*Mastadenovirus*	Human adenovirus A (3)
		Human adenovirus B (10)
		Human adenovirus C (4)
		Human adenovirus D (32)
		Human adenovirus E (1)
		Human adenovirus F (2)

[*] Three other genera not shown – viruses not infecting humans.

Cidofovir is a cystidine nucleoside and phosphonate analogue with both in-vitro and in-vivo activity against a spectrum of viruses, including herpesviruses and adenoviruses.[31]

Real-time quantitative PCR detecting adenovirus genome in whole blood has transformed the monitoring of these infections in the post-transplant period. If anti-viral therapy is required, weekly quantitation of viral load helps to assess therapy. From experience, such treatment schedules may last many weeks.

Herpesviridae

Herpesvirus infections remain a significant cause of respiratory morbidity in iatrogenically immunosuppressed individuals despite advances in diagnosis and anti-viral therapy. The herpesviruses most involved in respiratory complications during the post-transplant period are cytomegalovirus (CMV), herpes simplex (HSV) and varicella-zoster virus (VZV). The roles of human herpesvirus-6 (HHV-6), HHV-7 and HHV-8 remain substantially undefined. There may be complex interactions between these viruses, for example, between CMV and HHV-6, to cause respiratory disease.

The Herpesviridae are a large family of enveloped double-stranded DNA viruses. The genome is tightly wound and enclosed in an icosadelthedral capsid 100–110 nm in diameter. An envelope surrounds the capsid from which glycoprotein spikes project providing antigenic determinants of the individual members of the group. A feature common to all of these viruses is that, following infection, they establish latent infection and persist for life in the host. Re-activation with production of progeny virus may occur at intervals, especially during periods of immunosuppression, to produce recurrent infection. The viruses have a comparatively complex genome coding for many enzymes involved in nucleic acid metabolism.

The ICTV divided the Herpesviridae family into sub-families on the basis of differences in the biological properties of the various viruses (Table 4).

The control of CMV, HSV and VZV infections in immunosuppressed patients has relied on the appropriate use of anti-viral drugs such as aciclovir and ganciclovir. More recently, live varicella vaccines have been licensed for the prevention of varicella. These vaccines, as well as heat-inactivated formulation, have also been found to enhance immunity against VZV in healthy persons. Inactivated varicella vaccine given before stem-cell transplantation and during the first 90 days thereafter reduces the risk of zoster.[32] The protection correlates with reconstitution of CD4 T-cell immunity against VZV.

In the last few years, qualitative and quantitative assays for the diagnosis of CMV and Epstein-Barr virus include estimating viral load by real-time PCR detectable in whole blood. Similarly, quantitation of HHV-6, 7 and 8 DNA is being investigated to define their pathogenic role. Clinical cut-offs or threshold values for appearance of clinical symptoms are being defined for CMV and for Epstein-Barr virus. These values represent the basis for the selection of limits of viral load for initiation of anti-viral treatment, according to pre-emptive therapy strategies.[33] Molecular amplification assays have superseded viral culture for the diagnosis of HSV and VZV infections.

SUMMARY

The greater availability of molecular amplification and sequencing technology is providing data for measuring viral relatedness and diagnostic tools for infections that would otherwise be undetected. Such elusive infections include those caused by coronaviruses, human metapneumovirus and HHV-6, 7 and 8. Molecular amplification techniques are generally restricted to regional centres and are unlikely to provide the rapid diagnosis required for utilising the newer anti-viral drugs or for control of infection procedures. Point-of-care-testing is having its impact on these fronts.

The development of new vaccines for the paramyxoviruses show promise and will ultimately provide huge public health benefits. Anti-viral drugs appear to be the best option for treating enterovirus, rhinovirus and adenovirus infections; the number of serotypes precludes vaccine development.

Immunocompromised patients require regular quantitative real-time PCR monitoring for evidence of disseminated

Table 4 The Herpesviridae (only viruses infecting humans and excluding zoonotic infections).

Family	Herpesviridae	Species	Trivial name or acronym
Sub-family	Alphaherpesvirinae		
Genus	Simplexvirus	Human herpesvirus 1	Herpes simplex 1
		Human herpesvirus 2	Herpes simplex 2
	Varicellovirus	Human herpesvirus 3	Varicella-zoster virus
Sub-family	Betaherpesvirinae		
Genus	Cytomegalovirus	Human herpesvirus 5	Cytomegalovirus
	Roseolovirus	Human herpesvirus 6	HHV-6
Sub-family	Gammaherpesvirinae		
Genus	Lymphocryptovirus	Human herpesvirus 4	Epstein-Barr virus

viral infection caused by adenoviruses and members of the herpesvirus family. Intravenous broad-spectrum anti-viral drugs appear to be the only therapeutic option.

REFERENCES

1. The International Committee on the Taxonomy of Viruses website: http://www.ncbi.nlm.nih.gov/ICTV/

2. Collins Pl, Chanock RM, Murphy BR. Respiratory syncytial virus. In: Knipe DM, Howley PM (eds) *Fields Virology*, 4th edn, Volume 1. Philadelphia: Lippincott Williams & Wilkins, 2001; pp. 1443–1486.

3. Chanock RM, Murphy BR, Collins PL. Parainfluenza viruses. In: Knipe DM, Howley PM (eds) *Fields Virology*, 4th edn, Volume 1. Philadelphia: Lippincott Williams & Wilkins, 2001; pp. 1341–1379.

4. Johnson PR, Collins PL. The fusion glycoproteins of human respiratory syncytial virus of subgroups A and B: sequence conservation provides a structural basis for antigenic relatedness. *J Gen Virol* 1988; **69**: 2623–2628.

5. Cane PA, Matthews DA, Pringle CR. Identification of variable domains of the attachment (G) protein of subgroup A respiratory syncytial viruses. *J Gen Virol* 1991; **72**: 2091–2096.

6. Cane PA, Pringle CR. Molecular epidemiology of respiratory syncytial virus: rapid identification of subgroup A lineages. *J Virol Methods* 1992; **40**: 297–306.

7. Martinello RA, Chen MD, Weibel C, Kahn JS. Correlation between respiratory syncytial virus genotype and severity of illness. *J Infect Dis* 2002; **186**: 839–842.

8. Murphy BR, Collins PL. Live-attenuated virus vaccines for respiratory syncytial virus and parainfluenza viruses: applications of reverse genetics. *J Clin Invest* 2002; **110**: 21–27.

9. Collins PL. Rational design of live-attenuated recombinant vaccine virus for human respiratory syncytial virus by reverse genetics. *Virology* 1999; **54**: 423–451.

10. van den Hoogen BG, de Jong JC, Groen J *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001; **7**: 719–724.

11. van den Hoogen BG, Bestebroer TM, Osterhaus DME, Fouchier RAM. Analysis of the genomic sequence of a human metapneumovirus. *Virology* 2002; **295**: 119–132.

12. Peret TCT, Boivin G, Yan L *et al.* Characterization of human metapneumovirus isolated from patients in North America. *J Infect Dis* 2002; **185**: 1660–1663.

13. Potter CW. Influenza. In: Zuckerman AJ, Banatvala JE, Pattison, JMR (eds) *Principles and Practice of Clinical Virology*, 4th edn. John Wiley, Chichester. 2000; pp. 253–277.

14. Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. *Clin Microbiol Rev* 2001; **14**: 129–149.

15. Peiris M, Yuen KY, Leung CW *et al.* Human infection with influenza H9N2. *Lancet* 1999; **354**: 916–917.

16. Monto AS, Robinson DP, Herlocher ML *et al.* Zanamivir in the prevention of influenza among healthy adults. *JAMA* 1999; **282**: 31–35.

17. Treanor JJ, Hayden FG, Vrooman PS *et al.* Efficacy and safety of oral neuraminidase inhibitor oseltamivir in treating acute influenza. *JAMA* 2000; **283**: 1016–1024.

18. Pillay D. Developments in the treatment of acute viral infections: what's the problem? *Curr Opin Infect Dis* 2002; **15**: 591–592.

19. Minor PD, Morgan-Capner P, Muir P, Enteroviruses. In: Zuckerman AJ, Banatvala JE, Pattison, JMR (eds) *Principles and Practice of Clinical Virology*, 4th edn. John Wiley, Chichester. 2000; pp. 428–434.

20. Chonmaitree T, Mann L. Respiratory infections. In: Rotbart HA (ed) *Human Enterovirus Infections*. Washington DC: ASM Press, 1995; pp. 255–270.

21. Ishiko H, Shimada Y, Yonaha M *et al.* Molecular diagnosis of human enteroviruses by phylogeny-based classification by use of the VP4 sequence. *J Infect Dis* 2002; **185**: 744–754.

22. Shafran SD, Halota W, Gilbert D, Bernstein J, Meislin H, Reiss M. Pleconaril is effective for enteroviral meningitis in adolescents and adults: a randomised placebo-controlled multicenter trial. In: Proceedings of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2002; Abstract 1904.

23. Falsey AR, Walsh EE, Hayden FG. Rhinovirus and coronavirus infection-associated hospitalisations among older adults. *J Infect Dis* 2002; **185**: 1338–1341.

24. Tyrrell DAJ, Bynoe ML. Cultivation of a novel common cold virus in organ cultures. *BMJ* 1965; **1**: 1467–1470.

25. Thiel V, Herold J, Schelle B, Siddell SG. Infectious RNA transcribed in vitro from cDNA copy of the human coronavirus genome cloned in vaccinia virus. *J Gen Virol* 2001; **82**: 1273–1281.

26. Vabret A, Mouthon F, Mourez T, Gouarin S, Petitjean J, Freymuth F. Direct diagnosis of human coronaviruses 229E and OC43 by the polymerase chain reaction. *J Virol Methods* 2001; **97**: 59–66.

27. Flint SJ, Enquist LW, Krug RM, Racniello VR, Skalka AM. Genome replication strategies: DNA viruses. In: *Principles of Virology – Molecular Biology, Pathogenesis, and Control*. Washington DC: ASM Press, 2000; pp 323–324.

28. Wadell G. Adenoviruses. In: Zuckerman AJ, Banatvala JE, Pattison JMR (eds) *Principles and Practice of Clinical Virology*, 4th edn. John Wiley, Chichester. 2000; pp. 310–312.

29. Ryan MAK, Grapy GC, Smith B, McKeehan JA, Hawksworth AW, Malasig MD. Large epidemic of respiratory illness due to adenovirus types 7 and 3 in healthy young adults. *Clin Infect Dis* 2002; **34**: 577–582.

30. Howard DS, Gordon L, Phillips II *et al.* Adenovirus infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 1999; **29**: 494–501.

31. Hoffman JA, Shah A, Ross LA, Kapoor N. Adenoviral infections and a prospective trial of Cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 388–394.

32. Atsuko H, Asanuma H, Rinki M *et al.* Use of an inactivated varicella vaccine in recipients of hematopoietic-cell transplants. *New Engl J Med* 2002; **347**: 26–34.

33. Gerna G. Advances in diagnosis of herpesvirus infections: clinical and therapeutic correlations. *Curr Opin Org Transplant* 2002; **7**: 308–313.